UTILIZATION AND LIMITATIONS OF SOIL HEALTH METRICS IN MISSOURI CORN PRODUCTION DECISIONS

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By

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The undersigned, appointed by the dean of the Graduate School, have examined the dissertation entitled

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presented by Jeffrey D. Svedin,
A candidate for the degree of Doctor of Philosophy, Natural Resources, and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATIONS

To

My beautiful and loving wife

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First, I must thank my Heavenly Father and Savior Jesus Christ to whom I acknowledge for every good decision and major accomplishment in my life. I am filled with gratitude for their abundant mercy and love.

I remember a distinct thought my senior year of high school that only someone truly idiotic would be willing to go 10+ more years of school just to add PhD to the end of their name. Well, 11 years after high school, I guess I was that special kind of crazy. Thankfully, my attitude has changed, and I could not have chosen a more worthwhile and rewarding pursuit. However, it would be a large leap of bravado to say that I have traveled this journey alone. The saying goes that it takes a village to raise a child, and I think that saying works well for finishing a PhD. There is no way that this 'child', me, could have finished my PhD without my own support 'village'.

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ABSTRACT

Soil health benefits are widely acknowledged but empirically-vetted connections to agronomic outcomes remain absent. Therefore, recommendations for on-farm soil health assessments and interpretation remain ambiguous. Empirical connections to two major outcomes remain absent, specifically row crop productivity and fertilizer recommendations. This dissertation investigates potential benefits from incorporating soil health indicators with established phosphorus (P) and potassium (K) fertilizer recommendations, evaluates links between soil health indicators and corn grain productivity, and identifies optimal sampling depths and regional sensitivity to common conservation practices for seven unique soil health indicators. All results and conclusions derive from a dataset collected over three seasons (2018-2020) including 446 sample locations collected from 101 Mid-Missouri commercial row crop systems. Current P and K fertilizer recommendations accurately identified where fertilizer improved yield with 42 and 34% accuracy, respectively. No significant or measurable benefit occurred from incorporating soil health indicators with established P and K soil nutrient analysis when identifying nutrient deficiencies. Investigations into general productivity discovered an empirical relationship between potassium permanganate oxidizable carbon (or POXC) and grain yield. This relationship identified a POXC value of > 415 mg kg soil^{-1} where corn productivity was optimized. Further, POXC outperformed all other established soil analyses in predicting corn grain yield. Finally, regional sensitivity analysis of soil biological indicators of soil health identified important environmental and soil properties to consider when interpreting soil health assessments in Mid-Missouri. Recommendations were unique for each soil health assessment, with specific conservation practices and

optimal sampling depth. In total, these results provide the needed groundwork connecting soil health with agronomic outcomes to support on-farm soil health interpretations.

DISSERTATION INTRODUCTION

Soils are the overlooked backbone of regional, national, and local beneficial ecosystem services. Soils regulate the hydrologic cycle, facilitate nutrient cycling, provide a medium for food production, and is a habitat for plant and microbial populations. However, anthropogenic stewardship has historically accelerated soil degradation processes. A disrupted soil system results in a disrupted hydrological cycle, soil erosion disrupting nutrient cycles and displacing them into sensitive aquatic ecosystems, and the modification of plant and microbial communities. Often anthropogenic impacts on soils are best observed when they are negative, such as the effects of the Dust Bowl, or the estimated loss of half of the topsoil in some Missouri soils during the last 200 years. These impacts from poor management decisions not only have negative impacts on the surrounding ecosystems, but effect on-farm productivity and sustainability.

In recent decades, efforts have been underway to measure, evaluate, and interpret the current state of soil processes to provide these ecosystem services. Simply, it is an evaluation of the soils ability to *function* or an assessment of a *soil's health*. At the heart of these evaluations are soil biological indicators, because of the cascading effects on soil processes and dynamic sensitivity to soil management. However, developing these assessments are challenging because of the complex interactions between soil formation factors and processes, management impacts, and the unique objectives of each assessment. Thus, widespread on-farm soil health evaluations remain elusive. Therefore, the corresponding objectives of this dissertation are to address some of the current questions and critiques regarding on-farm soil health utilization and interpretation.

Chapter 1 outlines the research objectives of the dissertation while providing a literature review of soil health evaluations, current soil biological indicators of soil health, and soil fertility recommendations. Chapter 2 seeks to address a major question regarding soil fertility and soil health assessment. This is accomplished by reviewing the effectiveness of current P and K fertilizer recommendations and evaluating whether integrating soil health assessments into soil fertility recommendations improves current P and K fertilizer recommendations. Chapter 3 addresses current criticisms that soil health assessments are not well connected to relevant agronomic outcomes, specifically grain productivity. Finally, Chapter 4 investigates questions of scalability of identified plot level relationships between soil biological indicators and management practices when employed regionally across a diversity of environments and soils.

Chapter 1:

Literature Review

Soils are composed of biological, chemical, and physical properties which interact to govern and facilitate soil processes that lead to beneficial functions. At regional scales these functions provide essential services for an ecosystem. Soil health assessments generally measure a suite of individual chemical, physical, and biological tests to determine the status of current soil functions (Andrews et al., 2004; Moebius-Clune et al., 2016). Development of these evaluations have been underway for decades, but biological assessments emerged as effective indicators to determine the 'health' of a soil (Karlen et al., 2019; Ndiaye et al., 2000).

The beginnings of soil biological assessments can be traced back to 1916, where organic amendments were used to investigate carbon (C) and nitrogen (N) ratios (Brown & Allisons, 1916). Over 100 years after these first initial investigations, the official USDA-NRCS definition of soil health is "the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans." These early investigations remain the heart of many complex soil health research questions today with empirical connections between soil assessments and soil functions remaining largely understood (Karlen et al., 2019; Yang et al., 2020). Considerable debate remains as how to effectively measure, evaluate, and communicate soil functions (Bünemann et al., 2018; Karlen et al., 2019; Norris et al., 2020).

Looking to the future, it is projected that soil health will move beyond an agronomic perspective and develop a comprehensive management view of soil's many functions to promote effective economic, environmental, and socially acceptable soil management practices (Karlen et al., 2019). Current national and regional efforts are investigating soil health impacts on cropping systems and identifying indicators that are sensitive to management practices and reflect soil functions (Norris et al., 2020). These efforts will yield management sensitive indicators that measure soil function to inform environmental threats and ecosystem services (Bünemann et al., 2018). This literature review provides a brief summarization of the current research

1.1 Relating Soil health Indicators with Soil Function

Promoting soil health emphasizes conservation efforts to increase soil organic matter because of its cascading beneficial effect on soil processes (Karlen et al., 2019). Over time, soil conservation efforts to conserve or improve soil organic properties have coalesced around several main principals, which include:

1) Reducing tillage intensity and frequency;
2) Diversifying crop rotations, including the use of perennial systems;
3) Removing periods of fallow by incorporating cover crops;
4) Returning and retention of crop residues; and
5) Site-specific soil and crop management practices to increase soil organic carbon (SOC).

These practices improve overall soil function by promoting specific agroecosystem functions including soil aggregation, water infiltration and storage, chemical buffering, nutrient cycling, and physical structure for plant growth.

Traditional soil analysis estimates total organic matter, which does not readily respond to management changes. In contrast, soil health indicators were developed to reflect short-term changes in soil properties as a function of management changes, and therefore directly or indirectly measure the labile C pool (Dou et al., 2008; Bongiorno et al., 2019). Constituents of the labile C pool are theorized as water soluble and readily available for microbial turnover and nutrient cycling (Ghani et al., 2003; Singh et al., 2018). These properties make the labile C pool, or biological properties associated with this pool, ideal sample targets to evaluate a soil's function to supply plant essential nutrients. Haynes (2005) went as far as to state labile C pool measurements as central to evaluate soil quality and nutrient cycling in agricultural soils and Franzluebbers (2016) indicated these tests should be included in regular soil fertility testing.

Choosing analyses to include in soil health evaluations has proven difficult, as they must meet a large set of criteria. These criteria have been discussed and debated but largely these tests are i) sensitive to management practices, ii) well correlated with specific soil functions, iii) reflect beneficial ecosystem processes, iv) provide useful information to land managers, and v) easy and inexpensive to measure (Doran & Zeiss, 2000). Additionally, they must be adaptable for high throughput laboratories where traditional soil-testing has occurred (Franzluebbers, 2016; Hurisso et al., 2018b). The following will discuss selected biological tests which reflect direct measurement of properties associated with the labile C pool. These tests are all candidates for commercial laboratory test adoption, and several are included in soil health evaluations such as the Cornell Assessment of Soil Health. Additionally, the NRCS Soil Health Technical Note (NO. 450-TCH-3, 2018) references each of these as direct indicators of nutrient cycling, C food sources, or general reflection of microbial activity.

1.1.1 Soil Respiration

Soil respiration is one of the most employed indicators of soil biological health (Bünemann et al., 2018). Various soil processing and analysis protocols are available, though all involve rewetting of air-dried soil and capturing and measuring the flush of CO_2 released during the predetermined incubation period (Franzluebbers et al., 1996, 2000; Haney et al., 2018; Franzluebbers & Veum, 2020). In the literature, this analysis is sometimes referred to as soil respiration, mineralizable C, or soil-test biological activity (Moebius et al., 2007; Hurisso et al., 2018a; Wade et al., 2018; Franzluebbers & Assmann, 2020). For the duration of this paper, the term soil respiration is used when referring to this measurement.

Soil respiration is broadly defined as the amount of organic C mineralized to CO_2 given a certain time, temperature, and moisture (Diederich et al., 2019). It reflects two portions of soil C dynamics, the availability of labile C sources (i.e., the energy) and general microbial activity. It is commonly utilized to reflect microbial turnover and estimate nutrient mineralization (Gregorich et al., 1997; Franzluebbers et al., 2018; Franzluebbers & Pershing, 2020). Additional applications include soil productivity (Culman et al., 2013; Wade et al., 2020a), changes in soil function induced by management practices (Culman et al., 2013), and potential soil microbial activity (Franzluebbers et al., 2000; Franzluebbers, 2020a). Improving soil respiration with management changes can be complicated by native soil and environmental properties (Dou et al., 2008; Ladoni et al., 2015; Franzluebbers & Assmann, 2020; Franzluebbers & Pershing, 2020).

Hurisso et al., (2016) summarized that soil respiration rate reflects management practices that stimulate or promote mineralization of soil nutrients. Example management practices that should affect soil respiration rates include conventional tillage, manure application, perennial crop, and leguminous cover crops. To this end, Franzluebbers et al., (2020) observed that 3-day respiration rates were greater in an integrated livestock and organic system when compared with a conventional tillage in corn-wheat (*Triticum aestivum* L.) -double cropped soybean (*Glycine max* L.) and wheat – sorghum [*Sorghum bicolor* (L.) Moench] rotation with rye cover crop proceeding corn production. Culman et al., (2013) observed that respiration was three times more sensitive to crop rotation than other management practices, likely because microbial activity is sensitive to residue quality.

During a temporal sampling experiment, Diederich et al., (2019) observed that respiration rates were greater in forage cropping systems compared to grain based systems, with no significant difference between grain systems with various conservation practices. They concluded that increasing the labile C pool in a US Midwest Mollisol required a change in system rather than a simple change in practice. Caudle et al,. (2020) observed the opposite with respiration rates nearly two times greater in a grain crop system compared to a forage based system. This might explain observations by Roper et al., (2017) who observed a significant difference in soil respiration between cash crop conservation management systems, but only at one of three long-term research sites included in the study. Roper et al., (2017) concluded that further investigations would be required to ensure scalability of current soil respiration relationships to all soils and environments.

Soil respiration has received the greatest amount of attention in incorporating a biological test into fertilizer recommendations (Haney et al., 2008; Franzluebbers, 2016). Marumoto et al., (1982) suggested the possibility of using soil respiration from rewetted soil to estimate mineralization of soil N and P. Hurisso et al., (2016) observed that soil respiration reflected short-term mineralization and could be a useful indicator for short-term nutrient availability. More recently, Franzluebbers & Pershing (2020) suggested merging soil respiration with STK and STP analysis as an overall fertility assessment. Culman et al., (2013) asserted that soil respiration was a good indicator of overall agronomic productivity. However, this was contradicted in a regional analysis of the US corn belt where soil respiration was more related to inherent site characteristics rather than relative yield (Wade et al., 2020a). Further work is needed to tease out the nuances of soil respiration's connection to agronomic productivity and nutrient availability. Field calibration is especially needed to quantify theorized connections between soil respiration and fertilization recommendations.

Incorporating soil respiration into fertilizer management has generally coalesced around predicting N fertilization decisions (Franzluebbers, 2016; Franzluebbers & Pershing, 2020). Soil respiration is utilized as an indicator of N mineralization and N recommendation rates are derived from these respiration rates. But soil respiration is inconsistent in improving N fertilizer recommendations across environment and climate conditions (Franzluebbers, 2018, 2020b; Yost et al., 2018; Bean et al., 2020; McDaniel et al., 2020). Further work is required to connect soil respiration to N recommendation and other plant essential nutrients. Further, SR research has overlooked improving other macronutrient fertilizer recommendations. Exploring potential relationships with P, K, and S are justified as they constitute regular fertilizer applications with economic demands and pollution concerns (Sharpley et al., 1998). Sulfur is especially interesting because the nutrient cycle is largely driven by microbial processes (Schoenau & Malhi, 2015) and is increasingly deficient in cropping systems (Haneklaus et al., 2015).

1.1.2 Potassium Permanganate Oxidizable (Active) Carbon

Potassium permanganate oxidizable carbon (POXC) refers to a method established in 2003 which uses potassium permanganate to oxidize soil C as an indicator of "active carbon" (Weil et al., 2003). Traditional soil-testing measures total oxidation of organic C while POXC measures a partial, or incomplete oxidation (Culman et al., 2012). POXC analysis utilizes slightly alkaline K permanganate (KMnO4) to react with the most readily oxidizable forms of soil C to estimate labile C (Weil et al., 2003; Culman et al., 2013; Hargreaves et al., 2019). This is likely a relatively processed pool of labile soil C, which is sensitive to management and the nature of the present vegetation (Skjemstad et al., 2006; Hurisso et al., 2016). As such, POXC responds to management changes more quickly than total soil organic C, and is often used to evaluate short- and long-term impacts of crop and soil management (Melero et al., 2009; Culman et al., 2012; Plaza-Bonilla et al., 2014; Tatzber et al., 2015; Hurisso et al., 2016; Morrow et al., 2016; Ramírez et al., 2020).

POXC is sensitive to various conservation management practices in different environments. In Michigan, POXC values were observed to reflect current fertilizer management and rotational diversity, with greater POXC measured in corn-soybean-wheat rotation compared to continuous corn (Culman et al., 2013). In North Dakota, POXC values increased with conservation practices, but there was no difference when comparing no-till and strip-till practices (Awale et al., 2013). A similar observation was made in the southeastern US, where there was a difference when comparing no-till and moldboard plough, but no detectable differences when compared to other conservation tillage practices (Singh et al., 2020). However, there was a significant difference between conservation tillage practices when cover crops and double cropped wheat-soybeans were included with the no-till management (Singh et al., 2020). This observation coincided with Plaza-Bonilla et al., (2014) who concluded POXC was more sensitive to management practices when organic matter inputs are increased. This conclusion is harmonized with increased POXC measurements from a radish cover crop in the top 0-15 cm that extended into deeper horizons reaching 90-105 cm (Wang et al., 2017). In general, it was noted that POXC reflects management practices that promote the accumulation and stabilization of soil organic matter; and it might be a good indicator of long-term soil C sequestration (Hurisso et al., (2016). In concert with this conclusion, POXC has been used to indicate soils where improved soil organic matter management is likely to improve productivity (Lucas & Weil, 2012).

POXC is a chemically-defined fraction of soil C and as such it is thought to reflect the biologically active pool related to other biological tests (Wade et al., 2020b). It is related to soil respiration (Culman et al., 2013; Hurisso et al., 2016), aggregate stability (Fine et al., 2017b), and microbial diversity and abundance (Ramírez et al., 2020) in a variety of environments. However, while related to these other biological tests, POXC responds uniquely to C inputs and management practices. When comparing labile C measurements in 10 long-term European field experiment, POXC was the most sensitive labile C measurement to tillage practice and C inputs and was recommended as the labile C fraction measurement for soil health assessments (Bongiorno et al., 2019). Similar observations were made in Canada, where POXC was recommended as the most useful indicator for farmers to track improvements from soil health practices in organic farms (Hargreaves et al., 2019). In an analysis of the Cornell Assessment of Soil Health (CASH), POXC was found to be the best single predictor of soil health status when evaluated with the CASH score index (Fine et al., 2017a). Finally, after a comparison between biological tests at five study sites in the United States Pacific Northwest, Morrow et al., (2016) recommended POXC as an integral component of soil health assessment. However, POXC measurement values are related to inherent soil properties and further research is required to develop baseline or reference values for various soils (Caudle et al., 2020).

1.1.3 Autoclaved-citrate extractable (ACE) Soil Protein

Soil autoclaved citrate extractable (ACE) protein was developed and reported as the “easily extractable glomalin” procedure by Wright & Upadhyaya (1996). The analysis includes the addition of 0.02 mol L^{-1} sodium citrate (pH = 7) followed by autoclaving with protein extracts quantified using the Bradford or bicinchoninic acid assays. Initially reported as an extraction method for “glomalin”, a protein produced in large quantities by arbuscular mycorrhizal fungi (Wright & Upadhyaya, 1996), the “easily extractable glomalin” method was expanded to represent general organic soil proteins extracted using the ACE protein method (Rosier et al., 2006; Hurisso et al., 2018b). As such, ACE protein has replaced all future references of glomalin for the remainder of this paper. This test is already included in the CASH assessment (Schindelbeck et al., 2016) and this rapid analysis is creating significant interest in widespread adoption (Hurisso et al., 2018b). Interest in ACE protein is generated in two parts, i) as an indicator of N mineralization to be used as a tool to inform N fertilization; ii) as an indicator of soil health connected to soil organic matter and aggregation processes.

The general soil protein pool which ACE protein measures represents the largest pool of organically bound N in soil, and is considered a reservoir of N that could quickly be released through mineralization processes (Roberts & Jones, 2008; Hurisso et al., 2018b). Historically, commercial laboratories offered inorganic N (NO_3^- , NH_4^+) analysis and total N for fertilizer recommendations. The inorganic N pool is temporally variable and represents a fraction of the seasonally available N and while total organic N is a less seasonally variable pool, neither inorganic or total organic N have proved reliable for fertilizer recommendations (Culman et al., 2013). Therefore, measuring a stable and readily available pool of N might give greater insight into mineralizable N for fertilizer recommendations. Depolymerization of soil protein is widely considered the rate limiting step in N mineralization, and measuring soil protein has been compared to measuring the 'source' of mineralized N (Jan et al., 2009; Hurisso et al., 2018b). Autoclaved citrate extractable soil protein is an effective estimate of this pool of N with detection reported up to 78% of the total pool (Geisseler et al., 2019). However, in this same study, ACE protein did not correlate well with net N mineralization rates (10-week incubation time) across a diversity of climates in California. Similarly weak correlations between ACE protein and 7-d anaerobic N mineralization have been observed but were attributed to the high variability of the N mineralization method (Hurisso, Moebius-Clune, et al., 2018b). Further investigation of the nuances between ACE protein concentrations and mineralizable N are needed prior to fertilizer recommendation development. Despite the weak correlation with N mineralization, ACE protein remains useful for soil health assessment because of its sensitivity to management practices and its relationship with soil aggregation processes.

Autoclaved citrate extractable protein has demonstrated clear correlations with soil aggregate processes, specifically aggregate stability (Geisseler et al., 2019). Connections between aggregate stability and ACE protein were established quickly after development, with greater concentrations of ACE protein connected with greater aggregate stability (Wright & Upadhyaya 1998). Concentrations of ACE protein were significantly correlated with increased aggregate size, and it was concluded that the ACE protein pool acted as a microbial glue in soil aggregation (Wright et al., 2007). In a three-year transition from plough tillage to no-till and a grass-covered reference, a positive correlation coefficient of 0.78 between ACE protein concentrations and aggregate stability was observed (Wright et al., 1999). A similar correlation coefficient (r = 0.77) was observed after partitioning soil aggregates into various sizes under a diversity of tillage and cropping practices (Nichols & Millar, 2013).

Due to clear correlations with aggregate stability, ACE protein provides value from its sensitivity to management practices. Incorporating wheat and millet into a no-till crop rotation increased ACE protein concentration relative to traditional crop rotations (Wright & Anderson 2000). In Chile, Borie et al., (2006) observed no-till and reduced tillage systems led to increased ACE protein concentrations compared to a 20cm depth moldboard plough tillage. Rillig et al., (2003) observed greater concentrations of ACE protein in native forest soils compared to agricultural fields. In South Dakota, Nichols & Millar (2013) observed elevated ACE protein concentrations in perennial grazed rangeland systems compared to row crop systems. In the same study, wheat-corn rotations with conventional tillage and manure applications contained the greatest ACE protein with no difference between no-till and conventional tillage without manure. The addition of manure would naturally increase ACE protein as it contains a significant protein pool, but the authors did not explain why there was no difference between no-till and conventional tillage without manure additions. However, study of tillage effects on soil organic matter constituents and aggregate stability observed a 63% increase in aggregate stability which the author attributed to increased concentration of ACE protein, fungal populations, and wax compounds found in the no-till system (Pikul et al., 2009). Liebig et al., (2006) observed a 27% increase in ACE protein from reduced tillage and increased crop diversity; however, this observation only occurred at one of seven research locations. They concluded that inherent soil characteristics influence how ACE protein responded to management practices. This observation aligns with Fine et al., (2017) which observed regional soil characteristics, such as soil texture and climate, influence baseline ACE protein concentrations. Concentrations of ACE protein were significantly different among three US regions: Midwest, Northeast, and Mid-Atlantic and varied by soil textural grouping. For example, average regional concentrations varied by textural class with the greatest concentrations in the Northeast US region observed in coarse textural groups and the greatest concentrations in the Mid-Atlantic region found in medium textural groups.

1.1.4 Extracellular Enzymes

Microorganisms, and to a lesser degree plants, exude extracellular enzymes (EE) to facilitate the breakdown and recycling of C rich bio-macromolecules. Originally brought into agroecosystems to enrich soil fertility knowledge (Kuprevich & Shcherbakova, 1971), research exploring response of EE to soil management later shifted towards soil health (Dick, 1994). These enzymes have sensitivity to accumulation of organic C in surface soils from no-till, organic amendments, and cover crops that make them key candidates to reflect soil nutrient cycling functions (Dick, 1984; De la Horra et al., 2003; Melero et al., 2009).

β-Glucosidase catalyzes the hydrolysis of β-D-glucopyranosides in the final, rate-limiting step in the degradation of cellulose (Stott et al., 2010). Cellulose is the most abundant polysaccharide in the earth and β-glucosidase is the final step in providing simple sugars for the soil microbial population. As a component of the decomposition of SOM, β-glucosidase reflects a soil's capacity to break down plant residues and cycle nutrients. It is sensitive to soil and residue management changes (Miller & Dick, 1995; Deng & Tabatabai, 1996), responds to cover crops and organic amendments (Bandick & Dick, 1999), and can respond within 1-3 years after adoption of conservation or no-till practices (Roldán et al., 2005). Ndiaye et al., (2000) observed β-glucosidase and arylsulfatase activity were significantly greater after the second season of cover crops, while standard physiochemical soil tests did not respond to cover crop treatments even after 7 years of implementation. De la Horra et al., (2003) observed a 44% increase under no-till management in the top 0-5 cm in a no-till system. The sensitivity to management practices and soil functions led Stott et al., (2010) to propose β-glucosidase as a soil health indicator in the Soil Management and Assessment Framework (SMAF).

Other enzymes are integral to specific nutrient cycling. Arylsulfatase catalyzes the hydrolysis of arylsulfate by fission of the O-S bond and provides plant available SO_4^- (Spencer, 1958) and was discovered in soil by Tabatabai & Bremner (1970). Acid phosphatase is a phosphoric monoester hydrolase that acts on ester bonds to release orthophosphate (Deng & Tabatabai, 1997). Deng & Tabatabai (1997) demonstrated that both enzyme activities are more sensitive to organic additions than tillage management. Dick (1984) observed that 19 years of no-till led to amplified activity rates relative to tillage treatments. Deng & Tabatabai (1997) observed that arylsulfatase was more sensitive to the additions of mulching applications than tillage management with no significant mulch effect for acid phosphatase activity. Klose et al., (1999) observed that arylsulfatase activity increased with less tillage disturbance with the greatest activity observed in cereal-meadow or oats rotation. Finally, García-Ruiz et al., (2008) summarized that as tillage intensity increased, extracellular enzyme activity decreased and improvements to soil quality significantly slowed down. Fertilizer application can also inhibit extracellular enzyme activity, such as where P and S fertilization inhibited acid phosphatase and arylsulfatase activities (Baligar et al., 2005).

1.1.5 Soil Health Scores

Soil health measures the complex functions of a soil to evaluate the 'health' of the soil. Single indicators are inherently limited in their ability to measure the multitude of chemical, physical, and biological functions soils perform. Further interpretation is complex because of the interactions between soil formation factors and processes and genetic soil characteristics. For example, all soil biological tests are corelated with SOC; thus, in soils with greater baseline SOC (*e.g.* mollisols) the aforementioned soil biological indicators will be greater—but that does not indicate the relative soil health is greater. Therefore, regional interpretations must account for baseline physical, chemical, and biological characteristics.

Alternatively, developed indexes give recommendations from compiled analyses used to measure a suite of soil functions. Examples of this approach include the SMAF (Andrews et al., 2004), CASH (Moebius et al., 2007; Moebius-Clune et al., 2016), or Soil Health Assessment Protocol and Evaluation (SHAPE; Nunes et al., 2021). The SMAF was primarily developed as a tool to answer whether current management practices were improving, sustaining, or degrading soil function (Andrews et al., 2004; Karlen et al., 2019). The CASH and SHAPE evolved from the SMAF, but each tool incorporates a suite of physical, biological, and chemical analysis to assess the complex interactions of land management impacts on soil health (Andrews et al., 2004). The CASH and SMAF are somewhat restricted to the region their correlation data were developed while SHAPE was developed to overcome these regional deficiencies (Roper et al., 2017; Chu et al., 2019; Nunes et al., 2021). Work is ongoing to link these tools to specific ecosystem services and outcomes, including soil fertility recommendations.

1.2 An Introduction to Soil Health and Soil Fertility with their Possible Interactions

A doubling of global crop yield from 1960-2000 coincides with a seven- and three-fold increase in nitrogen (N) and phosphorus (P) fertilizer applications (Tilman et al., 2002). In the United States and England, 40-60% of recent corn (*Zea mays* L.) yield increases are attributed to fertilizers (Stewart et al., 2005). It is obvious that fertilization is fundamental in agricultural yield success worldwide. However, widespread fertilization in agroecosystems is leading to regional, local, and worldwide environmental issues (Fausey et al., 1995; Mueller & Helsel, 1996; Sharpley et al., 1998, 2018; Kleinman et al., 2011, 2019; Krempa & Flickinger, 2017). These positive, yet also adverse effects, place judicious fertilizer management at the heart of sustainable agroecosystems (Sharpley et al., 2015; Ros et al., 2020).

Sustainable fertilizer management has evolved around the 4R program. These R's include the right source of nutrients, at the right rate, at the right time, and in the right place (Johnston & Bruulsema, 2014). Addressing the right rate addresses two primary questions 1) How much of nutrient "x" will be plant available during the season? and 2) How much fertilizer should I place to maximize yield and limit environmental impacts? Public and private initiatives have been developed to address these two questions. The most widely adopted, whether directly or through modified recommendations, are Land Grant University fertilizer decision support recommendations. These tools are built on measuring plant available nutrients (soil-test values) and then through correlation and calibration field trials establishing yield responses to these soil measurements (Bray, 1954; Mehlich, 1984; McGrath et al., 2014).

Fundamental research evaluating soil-tests and yield relationships were developed decades ago under monoculture systems with regular tillage and fallow (Bray, 1954; Mehlich, 1984). Today's agriculture fields are managed under no-till or reduced tillage, and may include diverse crop rotations with cover crops grown between cash crops. These conservation management practices have multifaceted impacts on soil management including erosion prevention, alleviating weed and disease pressure, and improving soil biological activity (Dou et al., 2008; González-Chávez et al., 2010). Improved soil biological activity has been connected to greater nutrient cycling and some have suggested agroecosystems with improved conservation practices requiring less fertilizer (González-Chávez et al., 2010). Others suggest incorporating biological or soil health tests may improve fertilizer recommendations (Franzluebbers, 2016). However, while conceptual support exists, there is no empirical evidence regarding how biological tests can meaningfully direct fertilization decisions (Bünemann et al., 2018) Therefore, a significant need exists to investigate whether including additional soil health metrics, specifically biological soil tests, will improve current fertilizer decision-support tools.

1.3 Behind the Curtains of Fertilizer Recommendations

Soil-testing and fertilizer recommendations were established as commercial fertilizer became available and the agriculture industry asked a simple question, how much fertilizer should be applied to maximize yield? The subsequent fertilizer recommendations were developed with similar approaches, including nutrient extraction with corresponding calibrations to field plot trials (Voss, 1998; McGrath et al., 2014). Such fertilizer recommendation development began in the 1920s with the majority of field calibration work peaking during the 1950s and 1960s. Validation research has continued, but little has been conducted since the 1980s (Voss, 1998).

From this work, two primary recommendation philosophies emerged within the United States. Both these philosophies generally reflect the premise popularized as 'Liebig's law of the minimum' (Voss, 1998; McGrath et al., 2014). This law states that production is restricted by the most limiting nutrient for a specific plant's needs (Jungk, 2009; McGrath et al., 2014). Therefore, each plant essential nutrient must be at a plant specific sufficient level to maximize yield. However, at some point, adding this nutrient will no longer produce a yield response, leading to economic losses and environmental impacts. Therefore, these recommendation philosophies are centered around identifying definable soil-test levels below which crops will respond to fertilizer additions, and above which they likely will not respond to further application (Voss, 1998). These are commonly referred to as "critical values" as identified in Figure 1.1.

The 'build-up and maintenance' philosophy identifies a critical soil-test value below which increasing fertilizer rates are recommended to increase yield, and above this critical value fertilizer application rates should match crop removal (Voss, 1998). This approach was largely developed to identify yield relationships with soil-test P and K values with methods identified in Figure 1.1 (Bray, 1944, 1948, 1954). A variation to this was called the 'sufficiency' philosophy. This approach, as described by Olson et al., (1987), established "low", "medium", and "high" soil-test values with respective probability of crop response to applied nutrients [i.e., 'assured', 'possible', and 'unlikely', respectively (Voss, 1998)]. Both of these methods hinge on relating accurate yield response with fertilizer applications at various soil-test values; therefore, environmental sustainability of fertilizer inputs depend on the accuracy of soil-test values and yield response relationships.

Recent research demonstrated possible improvements in soil-test P (STP) and soil-test K (STK) recommendations. In Arkansas it was observed STP and STK recommendations only accurately predicted the correct crop response to fertilization at 38-50% of locations for P and 60-78% of locations for K (Fryer et al., 2019). Earlier, Heckman et al., (2006) observed yield responses to fertilization at only 17-43% of the 51 field sites with STP levels below state recommended optimal levels. They also observed yield responses to fertilization at sites above optimal and some excessively high STP (Heckman et al., 2006). In Minnesota, Randall & Evans, et al., (1997) observed similar yield response to P fertilization near the critical Bray STP 20 mg kg^{-1} soil (6 of 12 site years) and then at half the critical limit of 10 mg kg^{-1} soil (8 of 12 site years). In this same study, K fertilization increased yields 4 of 24 years at sites with STK greater than the critical limit (Randall et al., 1997a). Finally, Fulford & Culman (2018) observed yield responses to fertilizer at only 9 of 42 sites years and challenged the appropriateness of Ohio fertilizer P and K recommendation rates because fertilization at double the recommended rate (removal rate) failed to maintain STP and STK over nine years.

The question arises, in what environmental conditions do traditional soil-test recommendations fail, and why? A number of possible explanations exist. Fertilizer recommendation error could be introduced by applying state or regional soil-test values across diverse environments and soils (Dodd & Mallarino, 2005). Investigations have shown how build-up of soil nutrient levels and the associated removal with yield vary with soil properties and environmental conditions, with some soils not displaying yield decreases until up to 8 years after no fertilization (Randall et al., 1997b; a; Fulford & Culman, 2018). Minnesota observed up to 30% yield losses attributed to low STP and therefore identified a recommended STP critical range from 15-20 mg kg^{-1}. However, the critical values varied between calibration sites with optimums being identified as low as 12.7 and as high as 19.2 mg kg^{-1} (Randall et al., 1997b). Similar results were observed in Iowa when examining over a 30-year period yield response to STP. Here critical concentrations varied from 6-10 mg kg^{-1} at various research sites with different soils (Dodd & Mallarino, 2005).

Another issue is interpretation subjectivity. For example, in Iowa the STK recommendations were confirmed as being robust with accurate critical values when identifying profitability standards for corn production (Mallarino et al., 1991). Yet in Arkansas Fryer et al., (2019) observed fertilizer recommendations were highly accurate for identifying soil that did not require fertilizer P (100% accuracy), but suboptimal in accuracy (0-20%) at identifying soils responsive to P fertilizer application. They concluded Arkansas recommendations were skewed to minimize the risk of yield loss from under fertilization. Heckman et al., (2006) made a similar observation when concluding soil-test recommendations are often tailored to error toward over fertilization to avoid yield decreases from under fertilization. Yet, recommendations built for over fertilization can lead to financial and environmental costs to farmers and society (Mallarino et al., 1991b; Lemunyon & Gilbert, 1993; Sharpley et al., 1993, 1998; Randall et al., 1997b; Dodd & Mallarino, 2005; Dodd & Sharpley, 2015). These recommendation systems are especially environmentally and fiscally concerning.

Finally, in addition to environmental variability and some subjectivity in developing fertilization schemes, cropping systems and management have changed substantially during the last 50 years. During peak fertilizer rate recommendation developments, cropping systems were often monocultures with limited or no crop rotation, absent of cover crops, and regularly buried crop residue with intensive tillage (Voss, 1998; McGrath et al., 2014). These practices resulted in nutrient loss and severe soil erosion, degrading local and regional water quality and soil resources. Over time, governments and organizations have responded with many initiatives promoting the prevention of nutrient pollution and soil erosion by: (1) keeping the soil covered; (2) disturbing the soil as little as possible; (3) keeping plants growing throughout the year; and (4) diversifying crop species in the rotation (Dodd & Sharpley, 2015). These conservation practices have the overall goal of preventing soil erosion, improving soil conditions by building soil organic matter (SOM), and improving soil biological activity. Adoption of these practices has created a sharply contrasting agroecosystem when compared with the systems soil fertility recommendations were based on.

Generally, conservation practices have been promoted to benefit landowners through reduced fertilizer inputs, increased crop yields, improved nutrient cycling, and reductions in nutrient and sediment losses (Snapp et al., 2005; González-Chávez et al., 2010; Kuhn et al., 2016; Duncan et al., 2019). The improvement of soil chemical and physical properties with conservation practices have been well documented over the past 50 years (Veum et al., 2015; Baffaut et al., 2020). There is growing evidence that benefits from these practices derive from improved soil biological activity (Dou et al., 2008; Wilson et al., 2019). Many have concluded that current soil fertility physiochemical tests do not capture the improved dynamic soil biological properties in these conservation systems. However, it has yet to be demonstrated that these biological changes can be translated into reduced fertilizer demands. Benefits of improved biology are thus primarily conceptual, with little empirical evidence confirming soil fertility recommendations should be adjusted based on biological properties (Bünemann et al., 2018; Wade et al., 2020a). Vetted empirical evidence is required to support claims that improved soil biology reduces fertilizer demand.

1.4 Comprehensive Soil Sampling: Connecting Soil Health and Soil Fertility

Soil health and soil fertility evolved to accomplish unique objectives. Soil fertility was developed to answer the clear question of "how much fertilizer should I apply?", while soil health integrates soil chemical, biological, and physical components to answer, "how is this soil functioning?" (Doran & Safley, 2002; Andrews et al., 2004; Kibblewhite et al., 2008; Lynch, 2015; Fine et al., 2017b; Haney et al., 2018). These unique objectives have prevented the linkage of soil sampling and analysis approaches. Soil fertility reflects productivity and profitability with large-scale adoption while soil health reflects management changes with limited application to agronomic outcomes. Joining these approaches will provide benefits to both. Biological tests in soil health assessment respond to management improvements more quickly than standard physiochemical tests, while soil fertility tests provide actionable support tools that soil biological tests currently lack (García-Ruiz et al., 2008; Veum et al., 2015; Bünemann et al., 2018). With similar spatial, temporal, and lab analysis variability, these tests could easily be blended, particularly if improved management recommendations reduce economic costs [e.g., reduced fertilizer requirements (Hurisso et al., 2018a; Franzluebbers, 2020c)].

Reasonable evidence exists that conservation efforts alter nutrient cycling enough to justify using soil biology to inform fertilizer recommendation decisions. Klein & Koths (1980), Hargrove (1985), and González-Chávez et al., (2010) all observed enriched soil C dynamics in no-till systems and concluded that improved nutrient cycling should reduce fertilizer demand. Rheinheimer et al., (2019) observed a 31% greater bioavailability of organic P and twice the soil microbial biomass with no tillage. In Tennessee, incorporation of cereal rye, crimson clover, hairy vetch, and a soil health mix of cover crops increased plant available Mehlich-3 K (Chu et al., 2019). In Missouri, the incorporation of organic based fertilizers altered soil P cycling in long-term soil studies (Motavalli & Miles, 2002). When comparing historic soil fertility tests with current biological tests, McDaniel et al., (2020) concluded that N recommendations would improve with the incorporation of biological tests. Finally, Wade et al., (2020) observed that biologically healthier soils produce greater corn yields per unit of N fertilizer.

Each of these studies confirm postulated theories that improved biological activity and nutrient cycling should influence fertility decisions. However, biological tests still lack critical thresholds and decision support recommendations necessary for decision support systems (Mendes et al., 2019). Critical thresholds for biological tests are in initial stages of development. One example, in Brazil, is the establishment between a suite of individual biological tests and relative corn and grain yield (Lopes et al., 2013). Further investigation of these thresholds led to interpretive classes as a function of soil organic C and a soil sampling concept that combines soil health and soil fertility sampling (Castro Lopes et al., 2018; Mendes et al., 2019). Similar work was done in rice systems in three different soil orders, but critical values varied significantly among soil orders (Biswas et al., 2017).

These critical limits lay the groundwork for future soil health decision support systems, but do not reflect nutrient cycling but rather agronomic productivity. Therefore, quantitative connections between soil health and soil fertility largely remain absent.

My unique hypotheses are that established soil fertility physiochemical are not static, as historically utilized, but dynamic; and adjusted to reflect soil biological properties. Integrating soil health tests into soil fertility evaluations offers an opportunity to refine fertilizer recommendations to reflect modern cropping systems and recent improvements to assess soil biology.

1.5 Connecting Soil Health to Productivity

One of the major "*long-term goals of sustainable agriculture research"* is to connect labile soil C assessments to agronomic performance (Culman et al., 2013). However, only half of published research investigating soil health indicators report clear, absolute interpretations (Bünemann et al., 2018). Improved productivity is often implied by soil health literature, but productivity is rarely evaluated or reported in soil health assessments (Bünemann et al., 2018; Miner et al., 2020). Stewart et al., (2018) reported less than one-third of peer-reviewed soil health studies included productivity data. There is evidence of a connection between soil health metrics and corn grain yield (Culman et al., 2013; Hargreaves et al., 2019), but an interpretive framework for on-farm application is lacking.

Conceptual diagrams illustrating soil and crop management impacts on soil health are effective tools providing insight into cropping system impacts on soil processes and functions (Veum et al., 2014). However, conceptual illustrations lack the underpinning quantitative recommendations that translate conceptual illustrations into decision support systems. Current on-farm commercial utilization of soil health metrics uses two approaches: 1) comparing measured values between conservation management practices and conventional practices within a farm or region, or 2) evaluating potential degradation by comparing a cropping system with a "reference state" such as a fence line or local native vegetation to represent undisturbed soil (Veum et al., 2014, 2015; Chu et al., 2019; Hargreaves et al., 2019; Caudle et al., 2020). While effective and informative for on-farm comparisons, these approaches cannot be scaled to regional interpretations nor do they establish trends that provide a framework for practitioners to interpret soil health metrics across a diversity of cropping systems, management practices, or soils (Zuber et al., 2020). Soil health decision support systems are needed to provide context to identify deficiencies and the environmental and economic benefits associated with ameliorating the indicated deficiency. The critical need for developing these decision support tools is the identification of soil health assessment threshold at which grain productivity is optimized. Therefore, empirical identification of these potential thresholds is critical for further development of on-farm soil health interpretation. Delivering these regional thresholds will provide a framework in which to interpret and inform on-farm implementation of soil health and the groundwork for evaluating potential benefits in economic and environmental sustainability of conservation management practices.

1.6 Research Objectives

The purpose of this multi-pronged research was to:

1) Evaluate corn yield response to P and K fertilization as impacted by soil fertility and soil health indicators.

2) Investigate relationships between regional soil health analysis and corn grain productivity.

3) Report sensitivities between soil health indicator sampling depths and soil and crop management practices within governing physical and chemical soil characteristics.

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1.8 Tables and Figures

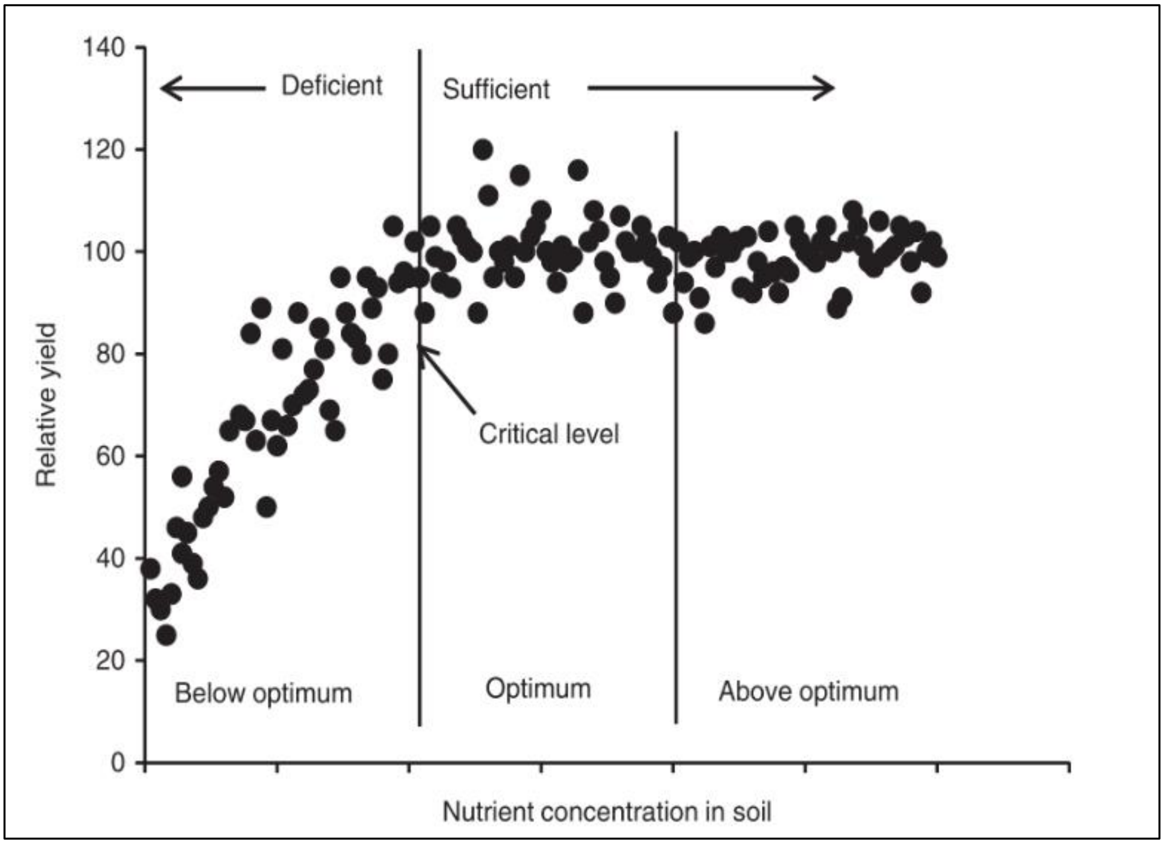

Figure 1.1 An example empirical relationship of relative yield response to soil nutrient concentration with "optimal" interpretation classes. Yield response to fertilizer above the "critical level" is unexpected but yield increases are expected with some level of certainty below that nutrient concentration threshold (McGrath et al., 2014).

Chapter 2:

Can Soil Biological Properties Improve Phosphorus and Potassium Corn Fertilizer Recommendations?

2.1 Abstract

Integrating soil health biological properties with soil fertility evaluations offers a unique opportunity to potentially refine fertilizer recommendations. The objectives of this research included: 1) evaluating current University of Missouri phosphorus (P) and potassium (K) fertilizer recommendations for corn (*Zea mays L.*), and 2) assess whether soil health biological metrics are effective indicators of yield response to P and K fertilization. In Missouri, 446 monitoring sites (148 m2) were implemented on 84 production corn fields over the 2018-2020 growing seasons. For each monitoring site, soil health and soil fertility samples were collected prior to planting, followed by application of non-replicated single-rate fertilizer treatments: 1) unfertilized control; 2) 112 kg ha^{-1} K_2O; and 3) 112 kg ha^{-1} P_2O_5. At monitoring sites below recommended critical soil test values, P (n=152) and K (n =86) fertilization increased yield at 42% and 36% of the sites respectively, with average yield increasing 10% and 11% for P and K, respectively. At the lowest fertility ratings, P and K fertilization increased yield at only 52 and 56% of sites, respectively, highlighting inherent inconsistency that exists with current recommendations. However, integrating soil health with soil fertility indicators failed to improve prediction of yield increases from P or K fertilization. Further, variable importance ranking confirmed that current physiochemical soil fertility tests remain the most effective factors identifying when fertilizer nutrients are necessary. Although soil health metrics offer insight into other agronomic or environmental benefits, established soil fertility evaluations remain the most effective tool for guiding P and K fertilizer decisions in Missouri corn production.

2.2 Introduction

Forty to sixty percent of current United States corn (*Zea mays* L.) yield is credited to nitrogen, P, and K fertilization (Stewart et al., 2005). Notwithstanding substantial contributions to productivity, offsite transport of fertilizer nutrients is leading to regional, local, and worldwide environmental issues (Kleinman et al., 2019; Smith et al., 2019). Continued environmental pollution, especially in freshwater systems, is leading to regional political pressure and restrictions on fertilizer application. Moving forward, sustainable agroecosystems require functional fertilizer recommendations that balance crop productivity and minimize environmental losses (Sharpley et al., 2015; Ros et al., 2020; Cassman & Dobermann, 2022).

Soil fertility testing is the bedrock of current crop fertilizer recommendations (McGrath et al., 2014). These decision support systems identify soil nutrient concentration thresholds, or critical concentrations, where additional fertilizer does not improve yield (Voss, 1998; McGrath et al., 2014). Basing fertilization decisions on these critical concentrations offers economic incentive to reduce unnecessary fertilization while preventing excessive application susceptible to off-site pollution (Hopkins & Hansen, 2019; Osmond et al., 2019). However, recent regional evaluations have highlighted needed improvements with some recommendation accuracies as low as 40% (Heckman et al., 2006; Fulford & Culman, 2018; Fryer et al., 2019). Investigating current inadequacies and improving these recommendations is one critical step in averting ongoing environmental degradation.

One plausible hypothesis for erroneous recommendations is the adoption of modern soil management practices. Many of the datasets that inform fertilizer recommendations date back over fifty years ago, often in monoculture fields with fallow between cash crops and regular tillage to prepare the seedbed and facilitate residue decomposition (Bray, 1948; Mehlich, 1984; Voss, 1998; McGrath et al., 2014). In contrast, many of the current recommendations are to improve soil health by diversifying crop rotations, adding cover crops, and minimizing tillage (Kibblewhite et al., 2008). These conservation practices improve physical, chemical, and biological soil properties, creating an environment different from when soil fertility recommendations were developed. Despite these changes in common management practices, soil fertility analysis and evaluations have largely remained unchanged.

Enhancing soil health properties improves nutrient cycling and reduces nutrient and sediment loss from erosion, leading to the promotion of these practices as a way to reduce fertilizer inputs and increase crop yields (Snapp et al., 2005; González-Chávez et al., 2010; Kuhn et al., 2016; Duncan et al., 2019). These improved soil processes are linked to enhanced soil biological activity (Dou et al., 2008; Wilson et al., 2019). This has led to the hypothesis that current physiochemical soil fertility evaluations do not reflect the beneficial improvements in biological properties from these conservation systems (Ndiaye et al., 2000; Mijangos et al., 2006; García-Ruiz et al., 2008; Franzluebbers, 2016). However, these advantages to fertilizer requirements remain largely conceptual for P and K fertilization, with the few empirical studies investigating these improvements focusing on nitrogen plant demand (McDaniel et al., 2019; Wade et al., 2020). Therefore, it remains unclear whether improving soil health affects crop nutrient demand and subsequent fertilizer requirements.

Integrating soil biological analysis with soil fertility evaluations offers a unique opportunity to refine fertilizer recommendations and investigate whether improved soil biological properties affect fertilizer requirements. Initial investigations integrating soil health and soil fertility in nitrogen recommendations have shown promise, with McDaniel et al., (2020) concluding incorporating biological tests would improve current recommendations and Wade et al., (2020a) observing biologically healthier soils produce greater corn yields per unit of applied N. Similar approaches integrating soil health with P and K recommendations are absent, leaving the asserted benefits of soil health unrealized. The objectives of this research included: 1) evaluating current University of Missouri P and K fertilization recommendations for corn, and 2) assessing whether soil health biological metrics are effective indicators of yield response to P and K fertilization.

2.3 Materials and Methods

2.3.1 Field Conditions and Experimental Design

Research was implemented on 84 fields in mid-Missouri across a diversity of management practices, climate patterns, and soils over three growing seasons (2018-2020). To evaluate response to P and K fertilization across these diverse environmental conditions, multiple fertilizer response trials were established on each of these fields. Each individual trial, called a "monitoring site", was a 148 m^2 and included four 37 m^2 non-replicated single-rate fertilizer treatments (Figure 2.1). Coordinates of each site center were measured using Trimble GeoXT 6000 and Geo 7x GPS devices with approximately 6-cm accuracy. With the center of the site identified, pre-cut webbing was stretched to define the site area. Using a hand-held compass, the sites were oriented on a north-south bearing regardless of field-location. Monitoring sites were established in unique environments and did not behave as replications of the same treatments; therefore, treatment randomization was not required to meet assumptions required in many randomized plot trials. The relatively small plot size allowed seamless implementation into commercial operations. Typical field trials included three to five sites per field, with 446 sites over the three growing seasons. Through communication with cooperating growers and applicators, sites were outlined with flags to ensure P, K, or S fertilizers were not applied to each site. Cooperating farmers selected hybrids, weed control, tillage, nitrogen fertilization, planting dates, and other practices based on their standard field management. Planting dates ranged from April 5-June 10.

2.3.2 Soil and Plant Sample Collection, and Fertilizer Treatments

Each monitoring site included a characterization of the soil profile and a suite of chemical, physical, and biological soil analyses (Table 2.1). Soil sampling occurred in the spring prior to planting (Mar-Apr). Eight to twelve 2.54-cm cores, to a depth of 15 cm, were collected within the site area for soil fertility and soil health analysis (Figure 2.1). At the time of sampling, each sampled core was split into two depths (0-5 cm depth and 5-15 cm depth) and composited into buckets. After gentle hand mixing of each sample depth, a 0.11 L sub-sample was collected from the 0-5 cm depth and 0.22 L subsample from the 5-15 cm depth and combined for a depth-weighted, composite soil fertility sample. The remaining 0-5 cm and 5-15 cm samples were stored in re-sealable zipper storage bags and stored in coolers for soil health analysis. A Giddings Model #5-UV / MGSRPSUV soil sampling machine (Giddings Machine Company, Windsor, CO) was used to obtain one 4.5-cm profile core to an approximate depth of 1 m, from which the soil profile was characterized, and sub-surface soil fertility assessments were measured. Each core was laid out on a processing table and characterized by visual and tactile properties into pedogenetic diagnostic horizons. Each core was characterized into four or five horizons, with a standardized surface 0-15 cm Ap horizon—to match the depth of the soil health and soil fertility samples. Soil samples were bagged and removed from the monitoring area before fertilizers were applied to treatment plots.

Following soil sampling, sites were sub-divided into four equal 6.1 by 6.1 m quadrats (37.2 m2). Fertilized treatments for the quadrats included the following: 1) control (i.e., no fertilizer treatment), 2) K treated with 112 kg ha^{-1} of K_2O as KCL (0-0-60), 3) P treated with 112 kg ha^{-1} P_2O_5 as triple super phosphate (0-46-0), and 4) sulfur (S) treated with 28 kg ha^{-1} of S as ammonium sulfate (21-0-0-24S; S results not reported in this analysis). An additional 25 kg ha^{-1} of nitrogen was applied as SuperU® (46-0-0) to treatments one, two, and three to match the nitrogen included in treatment four. An additional 40 kg ha^{-1} nitrogen (46-0-0) was applied at V6 to guard against late season nitrogen deficiencies from wet spring conditions in 2019 and 2020.

Soil fertility samples were air-dried and submitted for analysis to Ward Laboratories (Ward Laboratories, Kearney, NE). Soil fertility analyses (Table 2.1) were examined using published University of Missouri soil fertility recommendations (Buchholz et al., 2004). Soil health samples were broken into two depths (0-5 and 5-15 cm), stored in a cooler at 1.6° C, then processed by passing through a 1 cm screen, air-drying, and dry sieving through a 2 mm screen. For potassium permanganate oxidizable carbon (POXC) and SOC soils were ground to a powder prior to analysis. All biological soil analyses were completed in the USDA-ARS Soil Quality Lab on the University of Missouri Columbia Campus following methods listed in Table 1. The deep core soil characterization samples were air-dried and measured for bulk density and gravimetric soil moisture with the top three pedogenic horizons submitted for soil textural and fertility analyses (Ward Laboratories, Kearney, NE). Corn grain yield was hand harvested at maturity from an 11

m^2 area within each quadrat of each site, with ears collected and weighed in bulk using a Rapala ProGuide Digital Scale (Rapala, Minnetonka, MN). An eight-ear subsample was oven dried at 65°C to measure grain moisture. Grain yield was estimated at 15.5% moisture and cob weight subtracted using a grain to cob ratio of 0.89, calculated from a published dataset (Kitchen et al., 2017; Ransom et al., 2021). For each monitoring site, relative yield for each fertilized treatment was calculated by dividing the non-fertilized control yield by the fertilized yield, expressed as a percent (Eq. 1).

$$Relative\ Yield = \frac{Control\ Yield}{Fertilized\ Treatment\ Yield} * 100 \qquad (1)$$

The fertilized treatment yield was considered the fertilized treatment yield at the specific site, unlike other methods which use averages or maximums of fertilized treatments (Dodd & Mallarino, 2005). Without replication statistical evaluations of yield response at individual sites were unattainable, as such, a five percent yield increase was considered ‘responsive'. This benchmark was obtained by hand-harvesting a site that did not receive fertilization in ten corn fields (n =10) to estimate error in yield between harvested plots. The average variability between unfertilized plots was five percent.

Descriptive statistics of historic cropping system and soil properties were collected for each site (Table 2.2). Soil series, Major Land Resource Area, and drainage class information were collected from the Natural Resources Conservation Service (NRCS) Soil Survey Geographic (SSURGO) database based on site geospatial locations. Landscape positions of sites were identified during soil sampling (Table 2.2). The previous five years of cropping system management histories were collected including tillage, manure, cover crop, and crop rotation practices. Cropping systems practices were indexed relative to the guidelines outlined in Table 2.

2.3.2 Data Processing, Analysis, and Statistics

All statistical algorithms were fit using the ‘caret’ package in R statistical software (R Core Team, 2016). The dataset was randomly partitioned into calibration (90%) and validation (10%) datasets prior to analysis. Cross validation, tuning of internal parameters, and model development were conducted on the calibration dataset while evaluation of final model performance was assessed using the validation dataset. Tuning of internal model parameters used a range of values and a ten-fold cross-validation repeated ten times to ensure model optimization. Models were trained on nine of the ten folds, with the accuracy measured using RMSE between the predicted and actual values on the final tenth fold. Tuning parameters with the lowest RMSE across each of the 100 cross-validation folds were chosen for the final model. Evaluations and comparisons between models were conducted on the validation dataset with R^2 and Root-mean-square-error (RMSE) statistics.

Relative response to fertilizer application was the dependent variable for each of the model algorithms. Explanatory variables included standard soil fertility analysis, a suite of soil biological analyses, environmental factors, and management practices (Tables 2.1 and 2.2). All variables were not included in every model but were utilized in three separate scenarios: 1) standard soil fertility analyses only; 2) standard soil fertility and soil biological analyses; and 3) all available explanatory variables (i.e., all shown in Tables 1 and 2). Respectively, these three model scenarios will be referred to as soil fertility Model, Integrated Model, and Full Model. Nonparametric random forest algorithms were used for modeling relative yield response for each fertilizer treatment. The RMSE and R^2 statistics were used to evaluate whether integrating soil biological tests, environmental conditions, and management practices improved model performance (Ransom et al., 2019). Random forest algorithms are resistant to multicollinearity, but highly correlated variables can introduce bias in conditional variable importance (VIP) evaluations (Strobl et al., 2007). Therefore, for the Integrated and Full Models, total nitrogen and organic matter (OM) were not included because of the high correlation with SOC (Figure 2.2). Two conditional methods of VIP were utilized to evaluate VIP of model performance: 1) increase in mean square error, which measures the decay in model mean square error as an explanatory variable is randomly assigned and permutated over the dataset and 2) increase in Node Purity, which reflects the difference in the residual sum of squares at each split and summed over all splits and trees (Hastie et al., 2009; Genuer et al., 2010).

2.4 Results and Discussion

The distribution of sampled soil properties reflects the diversity of environments in which the monitoring sites were deployed (Figure 2.3). Standard physiochemical analyses in soil fertility evaluations (Table 2.1) were within established regional recommendations for grain crop production (Buchholz et al., 2004). Biological analyses do not yet have established regional standards, but values observed in this dataset were comparable to other regionally reported values (Zuber et al., 2020). The variability in the soil biological analysis values reflects the wide diversity of cropping system practices and edaphic environmental properties in which the sites were employed (Veum et al., 2015). Corn yields varied by year, with better yields in seasons with greater precipitation during crop growth (Figure 2.4). Across all observations, P and K fertilization did not significant increase yields, demonstrating the need to investigate site-specific factors that govern regional yield response to P and K fertilization (Dodd & Mallarino, 2005; Fulford & Culman, 2018; Fryer et al., 2019).

2.4.1 Evaluating Missouri Phosphorus and Potassium Fertilizer Recommendations

Yield response relative to soil test values for P and K (STP and STP) generally follow trends established by the University of Missouri fertility guidelines (Figure 2.5). The greatest yield increases, and percent of sites with a yield response, occurred where fertilizer recommendations indicated a deficiency in available P and K (Figure 2.5). Where STP was below the recommended critical concentration, P fertilizer improved yield at 42% of the sites with an average 10% yield increase (n = 152). Potassium fertilization yielded similar results, with yield increases at 36% of sites below established STK critical concentration with an average 11% increase in yield (n = 86). In the lowest fertility rating (Low and Very Low), over 50% of the sites responded to fertilizer application for both P and K respectively (Figure 2.5). These results emulate a regional Northeast USA assessment where P fertilization increased yield at 17-43% of sites below established critical concentrations (Heckman et al., 2006) and performed better than an Ohio study where P and K fertilization increased yield in five and four of 42 total site-years respectively (Fulford & Culman, 2018). At fertility ratings above the critical concentration (High, Very High, Extremely High) the percent of sites responding to fertilization were generally low (< 25%). These results follow the Dodd and Mallarino (2005) observation that the probability of fertilization increasing yield above the soil-test critical concentration is < 25%.

The low response in the "Medium" fertility ratings were unexpected and highlight the persistent uncertainty in fertilizer recommendations near recommended critical thresholds. These inconsistencies likely derive from bias during recommendation developments and the complexity of the dynamic soil-plant system (Fryer et al., 2019; Brouder et al., 2021). The uncertainty observed in regional P and K soil-tests involve complex interactions between environmental conditions, soil properties, and management practices. Long-term and thorough site-specific fertilizer response trials can provide effective soil-test prediction of yield response to P and K fertilization (Dodd & Mallarino, 2005; Schlegel & Havlin, 2017). These types of trials form the bedrock of P and K fertilizer recommendations (McGrath et al., 2014).

Several reasons why site-specific derived soil fertility calibration datasets do not translate to strong regional responses exist. Typical recommended soil fertility sampling protocols do not sample the full rooting depth, but rather rely on the topsoil sample (< 15 cm) as an indicator of potential nutrient supply. Plant roots are not limited to recommended sampling depths, and acquisition of deeper soil nutrients can overcome deficiencies indicated in the topsoil (Woodruff & Parks, 1980). This subsoil nutrient supply and root acquisition are contingent upon intrinsic soil formation factors which vary considerably between regional soil types and likely not well represented in current soil fertility recommendations. Deficiencies in other nutrients, such as N, will mask deficiencies in P and K (Hirniak, 2018), though I do not anticipate this effected this dataset. Phosphorus and K fertilizer responses are sensitive to soil conditions and properties which vary between research locations where the calibration data are collected (Randall et al., 1997a; b; Dodd & Mallarino, 2005). Finally, inherent soil properties that interact with P and K nutrient cycles, such as clay mineralogy, can influence the effectiveness of soil tests as an indicator of fertilizer requirement (Breker et al., 2019). This dataset includes over 20 soil types, with unique soil properties and response conditions to P and K fertilization, and likely contribute substantial unpredictability in the effectiveness of soil tests to estimate P and K crop supply during the growing season. These site-specific interactions are the principal justification for investigating multivariate approaches to improve current regional P and K recommendations in modern management systems (Fulford & Culman, 2018; Brouder et al., 2021).

2.4.2 Integrating Soil Health into Soil Fertility Recommendations

Each random forest model (SF, Integrated, and Full Models) performed poorly in predicting yield response to both P and K fertilization (Table 2.3). The relatively low R^2 and high RMSE for both the calibration and validation datasets indicate poor model performance and only allow for general model interpretations. Predicting yield response to K fertilization performed better than P fertilization, with greater R^2 in the calibration dataset and lower RMSE in the validation dataset. This is because of the stronger yield responses to K fertilization in low STK environments relative to yield increases to P fertilization in low STP environments (Figure 2.5). The addition of SH, management, and environmental factors improved the out-of-bag error R^2 values for the calibration dataset for K fertilization (Table 2.3). However, no substantial improvement in RMSE indicates the supplementary factors did not improve model accuracy. The addition of variables in the Integrated and Full models lead to model overfitting on the calibration dataset, and when applied to a unique dataset (validation dataset) prediction accuracy remained poor (Table 2.3). The Integrated and Full models for predicting yield response to P fertilization did not improve R^2 or RMSE, leading to the overall conclusion that soil health and management factors did not improve current soil fertility recommendations.

Poor performance is common in regional P and K fertility assessments, with similarly low R^2 values (R^2 = 0.09-0.28) in both Ohio and the Northeast USA (Heckman et al., 2006; Fulford & Culman, 2018). As discussed previously, the challenges in providing robust P and K recommendations are attributed to site-specific, complex management, climate, and soil interactions. Adding other soil analyses has been demonstrated to improve fertilizer recommendations (Wortmann et al., 2009), but the addition of soil biological tests did not improve P and K recommendations in this dataset (Table 2.3). This confirms other work where a commercially available soil biological test (Haney Soil Health Test) failed to improve estimations of plant available P (Singh et al., 2020). Three hypotheses explain why model performance did not improve when biological indicators were included: 1) the stated purposes of many biological tests are quite different than the purpose of soil fertility tests, 2) connections of soil health tests to P and K nutrient cycles are likely weak, and 3) biological and soil fertility connections are likely site-specific and degrade when employed regionally. Each of these will be discussed further.

Soil fertility analysis assesses physiochemical processes that govern labile P and K availability (Khan et al., 2014; Brouder et al., 2021) while soil health tests detect effects of management on soil biological properties (Karlen et al., 2019). Their associated recommendations reflect these differences, with soil fertility tests informing fertilizer management while soil health tests inform soil and general cropping system practices. There are conceptual connections to these evaluations, such as biological improvements in nutrient cycling (González-Chávez et al., 2010; Franzluebbers, 2016), but these connections are outside the scope of stated soil health purposes. Therefore, it is unsurprising that the soil health indicators did not improve P and K recommendations because it is simply outside the purpose of their development (Duncan et al., 2019).

Physiochemical, rather than biological, processes predominantly govern P and K nutrient supply to crops (Sharpley et al., 1993; Brouder et al., 2021). These biological relationships are underdeveloped, but are hypothesized to be connected with P and K nutrient supply to crops through two processes: 1) estimating P and K mineralization from organic material, and 2) indirect effects on physiochemical processes that govern labile P and K availability. The effects of soil biology on P and K mineralization are conceivably masked by the larger physiochemical pool source while any biological impacts on enhancing physiochemical nutrient supply are likely already captured in current P and K soil analysis. The disconnect between soil biological tests and major P and K nutrient cycles leads to weak relationships with yield response from fertilization which offers little value to soil fertility evaluations.

Finally, the complex and multi-factor relationships between biological properties, labile P and K, and yield are not yet developed and likely site-specific (Bünemann et al., 2018). Direct relationships between the chemical and soil biological analyses are not established, but the regional nature of this dataset suggests connections are weak (r = 0.16 - 0.32; Figure 2.2). This is because soil fertility and soil health analyses are sensitive to distinctive management practices (historic fertilizer application vs rotation, tillage management, cover crop, etc.). Correlated tests could also be interpreted to imply that information provided from the biological analyses has already been captured in the STP and STK estimates of plant nutrient availability. Or in other words, the biological analyses are redundant and do not provide unique information to improve identification of soils responsive to P or K fertilization. For example, POXC is believed to reflect an 'active' pool of carbon (C) that is easily accessible to microbial turnover, and it is likely that current STP and STK soil extractions adequately estimate P and K availability from this same pool; therefore, including POXC as a predictor of yield response to P or K fertilization is unnecessary. However, as previously stated, these connections remain conceptual and further empirical evidence is required to establish mechanistic relationships between these biological and physiochemical analysis.

2.4.3 Identifying Soil Analyses to Advise Fertilizer Recommendations

Variable importance analysis offers insight into the relative importance explanatory variables contribute to the overall predictive structure of a random forest model (Archer & Kimes, 2008; McDaniel et al., 2020). I effectively use VIP to evaluate which agronomic factors govern yield response to P and K fertilization. Two methods of VIP are reported to demonstrate the robust nature of the top individual predictor variables (Figure 2.6). Because neither the Integrated or Full model improve identification of yield response to P and K fertilization, only the soil fertility model VIP plots are reported.

For yield response to K fertilization, both VIP methods identified STK as the top predictor (Figure 2.6, panels C and D). This aligns with decades of research confirming STK is an effective too for evaluating soil fertility status in a diversity of environments and cropping systems (Mallarino et al., 1991; Vyn & Janovicek, 2001; Brouder et al., 2021). Dependent upon the VIP method, CEC and percent clay were the second most important variables (Figure 2.6; panels C and D). Both CEC and clay content are related to the major potential loss of K fertilizer to interlayer positions in phyllosilicate clay minerals, commonly recognized as K fixation (Khan et al., 2014; Brouder et al., 2021). Clay content is a direct measure of potential fertilizer loss to interlayer positions while CEC acts as an indirect measurement that is presently included as a site-specific adjustment to reflect this process in STK fertilizer recommendations (Buchholz et al., 2004). Other studies have identified pH and OM as factors that can improve K recommendations, but no mechanistic descriptions of why were provided (Wortmann et al., 2009). Both pH and OM contribute to bulk soil CEC which is why VIP identify CEC as the superior indicator to pH and OM in these analyses.

For yield response to P fertilization, the top variables in the VIP analysis were CEC and Bray STP (Figure 2.6, panels A and B). Both VIP methods identified CEC as the top indicator with the Bray STP test as the second variable governing yield response to P fertilization. Current Missouri recommendations only utilize the Bray STP estimate (Buchholz et al., 2004), implying CEC could improve P fertilizer recommendations (Figure 2.5). Soil CEC stems from clay mineralogy and soil OM (Williams, 1932; Solly et al., 2020) which govern three main mechanisms of P availability: 1) applied P fertilizer is susceptible to adsorption to clay mineral surfaces, 2) soil OM prevents adsorption through stable organic phosphate complexes and coating iron and aluminum oxides, and 3) soil OM provides an indication of potentially mineralizable P (Sanchez & Uehara, 1980). Cation exchange capacity was moderately correlated with both OM and SOC (Figure 2.2), which suggests CEC's hybrid sensitivity to both physical (clay percentage) and biological (OM, SOC) properties influences its effectiveness as an indicator of yield response to P fertilization.

The inclusion of STP as an effective variable was anticipated because of its use as the indicator for P fertilization and demonstrated effectiveness in identifying P deficient soils (Bray, 1944; Dodd & Mallarino, 2005). The VIP ranking of STP confirms its effectiveness as an indicator identifying P deficient soils, but the greater importance of CEC remains unknown. Common soil tests do not directly measure available P, but rather act as indicators of a soil's capacity to supply, or for plant roots to acquire, P for crop growth (Brouder et al., 2021). These mechanisms are impacted by other soil properties and the diversity of environments and soil properties likely affected the effectiveness of STP in identifying P sufficiency levels (Dodd & Mallarino, 2005). Organic P has been proposed as an unrecognized and underutilized method of improving P recommendations, because it comprises between 30-65% of total soil P (Dodd & Sharpley, 2015). This research does not support that assumption. While no direct measurements of organic P were included, three biological analyses were included as potential indicators of organic P nutrient supply to crops: acid phosphatase, POXC, and soil respiration. Acid phosphatase measures extracellular enzymatic activity releasing plant available phosphate from organic compounds (Acosta-Martínez & Tabatabai, 2011), POXC estimates the labile C pool which is readily available for microbial turnover and release of nutrients, and soil respiration reflects the mineralizable C and is considered an indirect measurement of microbial activity (Hurisso et al., 2016). Including these indirect measurements of organic P supply did not improve identification of P deficient soils (Table 2.3) and further research, with either direct measures of organic P or biological analyses linked to organic P pools, would elucidate the failure of these soil health metrics to improve soil fertility evaluations.

Integrating both soil fertility and soil health indicators failed to improve prediction of yield response to P and K fertilization; however, further research investigating links between these two soil evaluation methods and the biochemical processes that govern nutrient availability in soil are needed. These results again confirm the limitations in current soil fertility recommendations but refute the hypothesis that integrating currently available soil health indicators improves regional P and K fertilizer recommendations.

2.5 Conclusions

These results confirm the efficacy of University of Missouri fertilizer recommendations and highlight the current limitations in P and K recommendations. Soil-test estimation of yield response to fertilization was most accurate at low nutrient levels and exhibited diminished precision at or above established critical concentrations. Variable importance analysis confirmed the effectiveness of current soil-tests, and indicated CEC is an underutilized tool in P fertilizer recommendations. These results reflect challenges in developing regional recommendations that effectively operate across natural variability among a wide range of soil types, environmental conditions, and management practices. Integrating soil health indicators failed to improve current model identification of yield response to P and K fertilization. These findings found little support for using soil health metrics to identify crop fertilization needs. Although soil health metrics offer insight into environmental or agronomic benefits, established soil fertility analysis remains the most effective tool to guide P and K fertilizer decisions in Missouri corn production. Further research is needed to improve current P and K recommendations to ensure sustainable economic and environmental management of these nutrients.

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2.7 Tables and Figures

Table 2.1 Soil test abbreviations, common names, brief method descriptions, units, and primary references.

Abbreviation	Common Name	Brief Method Description	Units	Reference
Soil Fertility Measurements				
OM	Organic Matter	Loss on ignition organic matter	g 100 g^{-1}	Nelson & Sommers, 1996
STP	Bray 1 Phosphorus	Bray 1 phosphorus extraction	mg kg^{-1}	Frank et al., 1998
	Mehlich 3 Phosphorus	Mehlich 3 extraction of Phosphorus	mg kg^{-1}	Mehlich, 1984
STK	Soil Test Potassium	Extraction of base cations with ammonium acetate buffer solution at pH 7	mg kg^{-1}	Warncke & Brown 1998
STS	Sulfate Sulfur	Mehlich 3 Extraction of Sulfate-Sulfur	mg kg^{-1}	Mehlich, 1984
CEC	Cation Exchange Capacity	Sum of base cations	mel 100 g^{-1}	Burt and Soil Survey Staff, 2014
pH_{water}	pH	Soil pH measured in water, with electrode (1:1 w/w)		Coleman & Hargrove, 1984
% Clay	Particle Size	Hydrometer method	g 100 g^{-1}	Burt and Soil Survey Staff, 2014
Soil Biological Tests				
SOC	Soil Organic Carbon	Measured via combustion on LECO TruMac C/N combustion analyzer (LECO Corp., St. Joseph, MI, USA).	g 100 g^{-1}	Nelson & Sommers, 1996
TN	Total Nitrogen	Measured via combustion on LECO TruMac C/N combustion analyzer (LECO Corp., St. Joseph, MI, USA).	g 100 g^{-1}	Nelson & Sommers, 1996
POXC	Permanganate Oxidizable Carbon	Oxidation with 0.2 M $KMnO_4$ and shaken for 2 min at 240 oscillations per min with a 10 min settling time	mg C kg $soil^{-1}$	Weil et al., 2003
SR	Soil Respiration	4-day incubation with KOH alkali trap	mg CO_2 kg $soil^{-1}$	Moebius-Clune et al., 2016
ACE Protein	Soil/ Total / ACE Protein	Autoclaved citrate extractable (ACE) protein, 3 g soil with 24 mL $Na_3C_6H_5O_7$ buffer, autoclaved and quantified with Bradford BCA	mg g $soil^{-1}$	Moebius-Clune et al., 2016
	Acid Phosphatase	*p-nitrophenyl phosphate* substrate addition with 1 hr incubation at 36°C with *p*-nitrophenol (PNP) standard	µg PNP g $soil^{-1}$ hr^{-1}	Acosta-Martínez & Tabatabai, 2011
	Arylsulfatase	*p-nitrophenyl sulfate* substrate addition with 1 hr incubation at 36°C with *p*-nitrophenol (PNP) standard	µg PNP g $soil^{-1}$ hr^{-1}	Klose et al., 2011
	β-Glucosidase	*p-nitrophenyl-β-D-glucopyranoside* substrate addition with 1 hr incubation at 36°C with *p*-nitrophenol (PNP) standard	µg PNP g $soil^{-1}$ hr^{-1}	Deng & Popova, 2011

Table 2.2 List and description of environmental and management data collected and used in phosphorus and potassium random forest models predicting relative yield response to fertilization. All management data are reflective of the previous five years before implementation of fertilizer monitoring sites.

Variable	Description
Environmental Conditions	
Drainage Class	USDA NRCS Soil Survey Classifications: excessively drained well-drained, moderately well-drained, somewhat poorly drained, poorly drained
Soil Type	USDA NRCS Soil Survey Classifications
Major Land Resource Area	USDA NRCS geographically associated land resource units including: 107B, Deep Loess Hills; 112,113,116A, Claypan Areas; 107, Heavy Till Plain; 155B, 115C Central Mississippi Valley Wooded Slopes,
Landscape Position	General landscape positions including summit, back-slope, terraced back-slope, foot-slope, and floodplain steppe
Management Data	
Crop Rotation	Reflective of the past five years. Monoculture: corn after corn, Corn-Soybean: Corn after Soybean rotation, Diverse: any additional cash crop additions (wheat, triticale, etc.)
Manure Application	Reflective of the past five years. Heavy: 2+ years of manure application, Light: one year of manure application, None: no manure applied in last five years
Tillage Practices	Reflective of the past five years. Heavy: 3+ years of tillage, Light: 1-2 years of tillage, No-Till: Zero tillage in last five years
Cover Crops	Reflective of the past five years. Heavy: 2+ years of Cover Crop; Light: one year of cover crops; None: No cover crops in last five years

Table 2.3 Model statistics for random forest model algorithms with relative yield to phosphorus or potassium fertilization as dependent variables. Included explanatory variables includes suites of soil fertility, soil health, management and environmental variables that are identified in Tables 1 & 2. Eighty percent of the dataset was partitioned for model calibration with the remaining 20% used for validating developed models. RMSE was calculated from the difference between predicted error and observed error.

Dependent Variable and Model Title	Model Inputs	Calibration		Validation
		R^2	***RMSE***	***RMSE***
Relative Yield to Potassium Fertilization				
SF Model	Soil Fertility	10%	7%	5%
Integrated Model	Soil Fertility + Soil Health	15%	7%	6%
Full Model	Soil Fertility + Soil Health + Management + Environment	14%	7%	5%
Relative Yield to Phosphorus Fertilization				
SF Model	Soil Fertility	6%	6%	7%
Integrated Model	Soil Fertility + Soil Health	7%	6%	7%
Full Model	Soil Fertility + Soil Health + Management + Environment	4%	6%	7%

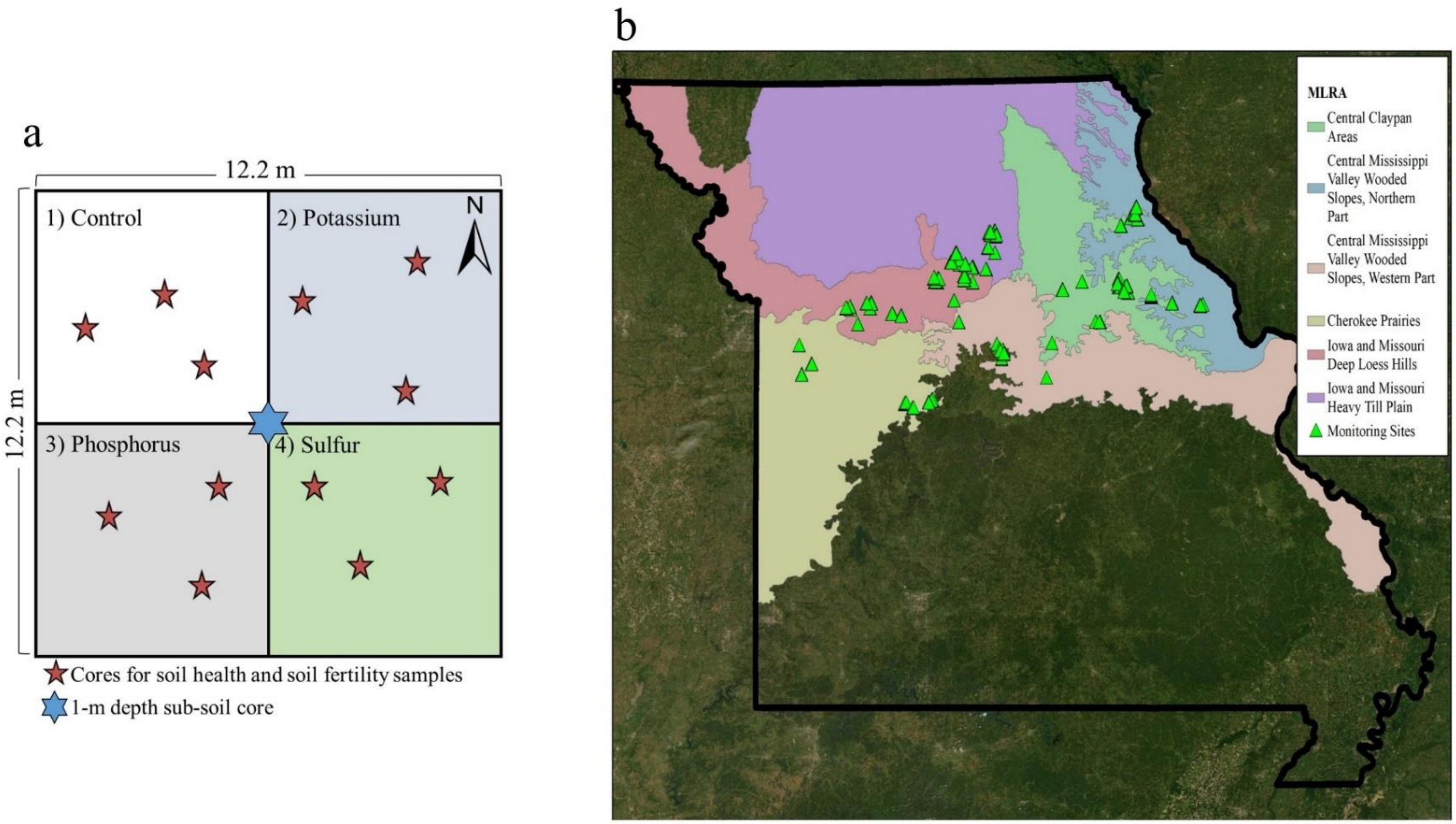

Figure 2.1 Panel a) monitoring site design, fertilizer treatments, and soil sampling scheme, and panel b) a map of Missouri soil regions by Major Land Resource Areas and geolocation of fields with fertilizer monitoring sites.

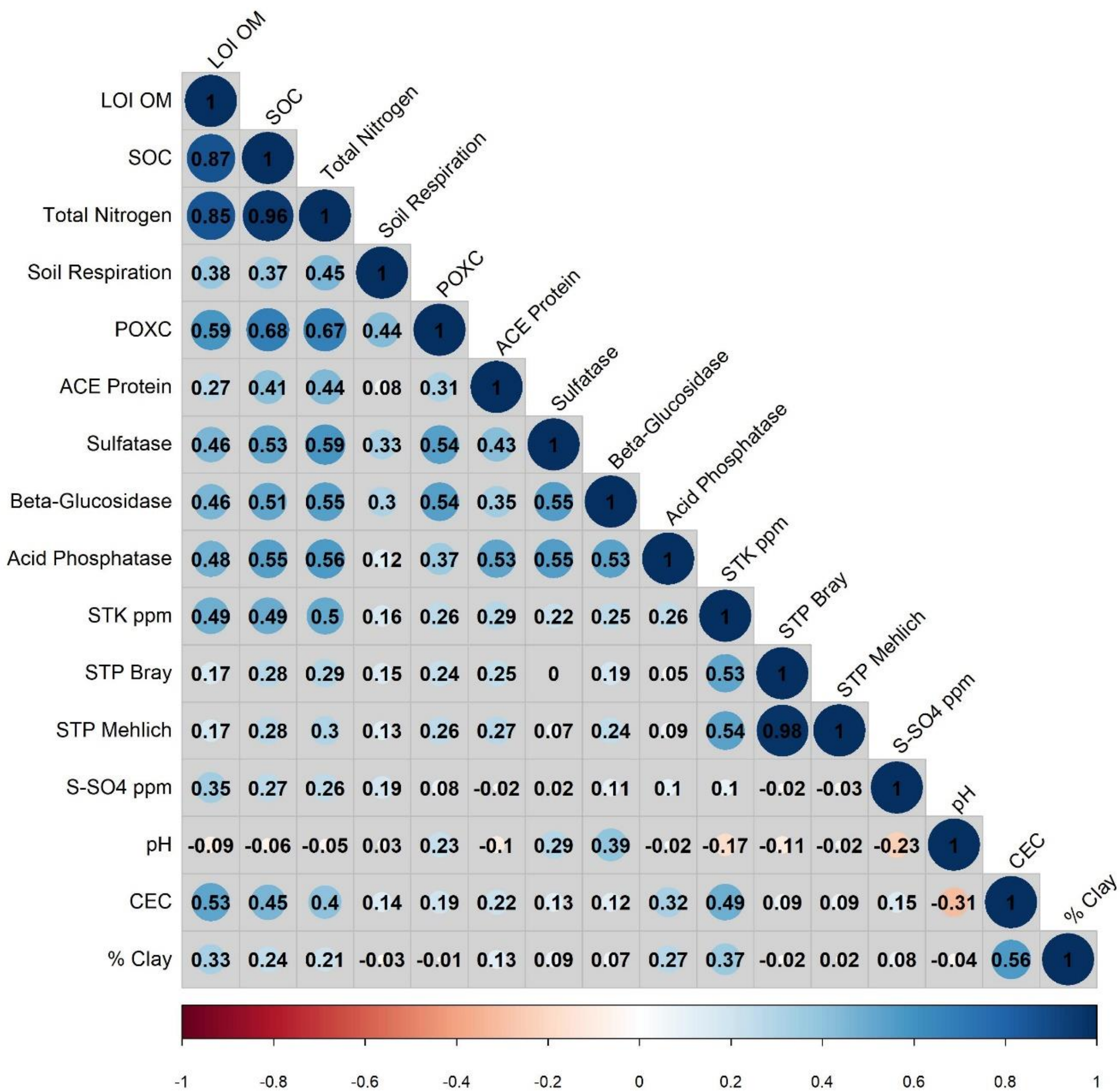

Figure 2.2 Pearson correlation matrix of soil analysis included in soil fertility and soil health evaluations. The size and color of circles reflect the sign and magnitude of the correlation between variables with coefficients identified within each circle. See Table 1 for analysis descriptions.

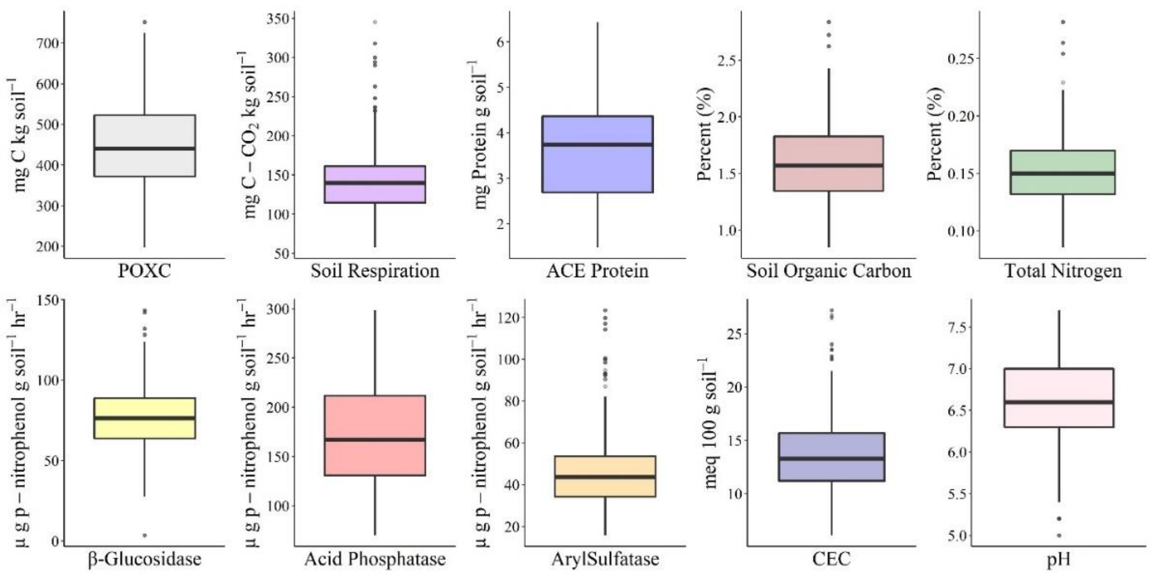

Figure 2.3 Boxplots of sampled soil fertility and soil health analyses. Abbreviations and descriptions are included in Table 1. Bold line in the middle indicates the median value, top and bottom of the boxes represent 75 and 25% of the data, respectively, while top and bottom of the whiskers represent 95 and 5 % of the data, respectively; outliers are represented by circles outside the whiskers.

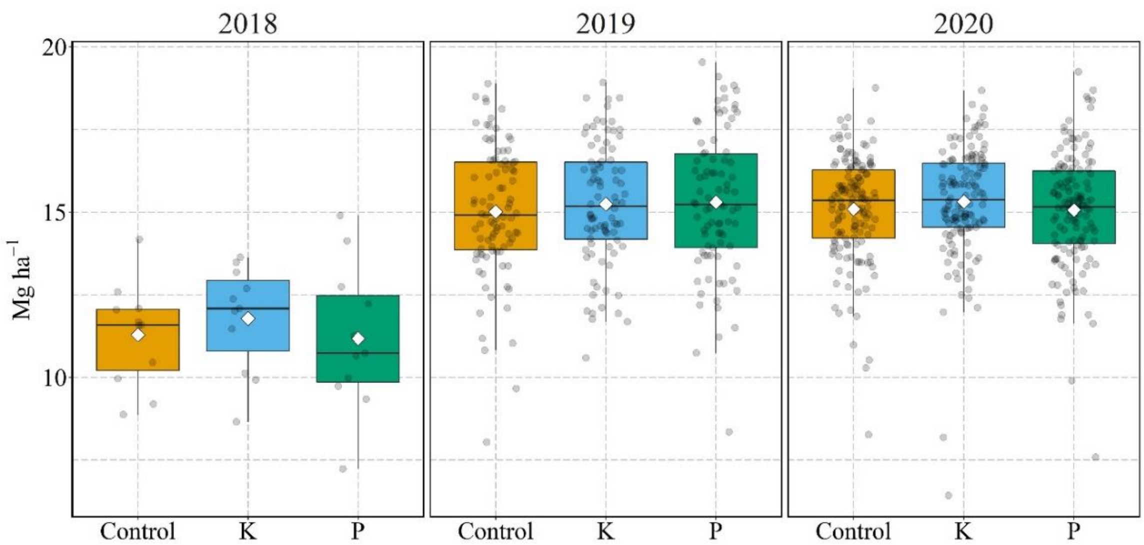

Figure 2.4 Boxplots of yield across treatments and seasons with means identified with white diamonds. Treatment fertilization included 1) no fertilization 2) 112 kg K_2O ha^{-1} 3) 112 kg P_2O_5 ha^{-1}. Bold line in the middle indicates the median value, top and bottom of the boxes represent 75 and 25% of the data, respectively, while top and bottom of the whiskers represent 95 and 5% of the data, respectively; outliers are datapoints outside the whiskers.

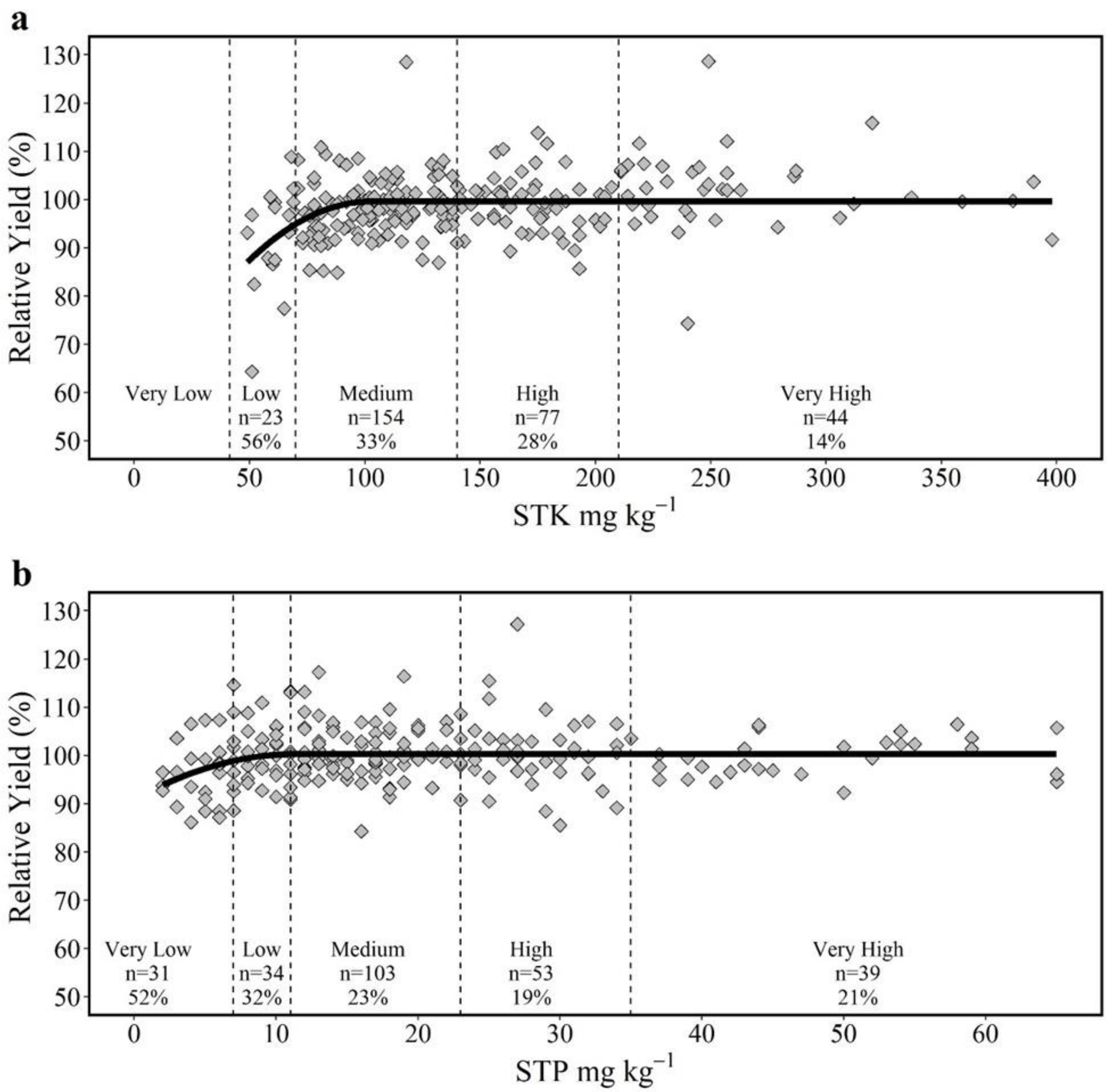

Figure 2.5 Relationships between a) STK and b) STP and relative corn yield response from fertilization across all experimental years. Vertical dashed lines represent University of Missouri soil fertility ratings. Fertility ratings are labeled with crop response considered unlikely for soil test values in the High rating categories. Under each rating label, the number of observations and percent of observations in that fertility rating with ≥ 5% yield increase are reported.

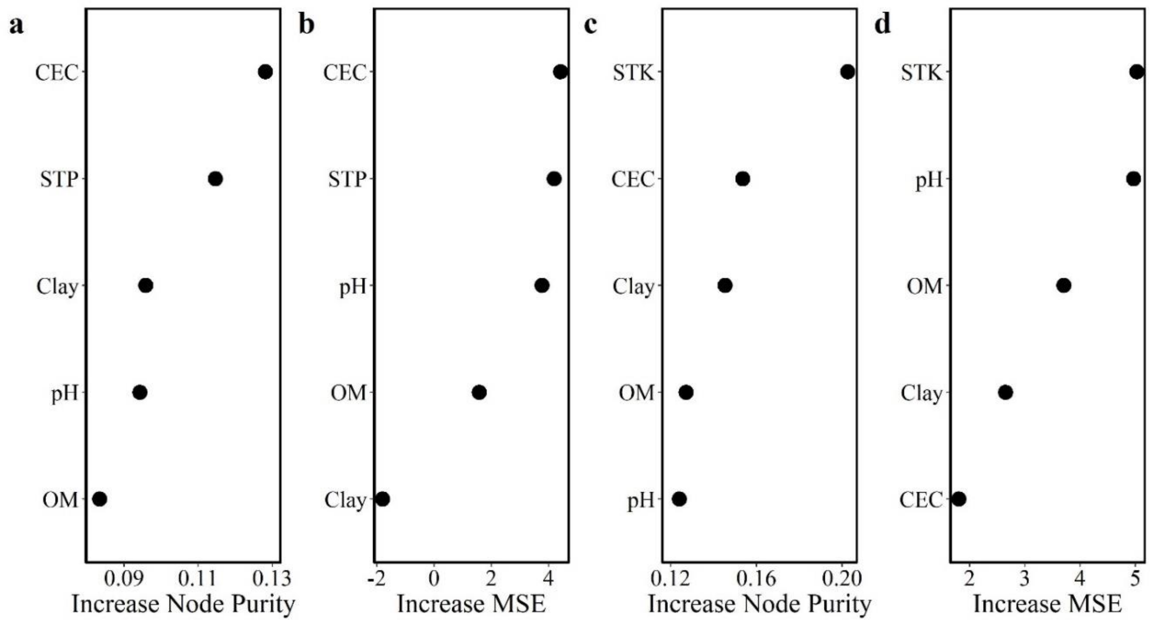

Figure 2.6 Variable importance plots for established random forest models that included soil fertility tests. Panels A and B reflect random forest prediction of relative yield response to P fertilization, panels C and D reflect random forest prediction of relative yield response to K fertilization. Two methods of VIP are displayed, panels A and C are the increase in node purity which reflect the reduction in the residual sum of squares at each split and summed over all splits and trees while panels B and D represent the percent increase in mean-square-error as the variable is randomly selected and permutated over the dataset while. For both methods, the greater the number the greater the relative importance in predicting yield response to fertilization.

Chapter 3:

Developing Agronomic Recommendations for Potassium Permanganate Oxidizable Carbon

3.1 Abstract

The absence of clear empirical relationships and recommendations between soil health and agronomic outcomes are a clear obstacle to wide-spread adoption of soil health assessments in commercial row crop systems. The objectives of this research included 1) determine whether soil health indicators are connected to corn (*Zea mays* L.) productivity in Missouri cropping systems, and 2) establish interpretive benchmarks for assessing and interpreting soil health indicators in Missouri corn cropping systems. The objectives were accomplished by collecting soil and corn grain yield at 446 monitoring sites (37 m^2) in 84 commercial production fields in 2018-2020 growing seasons. Soil health and soil fertility samples were collected prior to planting with corn grain yield collection occurring after corn maturity. These data, along with site-specific soil and weather data, were modeled using linear regression, conditional inference forest (CIF), and random forest (RF) methods. Random forest partial dependency plots were used to identify potential interpretive benchmarks for high importance factors, with corresponding decision and conditional inference trees to serve as a graphical framework of potential on-farm application of the identified top indicator variables. Root-mean-square-error of the three model approaches were similar (~1.4-1.5 Mg ha^{-1}) with distinct improvements in R^2 for the CIF (R^2 = 0.45) and RF (R^2 = 0.46) algorithms over the stepwise approach (R^2 = 0.30). Seasonal rainfall and soil health indicator potassium permanganate oxidizable carbon (POXC) were the only factors included as top tier factors governing grain productivity in each model approach. A potential POXC benchmark ~450 mg POXC kg $soil^{-1}$ was

observed through partial dependency analysis of the RF algorithm and confirmed and refined to 415 mg kg^{-1} for both a decision tree and conditional inference tree analyses. Little evidence was found connecting grain productivity with soil health indicators autoclaved citrate extractable protein and soil respiration. These findings demonstrate the unrealized importance of POXC on corn grain productivity and underscores the potential power of emerging indicators to assess and quantify soil health management effects on productivity. Identification and leverage of similar quantitative relationships with agronomic outcomes will spur on-farm soil health assessments and facilitate sustainable cropping systems.

3.2 Introduction

Soil health is defined as "the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans" (USDA-NRCS 2020). Per this definition, soil health metrics were developed to provide a holistic view of the complex, multi-faceted functions in the soil system. Farmer-level interest is clear, with the expansion of soil health analysis offered in soil test laboratories and increased interest in soil health management practices (Gruver and Weil, 2007; Moebius-Clune et al., 2016; Stewart et al., 2018). Parallel with this movement, significant research efforts have identified soil analyses that reflect improvements in soil biological properties from adoption of conservation land use practices. However, only half of published research investigating soil health indicators report clear, absolute interpretations (Bünemann et al., 2018). Establishing quantitative interpretative frameworks for soil health metrics is critical for widespread adoption.

Conceptual diagrams illustrating soil and crop management impacts on soil health are effective tools providing insight into cropping system impacts on soil processes and functions (Veum et al., 2014). However, conceptual illustrations lack the underpinning quantitative recommendations that translate conceptual illustrations into decision support systems. Current on-farm commercial utilization of soil health metrics uses two approaches: 1) comparing measured values between conservation management practices and conventional practices within a farm or region, or 2) evaluating potential degradation by comparing a cropping system with a "reference state" such as a fence line or local native vegetation to represent undisturbed soil (Veum et al., 2014, 2015; Chu et al., 2019; Hargreaves et al., 2019; Caudle et al., 2020). While effective and informative for on-farm comparisons, these approaches cannot be scaled to regional interpretations nor do they establish trends that provide a framework for practitioners to interpret soil health metrics across a diversity of cropping systems, management practices, or soils. (Zuber et al., 2020). Soil health decision support systems are needed to provide context to identify deficiencies and the environmental and economic benefits associated with ameliorating the indicated deficiency. Several frameworks are available to evaluate soil health including the Soil Management Assessment Framework (Andrews et al., 2004), Cornell University's Comprehensive Assessment of Soil Health (Moebius-Clune et al., 2016; Fine et al., 2017), the Haney Soil Health Tool (Haney et al., 2018), and the recently developed Soil Health Assessment Protocol and Evaluation tool (Nunes et al., 2021). These tools provide a framework for interpretation of soil health metrics, but do not provide specific recommendations for agronomic outcomes.

Improving soil health through conservation practices has been connected to enhanced nutrient cycling, greater productivity, reduced anthropogenic erosion, and improved water dynamics (Snapp et al., 2005; González-Chávez et al., 2010; Kuhn et al., 2016). However, the currently available decision support tools do not quantify the outcomes or benefits of improved soil health. Ideally, soil health assessments would offer quantified interpretations similar to soil fertility evaluations. For example, soil fertility decision support tools provide specific recommendations for a common management decision (fertilizer application) to accomplish a specific objective (grain yield). This type of decision support system spurred commercial adoption of soil fertility assessments which have become an invaluable on-farm tool to improve productivity and mitigate environmental impacts (Peck, 1990; Voss, 1998). The effectiveness of these recommendations can be attributed, in part, to simple quantitative benchmarks with direct connections to agronomic decisions and outcomes (Bray, 1944). Improved productivity is often implied by soil health literature, but productivity is rarely evaluated or reported in soil health assessments (Bünemann et al., 2018; Miner et al., 2020). Stewart et al., (2018) reported less than one-third of peer-reviewed soil health studies included productivity data. There is evidence of a connection between soil health metrics and corn grain yield (Culman et al., 2013; Hargreaves et al., 2019), but an interpretive framework for on-farm application is lacking. These nuanced relationships are regionally dependent (Oldfield et al., 2019) and further research is needed to confirm these observations while developing a quantitative interpretation that identifies conditions where improved soil health leads to improved productivity. Achieving these goals would launch soil health metrics from research-based applications to “boots-on-the-ground” agronomic implementation and recommendations. The objectives of this research were to 1) determine where soil health indicators are connected to productivity in Missouri corn cropping systems, and 2) establish interpretive benchmarks for Missouri corn production.

3.3 Materials and Methods

3.3.1 Site Description and Experimental Design

Research was implemented across 84 fields in mid-Missouri between 2018-2020. The research encompassed central Missouri (Figure 3.1) and spanned across six Major Land Resource Areas (MLRA) predominantly classified as Mollisol and Alfisol soils with predominantly smectitic clay mineralogy (NRCS-SSURGO). Soil conditions are variable with generally deep moderately or well drained soils located in the Deep Loess Hills (107B) or Heavy Till Plain (109) to poorly drained in the Claypan (113) and Wooded Slopes (115B; Table 1). Legacy impacts from current and historical cropping practices are abundant in the area, with estimates that claypan soils have lost nearly one-half of their original topsoil (Bird & Miller 1960). Further, addressing environmental concerns from offsite transport of sediment, nutrient, and herbicides from cropping fields into regional watersheds remain major research and state conservation objectives (Willett et al., 2012; Sadler et al., 2015; Baffaut et al., 2020).

Grain yield productivity data were extracted from an established soil fertility trial where three to six fertilizer response trials ('monitoring sites') were established in each field (Svedin et al., 2021). Each monitoring site (148 m^2), 446 sites total, was divided into four equal 37 m^2 non-replicated, single-rate, fertilizer treatments implemented prior to planting (Figure 3.1). A standardized plot plan was followed and included the following fertilizer treatments, 1) unfertilized control, 2) K treated with 112 kg ha^{-1} of K_2O using KCL (0-0-60), 3) P treated with 112 kg ha^{-1} P_2O_5 using triple super phosphate (0-46-0), and 4) sulfur (S) treated with 28 kg ha^{-1} of S using ammonium sulfate (21-0-0-24S). An additional 25 kg ha^{-1} of N was applied as SuperU® (0-0-46) to treatments one, two, and three, to match the N included in treatment four. An additional 40 kg ha^{-1} N as urea (46-0-

0) was applied at V6 in 2019-2020 to ensure sufficient N supply. Cooperating farmers selected hybrids, weed control, tillage, N fertilization, planting dates, and other practices for each individual field. Climate and soil conditions dictated planting dates, which ranged from April 5 to June 10. The reported results and conclusions reflect only the control treatment grain yield to avoid confounding results from fertilizer application, with the assumption that soil fertility tests would be sufficient to describe potential nutrient deficiencies.

Major Land Resource Area (MLRA) and drainage class information was collected based on geospatial location from the NRCS Soil Survey Geographic (SSURGO) database (Soil Survey Staff, Natural Resources Conservation Service). Landscape positions were identified during soil sample collection while cropping system management practices were collected from the previous five seasons. Historic cropping system practices included tillage, manure practices, cover crop incorporation, and cropping diversity (Table 3.1). Daily minimum and maximum temperature, precipitation, and day length were extracted based on monitoring site geospatial location from the Daymet 1 km^2 grid climate database (Thornton et al., 2020). Total seasonal rainfall was calculated as the total rainfall between planting date and 105 days after harvest. Shannon diversity index (SDI) measures rainfall distribution, and was calculated as

$$SDI = \frac{[-\sum pi \ln(pi)]}{\ln (n)}$$

where *pi* is the daily rainfall relative to the total rainfall in a given time (the growing season) and *n* is the total number of days. The SDI has been demonstrated to impact other management practices (*e.g.* nitrogen management) and affects potential soil infiltration and runoff (Ransom et al., 2019).

3.3.2 Soil and Plant Collection, Processing, and Analysis

Soil fertility, soil health, and pedogenic soil profile samples were obtained in spring prior to planting and fertilizer treatments (Mar-Apr). Soil fertility and soil health samples were collected from eight random 0-15 cm depth cores for each site. Soil fertility samples were air-dried and submitted for analysis to Ward Laboratories (Kearney, NE). A standard suite of soil fertility analysis was conducted, including: loss on ignition organic matter (Nelson and Sommers, 1996), soil test phosphorus (STP) following the Bray extraction methods (Frank et al., 1998), soil test potassium (STK) following the ammonium acetate extraction buffered at pH 7.0 (Warncke and Brown, 1998), cation exchange capacity (CEC) from the sum of base cations (Soil Survey Staff, 2014), and pH (1:1 w/w) measured in water (Coleman and Thomas, 1967).

Soil health samples were broken into two fixed-depth horizons, 0-5 and 5-15 cm, and stored in a cooler at 1.6° C. Samples were subsequently homogenized by passing through a 1 cm screen, air-dried, then dry-sieved through a 2 mm screen. These sampling depths were selected to reflect two approaches, 1) soil health metrics are often more sensitive to management changes at the 0-5 cm depth (Karlen et al., 2014), and 2) Missouri soil fertility sampling guidelines recommend a 0-15 cm depth (Nathan, et al., 2012). Soil biological analysis included soil respiration, soil autoclaved-citrate extractable protein (ACE Protein), potassium permanganate extractable carbon (POXC), soil organic carbon (SOC), total nitrogen, β-glucosidase activity (Deng and Popova, 2011), arylsulfatase activity (Klose et al., 2011), and acid phosphatase activity (Acosta-Martínez and Tabatabai, 2011). Analysis for soil respiration, ACE Protein, and POXC followed methods outlined in the Cornell Soil Health Assessment (CASH) protocols (Moebius-Clune et al., 2016).

Briefly, soil respiration was estimated by rewetting 20g of dry soil and measuring the release of CO_2 after a 4-day incubation period, ACE protein estimated by adding 20mM sodium citrate solution (pH 7) to 3.0 g of dry soil followed by autoclaving and mixing with 1mL of Pierce BCA protein reagent (Thermo Scientific). Finally, POXC was based on Weil et al., (2003) with 20mL of 0.02 mol L^{-1} $KMnO_4$ added to 2.5 g of dry soil, shaken for 2 minutes and allowed to settle for 10 minutes, after which 0.5 mL of supernatant was transferred to 49.5 mL of deionized water and sample absorbance read with a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA) at 550 nm (Moebius-Clune et al., 2016). All enzyme analyses were conducted by adding an enzyme-specific substrate to 1 g of dry soil, incubating for 1 hr at 36^{o} C, and measuring sample absorbance with a spectrophotometer at the specified wavelength (Cary 60 UV-Vis, Agilent Technologies). Soil organic carbon and total nitrogen were analyzed following (Nelson and Sommers, 1996) on a LECO Trumac C/N combustion analyzer (LECO Corp., St. Joseph, MI, USA). Soils were ground to a powder prior to analyze for POXC, SOC, and total nitrogen. All soil biological analyses were completed in the USDA-ARS Soil Quality Lab in Columbia, MO.

For soil profile characterization, a single 1.2 m deep and 4.086 cm diameter soil core sample was taken at the center of each monitoring site using a Giddings Model #5-UV / MGSRPSUV (Giddings Machine Company, Windsor, CO). Because these samples were collected in agricultural production fields, the first horizon was standardized to a 0-15 cm depth and assumed as the plough layer (Ap) horizon. Subsequent pedogenic horizons were characterized using visual and tactile clues with a maximum five horizons identified per soil core. Identified horizons were subsequently sampled and air-dried. Bulk density and soil moisture were collected for each sampled horizon, and the top three horizons analyzed for particle size (Soil Survey Staff, 2014) and the same soil fertility analysis previously listed.

Corn grain yield was estimated from an 11 m^2 area harvested by hand at grain maturity in each treatment. Harvested ears were collected and weighed in bulk using a Rapala ProGuide Digital Scale (Rapala, Minnetonka, MN) with an eight-ear subsample oven dried at 65°C to measure grain moisture. Grain yield was adjusted to 15.5% moisture and cob weight subtracted using a grain to cob ratio of 0.89, calculated from a published regional dataset (Kitchen et al., 2017; Ransom et al., 2021).

3.3.3 Data Processing, Analysis, and Statistics

Explanatory variables included the previously listed soil fertility and soil health analyses, coupled with site-specific seasonal climate factors, public soil information, and management practices (Tables 3.1 & 3.2). A combination of traditional statistics and nonparametric tools were used to optimize prediction of grain yield productivity to determine which parameters governed yield variability in this dataset. All statistical analyses were conducted in R statistical software (R Core Team, 2016). Stepwise linear regression, random forest (RF), and conditional inference forest (CIF) models were fit using the 'caret' package (Kuhn, 2017). The dataset was partitioned into calibration (80%) and validation (20%) datasets prior to analysis. The tuning of internal parameters and model development were conducted through a cross validation approach on the calibration dataset while final model performance was assessed using the validation dataset. Model statistics were evaluated by calculating root-mean-square-error (RMSE) and R^2 (Qin et al., 2018). Tuning of internal model parameters used a range of values and a ten-fold cross-validation repeated ten times to ensure model optimization. Models were trained on nine of the ten folds, with the accuracy measured using RMSE between the predicted and actual values on the final tenth fold. Tuning parameters with the lowest RMSE across each of the 100 cross-validation folds were chosen for the final model (Breiman, 2001; Ransom et al., 2019).

Stepwise regression was used as a traditional linear approach for predicting yield and identifying factors governing productivity (Culman et al., 2013). Cross validation was utilized to identify the optimized number of final predictor variables to be incorporated into the model. After determination of the optimal number of variables, the model was fit to the whole dataset to determine optimal factors for predicting grain yield. Nonparametric RF and CIF algorithms were utilized for modeling grain yield productivity to address some of the challenges in linear modelling approaches, such as assumptions regarding linear relationships and multicollinearity between predictor variables. The nonparametric algorithms provide a robust means of investigating the complex interactions between soil, environmental, and management effects upon grain yield without the same associated assumptions. Further, they provide novel variable importance measures that do not suffer from similar shortcomings of traditional variable selection methods (Cutler et al., 2007). The RF control parameters were *ntree* = 501 (number of trees to grow) with *mtry* = 1 through 8 and cross validation identifying *mtry* = 8 as the optimum number of variables to consider for splitting at each node. For the CIF control parameters, *ntree* = 500 and *mincriterion* = 0 (significance level for a split to occur) to grow out the maximum trees. Both RF and CIF are boosted approaches that build upon to decision trees (Random Forest) and conditional inference trees (Conditional Inference Forests) to improve accuracy at the expense of interpretability (James et al., 2000). Decision trees and conditional inference trees utilize recursive binary splitting to determine optimal splits of a feature space assembled by variables that influence grain yield productivity. The difference between decision and conditional inference trees is the criterion that constitutes a node split. For decision trees, the split is selected to maximize the information measured (reduce residual sum of squares) while conditional inference trees select splits with a test of significance (Hothorn et al., 2006). Each of these approaches are effective approaches that overcome the challenges in linear approaches (Cutler et al., 2007).

Variable importance evaluations were calculated for both RF and CIF models using the 'randomForest' and 'partykit' packages in R (Liaw & Wiener 2002; Hothorn et al., 2006; Hothorn & Zeileis, 2015). Two variable importance methods were utilized to evaluate important factors in the RF model 1) decay in mean standard error (MSE) which measures the decay in model accuracy as a variable is randomized, and 2) node purity, which measures the change in residual sum of squares at each node split weighted across the 501 decision trees in the random forest (Breiman, 2001). Highly correlated variables can interfere with variable importance evaluations or partial dependency evaluations in RF evaluations (Strobl et al., 2007; Elith et al., 2008). Therefore, loss-on-ignition organic matter and total nitrogen were not included because of their high correlation with SOC ($r > 0.90$). The CIF variable importance was specifically developed for the challenges in RF variable importance evaluations and potentially confirm variable importance from the other methods. Partial dependence plots were used to further explore the relationship between individual explanatory variables and predicted productivity. Given the complexity of the soil system, partial dependency plots were provided to obtain a description of some of the major aspects of the functional relationships between grain yield productivity and soil, management, and climate factors. They provide a graphic representation of the dependence of grain yield on individual predictor variables (*i.e*. soil test potassium, rainfall, etc.) after averaging out the effects of the other predictor variables in the model (Cutler et al., 2007). After the top indicators were determined, decision and conditional inference trees were fit using the identified variables (Tables 3.1 and 3.3) to serve as a graphic framework of potential on-farm application of the identified top indicator variables.

3.4 Results and Discussion

A wide range of soil conditions and environmental properties were observed. Soil fertility conditions were variable, with observed deficiencies and soil nutrient concentrations above recommended soil test values (Table 3.2). While no recommendations are currently available for soil health indicators, there was considerable variability in each indicator, similar to other published regional observations (Zuber et al., 2020). In 2018, weather conditions were substantially drier than the subsequent years resulting in yields considerably lower during that season. It is noteworthy that less than 10% of monitoring sites were executed in 2018; consequently this dataset predominantly reflects seasons of sufficient, or excessive rainfall.

3.4.1 Linear Relationships and Model Performance

In general, correlations between bivariate linear relationships were not strong between soil health indicators and grain yield (Figure 3.2). While several relationships were significant, the low r^2 values highlight the limitations of bivariate analysis in detecting complex relationships between soil properties and productivity. Relationships between yield and several soil health indicators (POXC, SOC, TN) were significant, but substantial variability remained ($r^2 \leq 0.06$) indicating these relationships are complex and multi factored, and therefore simple bivariate linear methods are inadequate. Reported correlation coefficients as high as 0.64 and 0.35 have been reported, such as in Michigan, USA (Culman et al., 2013) and in Honduras (r^2 = 0.74) for various soil health indicators (Stine and Weil 2002). However, these previously published relationships reflect long-term studies at single locations, which are ideal for identifying site-specific management impacts on soil health indicators, by controlling for the complex interactions introduced from sites in multiple climate and environmental conditions. These reported site-specific single-factor relationships likely degrade as more cropping systems, climate, and soil conditions are included in a dataset (Wade et al., 2020). Reducing sampling depth to 0-5 cm from the standard 15 cm sampling depth did not meaningfully impact the bivariate relationship for any of the soil health indicators (Figure 3.2).

Cross-validation with stepwise regression identified a five-variable model as the model with the lowest RMSE and highest R^2 (Table 3.3). Yet, both the CIF and RF models outperformed the stepwise linear regression prediction on the validation dataset, with modest reductions in RMSE and considerable increases in R^2 values (Table 3.3). The RF model outperformed the CIF model on the calibration dataset, but both performed similarly on the validation dataset with RMSE equal to 1.4 Mg ha^{-1}. It is worthwhile to note that while RF and CIF methods of deriving node-splits differ, they provided similar precision (Table 3.3). The improvements in reduced error and improved accuracy highlight the potential benefits of RF and CIF as alternatives to traditional approaches when examining non-linear and interacting factors in agricultural research. Model performances were moderate (R^2 < 0.50) but acceptable considering parameters only included soil properties, limited management practices, and precipitation patterns. Further data collection including factors such as corn variety, weed and pest pressure, nitrogen management, and final stand would potentially further improve model performance.

3.4.2 Identifying Yield Governing Factors

After identifying the optimal five variable limit, a stepwise regression model with a five variable limit was fit to the whole dataset to identify key factors governing grain yield (Table 3.4). The final model performed modestly (R^2 = 0.32) with the top variable subset including total rainfall, CEC, POXC, and MLRA (Table 3.4). Seasonal rainfall, CEC, and MLRA currently have well established links to grain productivity. Precipitation patterns drive productivity through absence, or supply, of plant available water and its subsequent effects upon fertilizer nitrogen losses (Tremblay et al., 2012; Li et al., 2019). Cation exchange capacity reflects both physical (soil texture) and chemical (soil exchange sites) soil properties and is linked to grain productivity through the storage and supply of essential plant nutrients. POXC is a relatively processed portion of the labile carbon (C) pool that has been connected to corn grain yield and is sensitive to management practices that promote soil C stabilization (Weil et al., 2003; Lucas and Weil, 2012; Hurisso et al., 2016). Finally, MLRA echoes the effect of parent material and soil formation processes on grain productivity (NRCS-USDA Agricultural Handbook 296). The inclusion of POXC confirms other reports that it is a superior indicator of productivity than current standard measurements of organic matter (Stine and Weil, 2002; Culman et al., 2013; Wade et al., 2020).

Variable importance in the random forest algorithm was calculated by measuring the decay in model accuracy as a single variable's values are randomly permutated (Breiman, 2001). An increase in MSE indicates greater error is introduced when that specific variable is randomly permutated (Figure 3.3). Model error increased most for seasonal rainfall, SDI, POXC, and planting date, with model error over 50% more for these four variables than the other explanatory variables. Four variables again clearly separated from the remainder of the explanatory variables in the CIF variable importance evaluations: seasonal rainfall, SDI, MLRA, and POXC. The CIF variable importance is not included as a superior method to identify variable importance, but rather as further evidence confirming the importance of high impact factors upon corn grain productivity. The inclusion of SDI, rainfall, and POXC in both the RF and CIF evaluations suggests a strong connection with grain yield. Further, the importance of MLRA as the fifth most important variable in the RF ranking compliments the stepwise regression approach (Table 3.4).

It is also worthy to note established soil fertility measurements were influential upon grain yield. Both CEC and STK were included in the top ten variables for both the RF and CIF variable evaluations, while STP was included in the top five indicators of the CIF evaluation (Figure 3.4). Soil test K and STP estimate the potential seasonal soil supply of P and K nutrients, while CEC reflects the potential storage of nutrients and, in Missouri, is closely related to clay content (Bray, 1944, 1954; Solly et al., 2020). ACE protein, a soil health indicator, was also included in the top ten variables for both RF and CIF variable importance evaluations (Figures 3.3 & 3.4). While not included as the “top” indicators of productivity, these indicators constitute a secondary tier of influence. The omission of soil respiration as a top or secondary indicator of grain yield was surprising, considering its reported links to yield production and suggestions it be included in regular soil sample analysis (Culman et al., 2013; Hurisso et al., 2016; Franzluebbers, 2016; Adhikari et al., 2021). In another regional assessment of soil health indicators and agronomic outcomes, Wade et al., (2020) observed that soil respiration reflects characteristics that are inherently site specific, and when employed across a diversity of environments this relationship with productivity is diminished. The results from this dataset confirm this observation. It should be noted that these results reflect seasons with adequate precipitation and the relative importance of soil respiration could be highlighted during seasons of water stress.

Planting date, MLRA, and SDI were included as top tier indicators but not in each evaluation method (Table 3.4, Figures 3.3 & 3.4). Planting dates set annual yield potential with cascading effects on yield from interactions with weather determining final stand counts and whether plant growth stages align with critical precipitation patterns (Van Roekel and Coulter, 2011; Baum et al., 2019). Shannon diversity index reflects the distribution of rainfall and potential infiltration into the soil surface. For example, a poor SDI indicates high intensity rain events over a short time, which leads to less potential soil infiltration and soil water storage for crop use. Finally, MLRA reflects soil forming factors and processes and their relative impacts upon physical, chemical, and biological soil properties that govern grain yield. The exclusion of MLRA, planting date, and SDI from top tier indicators in variable importance evaluations derive from unique method assumptions and mathematical approaches used to determine important relationships between independent and dependent variables. These various methods are not provided to support one method, but rather as confirmation of the robust nature of the independent variables' impact upon productivity.

Only seasonal rainfall and POXC were included in the top tier in every method of identifying impactful factors on grain yield (Table 3.4; Figures 3.3 & 3.4). The inclusion of rainfall is unsurprising because of its previously discussed relationship between water supply and grain yield. Excessive rainfall or drought are the top two drivers of yield loss from extreme events in the US (Li et al., 2019). Surprisingly, POXC, rather than any other established soil fertility or soil health indicator, demonstrated the strongest quantifiable link with grain yield. This was not expected because of the well-established connections between productivity and soil fertility assessments and the poor bivariate relationship between POXC and grain yield (Figure 3.2). This demonstrates the unrealized importance of POXC on corn grain productivity and further underscores the potential power of emerging indicators to assess and quantify soil health management effects on productivity.

3.4.3 Benchmarks for Interpreting Soil Health Metrics

Stepwise regression identified POXC, rainfall, CEC, and MLRA as important indicators of grain yield. While useful, the practical on-farm application of these model coefficients is limited to “positive” or “negative” impacts on grain yield. The coefficients do not provide a framework to interpret whether a certain POXC, CEC, or rainfall amount can maximize yield. Therefore, there is limited on-farm utility of these coefficients. While specific coefficients are not provided in variable importance evaluations, external partial dependency plots (PDP) offer insight into the input-output variable relationships and provide a useful basis for interpreting the relationship of these factors with grain yield (Friedman, 2001; Elith et al., 2008). Partial dependency plots further provide insights into possible thresholds for an indicated variable and the relative impact upon prediction of a response variable (Cao et al., 2015; Zeng et al., 2017; Lawrence et al., 2021).

The PDP plots confirmed the findings of the variable importance evaluations with weather related factors (Seasonal rainfall and SDI) resulting with the largest increases (1.0-1.5 Mg ha^{-1}) in predicted yield (Figure 3.5). Both seasonal rainfall and SDI follow S-shaped membership curves (Figure 3.5) where optimal yield occurred at seasonal rainfall above 500 mm and SDI above 0.70 (Figure 3.5) with the lowest yield below 300 mm seasonal rainfall and SDI below 0.60 (Figure 3.2). This relationship between yield and seasonal rainfall was expected, with yield increases no longer occurring once the necessary rain was achieved for optimal production. I expect the lower SDI values reflect high intensity spring rain events which negatively impacted seedling emergence and vigor and subsequent stand counts and final yield. Planting date itself was included as an important variable in the variable importance evaluations, but the indicated effect on yield is minimal unless planted after d 150 (May 30th; Figure 3.5). This date is later than other Missouri reports that observed 10% yield decreases beginning May 11th and 22% yield decreases by May 31st (Wiebold and Massey 2012). However, this corresponds well with other reports that optimal planting for the US Corn Belt is typically late April with yield declines beginning in late May (Nafziger, 1994) that can reach up to 15-30% if delayed more than 4 weeks (van Roekel and Coulter, 2011). Missouri seasonal climate variability is substantial and further research is required to verify the seasonal stability of these observed trends. As previously mentioned, yield was collected predominantly in sufficient or excessive seasonal rainfall, any discrepancies with other reported trends are likely because native to the environmental and climate conditions from 2018-2020.

The PDP relationship between the soil fertility tests (STP, STK) behaved similarly to current recommendations (Figure 3.5). Typical P and K fertilizer recommendations are based on fitted quadratic curves between relative yield and soil test (STP and STK), where fertilizer application is not recommended above the fitted curve plateau, referred to as the critical concentration (Dodd and Mallarino, 2005). The PDP relationships for STP and STK mimic quadratic response curves, with yield increases plateauing near established University of Missouri critical concentrations (Figure 3.5; current critical concentrations identified with vertical red dashed lines). The similarities between the PDP plots and established STP and STK recommendations lends credibility to optimizing yield based on these soil tests and adds prospective reliability to the other reported PDP relationships (Figure 3.5).

Soil health PDP plots each demonstrated distinct relationships with grain yield (Figure 3.5). POXC demonstrated an S-shape membership curve with optimal yield observed above ~450 mg kg^{-1} and the lowest yields below 300 mg kg^{-1} (Figure 3.5). The relative yield gains from POXC were not as large as climate (0.6 Mg ha^{-1}), but were the largest of all other soil analyses (Figure 3.5). Yield and ACE protein demonstrated a generally positive relationship (Figure 3.5). There was an indicated plateau near 4 mg g soil^{-1}, but the relative yield increase (0.3 Mg ha^{-1}) is nearly half of the POXC yield effect. ACE protein has not been historically utilized to estimate productivity, but rather is connected to the soil protein pool, potentially available organic nitrogen, and aggregate stability (Wright et al., 1999; Rosier et al., 2006; Hurisso et al., 2018; Geisseler et al., 2019). The positive link between ACE protein and productivity could reflect the supply of nitrogen through mineralization, or indirect links between aggregate stability and grain productivity. Finally, the soil respiration effect on grain yield was minimal, without a strong observable relationship (Figure 3.5).

Both soil respiration and POXC have been previously related to grain yield while ACE protein is typically utilized to estimate potentially available organic nitrogen pool or the impacts of management practices on soil properties. In one analysis, soil respiration and POXC were identified as the top two variables in predicting yield (Hurisso et al., 2016). Further, at a long-term research site, soil respiration was identified as a more sensitive indicator of corn productivity and agronomic performance than POXC and other commonly employed soil organic matter methods (Culman et al., 2013). That trend is contrary to the reported results here (Figures 3.3 and 3.4). While both soil respiration and POXC measure distinct portions of the labile C pool, soil respiration also reflects soil metabolic potential (Hurisso et al., 2016). Soil metabolic potential is highly sensitive to site-specific soil conditions (texture, structure, soil organic matter, etc.) and Wade et al., (2020) observed the site-specific characteristics of soil respiration connections with corn productivity are diminished when employed regionally. I expect the regional nature of this dataset rendered soil respiration as an ineffective indicator of corn grain yield. It should be noted that evaluations of POXC and soil respiration typically only include total soil C as a covariate (Stine and Weil, 2002; Lucas and Weil, 2012; Culman et al., 2013; Hurisso et al., 2016; Singh et al., 2020). Expanding explanatory variables to include weather, management, and soil fertility factors in a universal approach suggests the POXC relationship with yield is robust, while the role of soil respiration may have been diminished by the influence of other factors (*e.g*. weather, soil fertility, management practice). In total, these results indicate soil respiration may not be an effective regional predictor of grain yield and may best serve as a side-by-side comparison or as an indicator of other agronomic outcomes (*e.g*. nitrogen mineralization). In contrast, this study confirms that POXC is an effective regional indicator of corn grain productivity and provides, for the first time, a potential benchmark for agronomic interpretation of POXC measurements.

3.4.4 Potential Application of the POXC Benchmark

The regression and conditional inference trees yielded similar RMSE and R^2 values on the validation dataset and performed the poorest of the previously evaluated model approaches (Tables 3.4 & 3.5). Both approaches included only two variable splits with the primary difference being the primary node split. SDI was the primary split in the decision tree, and seasonal rainfall was the primary split for the conditional inference tree (Figure 3.6). In both evaluations, POXC values of 415 and 416 mg kg soil^{-1} were identified as a second split (Figure 3.6). This split criterion corresponds with the PDP plots which indicate a noticeable increase in yield near this POXC value (Figure 3.5). The 1.0 Mg ha^{-1} difference between terminal nodes after the POXC split in the conditional inference plot correspond well with the observed PDP analysis (Figures 3.4 and 3.5). These results could simply be summarized by stating that the most productive yields occurred in environments with sufficient rainfall with less intense precipitation events where POXC was above 415 mg kg^{-1} (Figure 3.6). These results confirm the previous conclusions that precipitation factors are the primary drivers of productivity and POXC is an effective tool to evaluate productivity once sufficient precipitation variables are satisfied (Figure 3.3 & 3.4). It is important to note that decision tree and conditional inference tree analyses provide clearly understood results with easily interpreted graphical representations (James et al., 2000). However, this approach does not provide the same level of precision as linear regression, RF, and CIF approaches. Therefore, rather than generating a universally applicable decision support tool, the decision and conditional inference trees provided a potential framework for application of the observed weather and soil health benchmarks.

The results address two major critiques of soil health assessments 1) including empirically derived relationships with agronomic outcomes and 2) the pressing need for interpretive baselines for soil health indicators (Bünemann et al., 2018; Caudle et al., 2020). Further, it is the first step to providing a necessary baseline for connecting labile soil C assessment to agronomic performance, one of the *"long-term goals of sustainable agriculture research"* (Culman et al., 2013). Further research is needed to investigate the robust nature of this benchmark within and outside Missouri corn cropping systems and potential interactions with natural edaphic and management practices (Zuber et al., 2020). Further, it is uncertain whether POXC is mechanistically connected to grain productivity, or it is connected to other soil processes that improve productivity. Current assessments of POXC conclude that it is one of the most sensitive fractions of the labile soil C pool (Bongiorno et al., 2019), reflects general soil health (Fine et al., 2017), and is recommended as an integral soil health component (Morrow et al., 2016). POXC is related to a relatively processed portion of the labile soil C pool that is sensitive to management practices that promote soil C stabilization (Culman et al., 2012; Hurisso et al., 2016) which suggests POXC reflects the agronomic production benefits associated with reducing tillage practices. I would reiterate that while POXC is sensitive to biological soil health status, it is not a universally equipped indicator for evaluation of all aspects of soil health (Karlen et al., 2019). These results are not proposed to replace current and effective decision tools that evaluate soil management affects upon soil functions, and I expect these thresholds or relationships could be incorporated into established regional soil health assessments in the future (Andrews et al., 2004; Moebius-Clune et al., 2016; Nunes et al., 2021). Rather, these results are proposed to empirically demonstrate the agronomic benefits of enhancing soil health indicators and provide an interpretive framework for practitioners to incentivize adoption of conservation management practices and soil health assessments.

3.5 Conclusions

These results provide a plausible framework for interpreting soil health indicators within the context of environments, management practices, and soil conditions found in Missouri corn cropping systems. These results report POXC as a more sensitive predictor of corn grain yield than both traditional measures of soil organic matter and soil fertility analyses. Further, a clear POXC benchmark is reported whereupon corn grain yield was maximized. Further evidence is required to validate this benchmark within and outside of Missouri corn cropping systems across soil and weather conditions. This study found little to support the use of soil respiration as an indicator of corn grain yield under these conditions and only identified a weak relationship with ACE protein. Future work is needed to explore the specific soil properties and processes represented by POXC and how POXC contributes to grain yield productivity. This will inform whether yield directly responds to improvements in POXC, or whether POXC serves as a proxy or indirect measure of other soil properties that enhance the growing environment. These results also demonstrate the advantages of using statistical non-parametric approaches to understand relationships between soil health indicators and productivity. Overall, this provides the first empirical relationship between POXC and corn grain yield that is uniquely designed to inform on-farm decision support systems. Identifying and leveraging similar quantitative soil health relationships with economic incentives will aid in incentivizing and spurring on-farm adoption of conservation management practices.

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3.7 Tables and Figures

Table 3.1 Descriptions of management and environmental variables included in evaluation of grain productivity. All management practices reflect the previous five years of management history. Major Land Resource Area descriptions are available through the USDA Natural Resources Conservation Services.

Variable	Description	n
Major Land Resource Areas		
113	Central Claypan Areas	173
107B	Iowa and Missouri Deep Loess Hills	132
109	Iowa and Missouri Heavy Till Plain	79
115A,B	Central Mississippi Valley Wooded Slopes, Western Part	53
Tillage		
Heavy Tillage	Three or more years of tillage	171
Light Tillage	Two years or less of tillage	15
No-Till	No Tillage operations	251
Cover Crops		
Heavy	Two or more years of planted cover crops.	138
Light	One year of planted cover crops.	98
No Cover Crops	No planted cover crops.	201
Cropping Rotation		
Corn-Soybean	Corn soybean rotation.	362
Diverse	The incorporation of any other cash crops aside from corn and soybeans (cover crops not included).	62
Monoculture	A single crop grown each season.	8
Manure Management		
Heavy	Two or more years of manure application	30
Light	One year of manure application	41
None	No manure application in previous five seasons	366

Table 3.2 Variable descriptive statistics for soil analysis, climate, and management practices.

Variable	Units	Mean	Median	St. Dev	Min	Max
Grain Yield	Mg ha^{-1}	15.9	16.1	1.97	8.84	20.2
Soil Fertility Analysis						
pH		6.6	6.6	0.5	5.0	7.7
Organic Matter	%	3.3	3.2	0.59	2.0	5.3
Soil Test P (STP)	mg kg^{-1}	22	17	19	2.0	168
Soil Test K (STK)	mg kg^{-1}	144	129	70	49	544
CEC	meq 100 g soil^{-1}	14	13	3.7	6.1	27
Sulfate-S	mg kg^{-1}	7.4	7.2	2.3	1.8	16
Sand	%	18	17	5.3	4.0	58
Silt	%	55	57	7.6	21	73
Clay	%	27	26	6.9	11	73
Soil Health Analysis						
Beta-Glucosidase	µg PNP g soil $^{-1}$ hr $^{-1}$	77	77	20	4	163
Acid-Phosphatase	µg PNP g soil $^{-1}$ hr $^{-1}$	166	158	50	66	294
Arylsulfatase	µg PNP g soil $^{-1}$ hr $^{-1}$	47	44	19	18	123
Soil Respiration	mg C-CO_2 kg soil^{-1}	144	141	41	49	318
ACE Protein	mg g soil^{-1}	3.6	3.7	1.1	1.5	6.6
POXC	mg kg soil^{-1}	445	440	105	198	752
SOC	%	1.6	1.5	0.35	0.85	2.8
Total N	%	0.15	0.15	0.03	0.09	0.28
Cropping System Management						
Planting Date	Ordinal Day	129	131	18	96	160
Climate and Environment						
Total Rainfall	mm	230	241	77	43	394
Shannon Diversity Index		0.63	0.63	0.06	0.43	0.74
Abundantly Well-Distributed Rainfall		149	147	58	24	279

Table 3.3 Reported statistics for training and testing datasets for three statistical methods.

	Training		Testing	
Model	**R^2**	**RMSE**	**R^2**	**RMSE**
		Mg ha^{-1}		Mg ha^{-1}
Stepwise Linear Regression	0.30	1.7	0.30	1.5
Conditional Inference Forest	0.33	1.7	0.45	1.4
Random Forest	0.45	1.5	0.46	1.4

Table 3.4 Stepwise linear model results fit to the whole dataset with reported model statistics and variable coefficients. The stepwise model fit was restricted to identify the five most significant variables. The optimal number of final variables was determined a priori through cross-validation (Table 3.3).

Coefficients	**Variables**	**Model Statistics**	
		p-value	R^2
12.3	Intercept	2.20E-16	0.32
-0.157	CEC		
0.00435	POXC		
0.00707	Rain		
0.956	MLRA Loess Hills		
-0.260	MLRA Heavy Till Plain		

Table 3.5 Reported statistics for nonlinear models, shown for both training and testing datasets.

	Training		**Testing**	
Model	**R^2**	**RMSE**	**R^2**	**RMSE**
Conditional Inference Tree	0.24	1.78	0.20	1.64
Regression Decision Tree	0.25	1.75	0.19	1.63

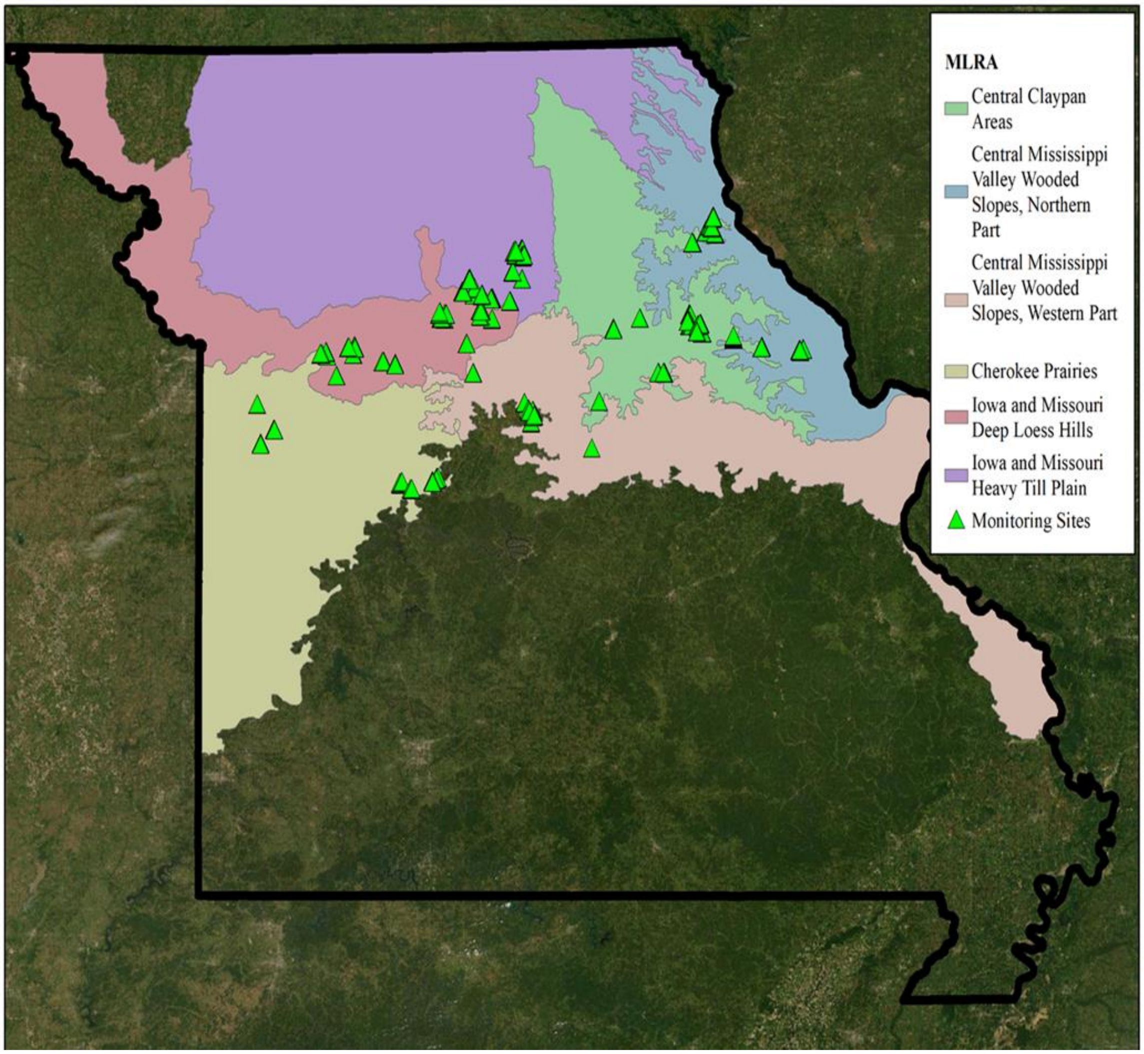

Figure 3.1 A map of Missouri soil regions by Major Land Resource Areas and geolocation of fields with established monitoring sites for yield collection.

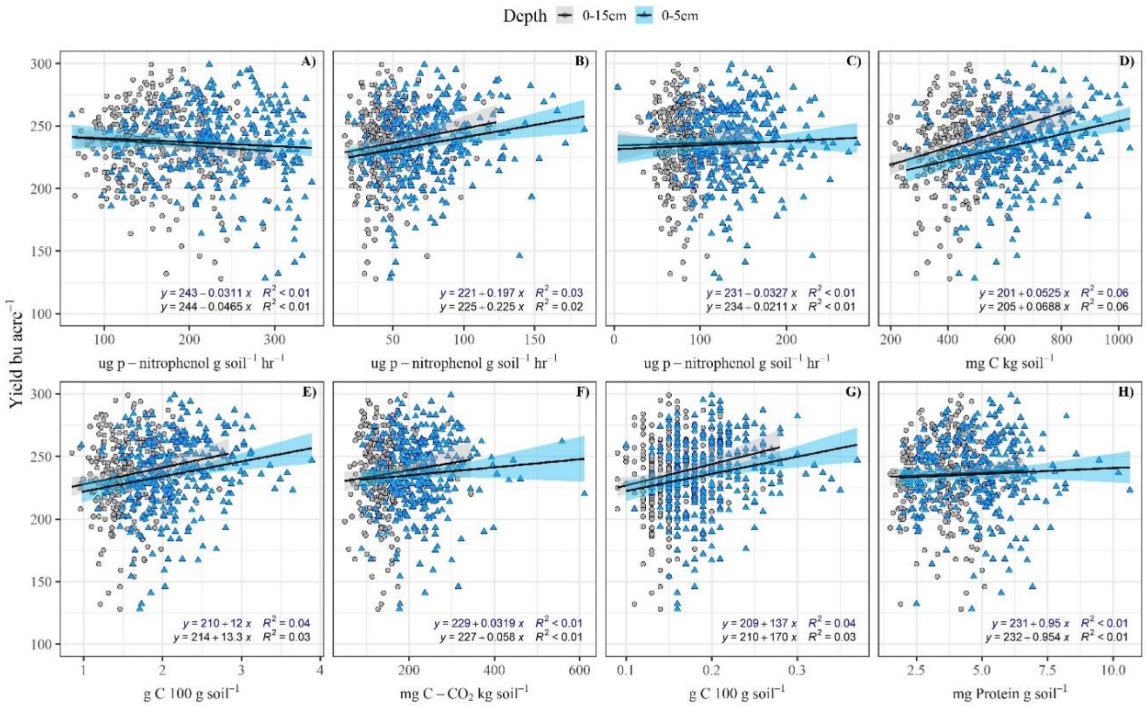

Figure 3.2 Linear relationships between soil health indicators (0-5 and 0-15 cm) and yield with reported model equations and r^2 statistics. Included soil health indicators are A) acid phosphatase B) arylsulfatase C) β-glucosidase D) permanganate oxidizable carbon, E) soil organic carbon, F) soil respiration, G) total nitrogen, and H) autoclaved citrate extractable protein.

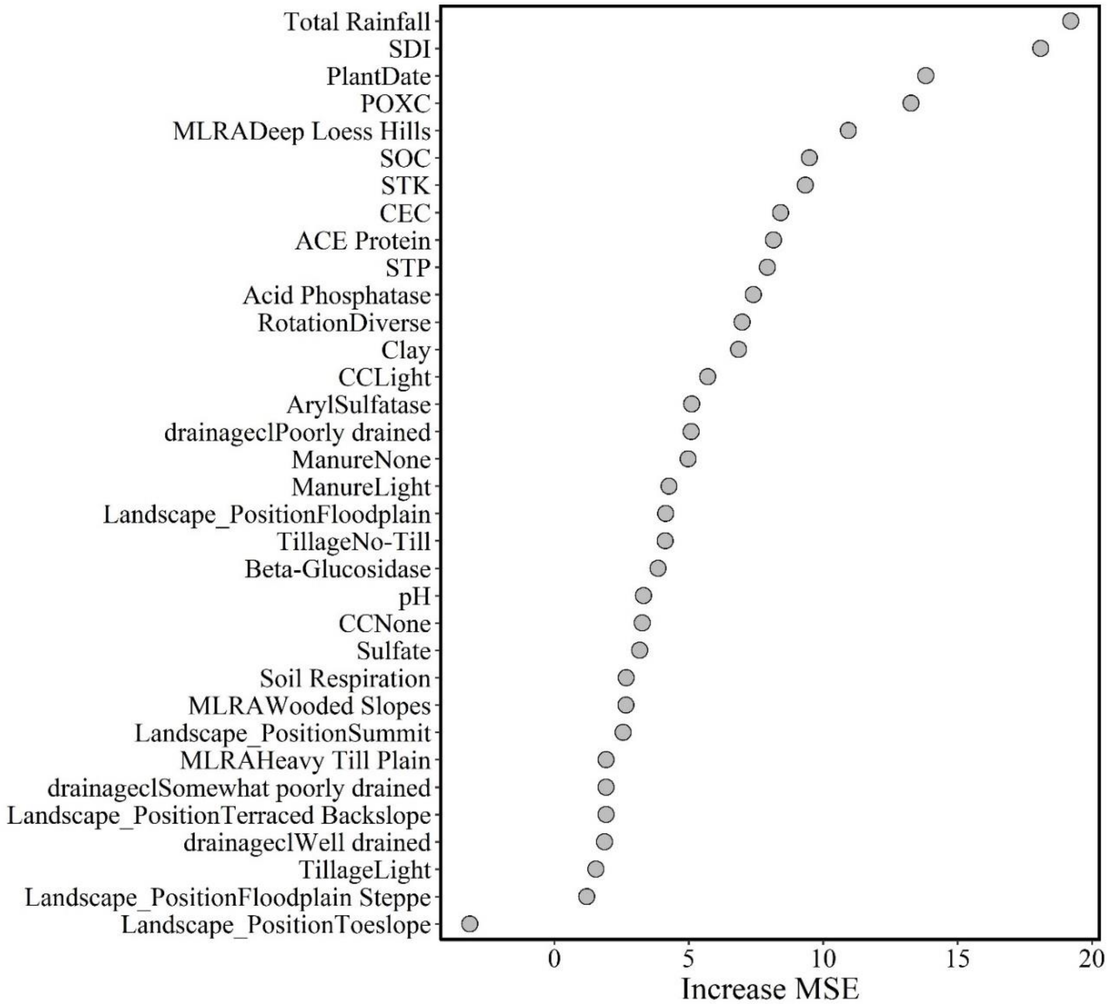

Figure 3.3 Variable importance rankings from the Random Forest results predicting corn grain yield at 445 monitoring sites in 89 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting productivity. All soil tests, management practices, and SSURGO data are included.

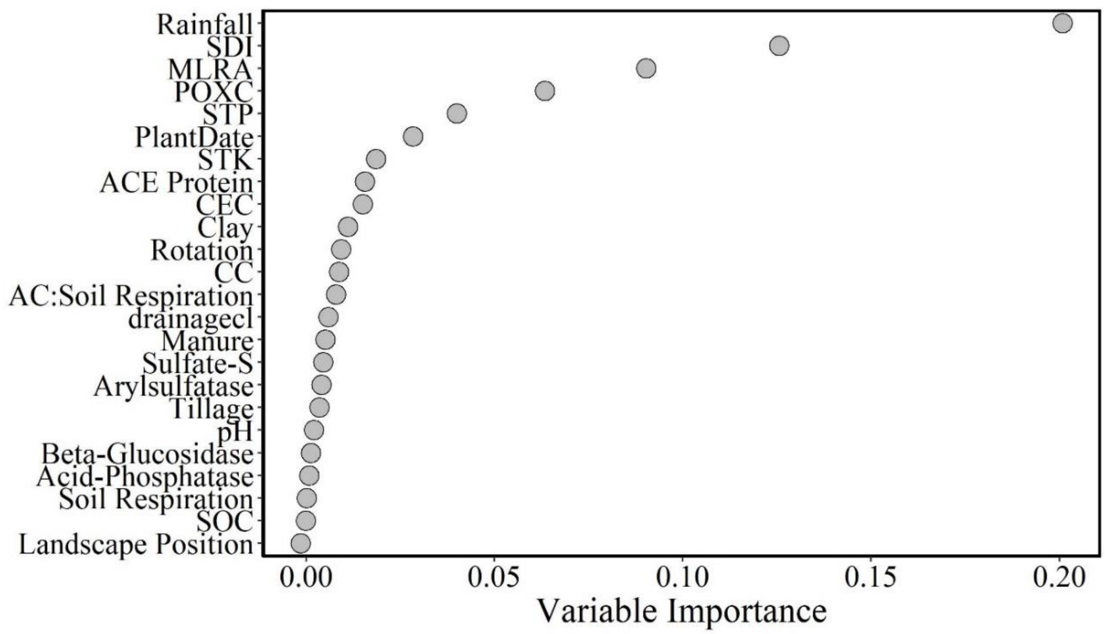

Figure 3.4 Variable importance rankings from the conditional inference forest (CIF) results predicting corn grain yield at 445 monitoring sites in 89 fields in Missouri. The greater the number and ranking, the more important the variable in predicting productivity. All soil tests, management practices, and SSURGO data are included.

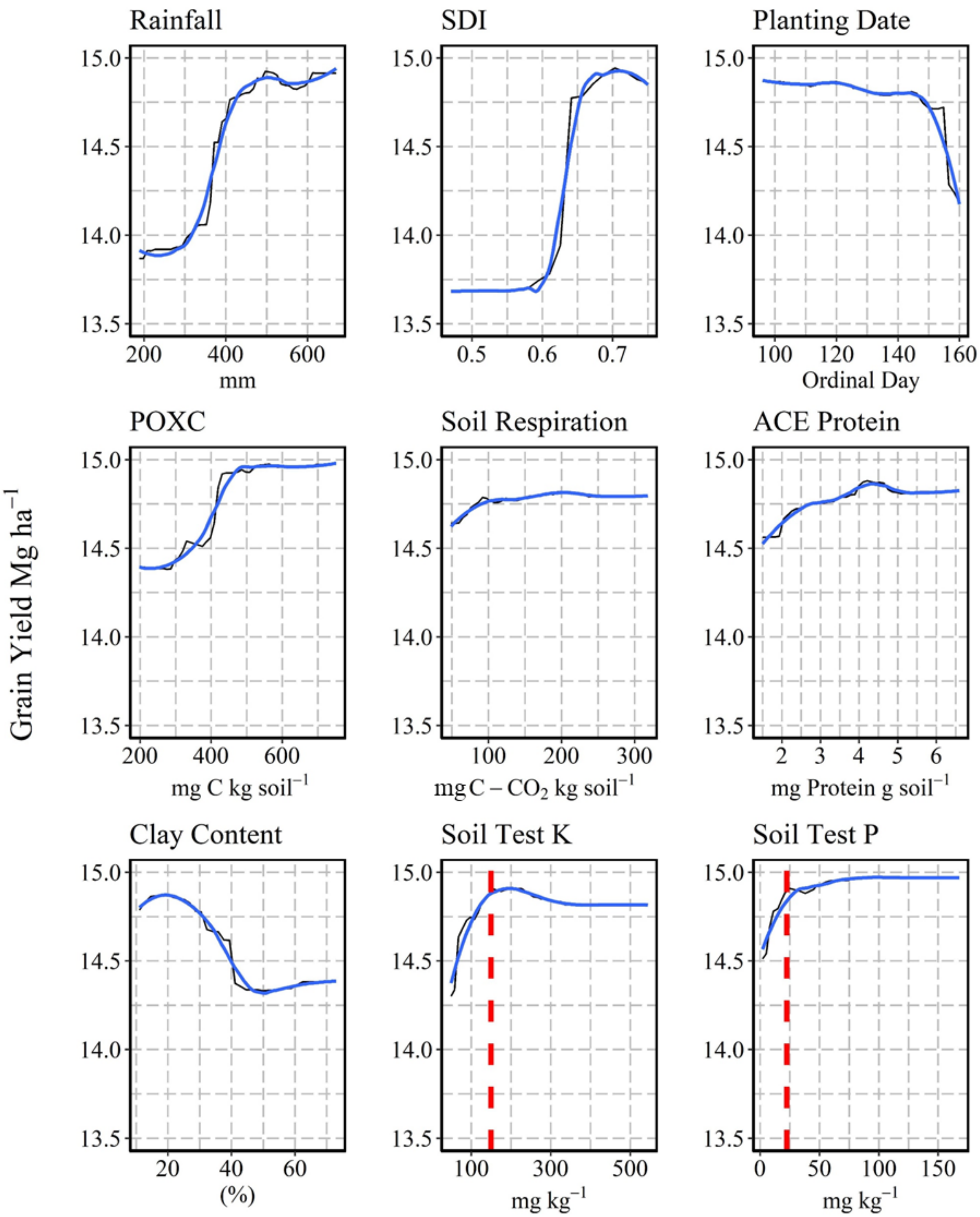

Figure 3.5 Partial dependency plots for climate, soil health, and soil fertility variables in predicting yield with the random forest algorithm. Blue lines reflect a LOESS smoothed line to highlight general trends. The y-axis is predicted yield from the random forest model with the x-axis reflects the range of values observed in this dataset for the indicated explanatory variable. The raw partial dependency relationship is indicated with the black line, with the overlaying blue line reflecting a smoothed function of the partial dependency relationship. Vertical red lines in soil test K and P plots are established regional critical concentrations to inform P and K fertilization.

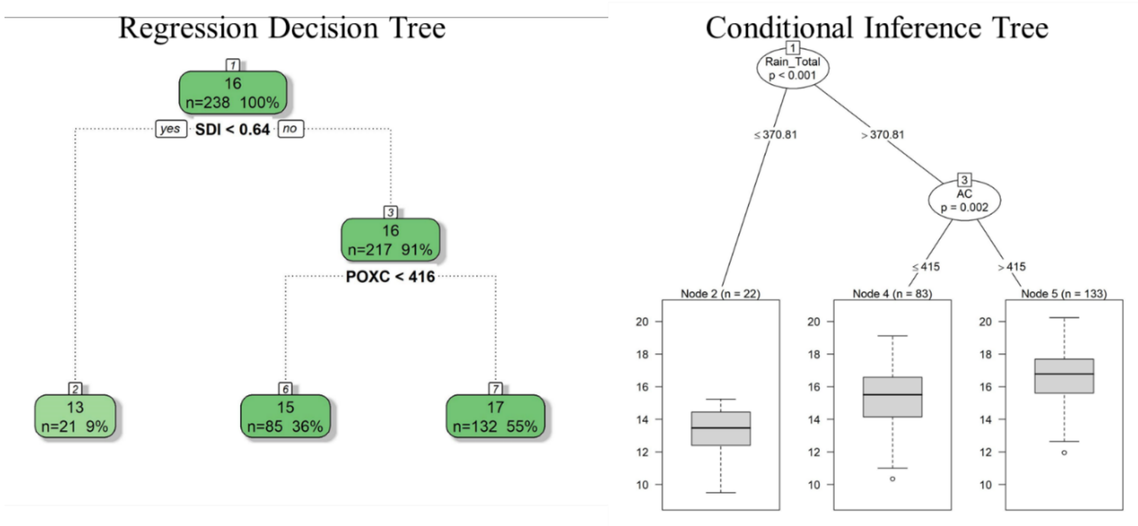

Figure 3.6 Decision and conditional inference tree for Missouri corn grain yield productivity. Splitting values are in the units of the parameter used for the split (Table 3.1), with n representing the number of observations included in each split and terminal node. Decision tree splits are conditional upon the minimization of residual sum of squares of the dataset while conditional inference trees are dependent upon an a priori significance level ($p < 0.05$). Boxplots for the conditional inference tree reflect the variability of yield (Mg ha^{-1}) at the terminal node with significant p-values identified at each node split.

Chapter 4:

A Regional Missouri Soil Health Assessment: Endemic and Management Effects when Interpreting Soil Health Metrics

4.1 Abstract

Effective regional soil health assessments hinge upon regionally robust soil analyses sensitive to critical soil processes and functions. Standardized soil health recommendations, sampling depths, and connections to conservation management practices are not yet fully developed. Therefore, objectives of this research included 1) identify important soil factor conditions to consider in regional assessments, 2) evaluate regional sensitivity of soil health indicators to manure application, tillage practice, cover crop implementation, and crop rotation, and 3) empirically evaluate sampling depth approaches. Between 2018-2020, soil samples were collected at two depths (0-5 cm; 0-15 cm) at 446 sample sites across 101 commercial row crop fields in Missouri. Random forest and least absolute shrinkage and selection operator (LASSO) were utilized to model selected soil health indicators with subsequent environmental and management effects determined by LASSO coefficients. Model R^2 varied (0.22-0.56) with soil respiration performing the poorest and potassium permanganate oxidizable carbon (POXC) the greatest. These results affirm the importance of interpreting soil health indicators within the parameterization of baseline soil organic carbon (SOC) measurements, soil texture, and soil formation factors and processes related to major land resource areas. Soil pH was identified as a major governing factor upon five of the seven soil health indicators—highlighting its important governing effect on biological processes and potential interference with established laboratory methods. The greatest and strongest crop

management effects upon soil heath indicators were related to field manure application with no-till and crop rotation relationships less robust but still effecting three of the seven indicators. Only soil respiration was regionally sensitive to cover crop practices. Finally, a framework is provided that identifies optimal sampling depths and regional management sensitivity for each soil health indicator. Overall, these results provide a needed framework for practitioners and soil test laboratories to consider when providing regional soil health evaluations.

4.2 Introduction

Effective soil health assessments hinge upon regionally robust soil analyses sensitive to critical soil processes and functions. Development of these indicators began in the 1990's (Karlen et al., 2019) with recent focus on biological assessments (Veum et al., 2015). On-farm interest is evident with recent public and private research initiatives (Karlen et al., 2017; Norris et al., 2020) and commercial laboratories now offering soil health biological soil analysis (Fine et al., 2017; Stewart et al., 2018; Wade et al., 2018).

Several management principals are recommended to improve soil health, including 1) diversifying plant communities, 2) minimizing of soil disturbance, 3) maintaining root growth throughout the season, and 4) maintaining soil cover to protect against disturbance (USDA-NRCS 2020). Integrating livestock grazing or manure inputs are recommended as well as a fifth soil health principal. Implementing these practices fosters soil health by preventing erosion processes, promoting soil biology, reduces losses and restoring soil organic carbon (SOC), stimulating nutrient cycling, and enhancing the hydrologic cycle (Doran and Safley, 2002; Doran, 2002; Lehman et al., 2015; Nunes et al., 2020). Recently, many soil biological indicators have been developed to track improvements from these management practices. These indicators are useful to assess management impacts upon nutrient cycling, soil C stabilization, aggregation processes, and soil microbial activity (Deng and Tabatabai, 1997; Veum et al., 2015; Hurisso et al., 2016; Geisseler et al., 2019). Empirical relationships between these soil biological indicators and the management practices are documented at plot or sub-field evaluations (Stine and Weil, 2002; Lucas and Weil, 2012; Culman et al., 2013; Veum et al., 2015). These results identified site-specific relationships that can be further verified in other environmental conditions and management practices. However, inherently these assessment conclusions are limited to the environmental conditions in which they are conducted. Ample evidence exists to show soil health indicators are sensitive to site-specific properties, such as soil texture, native SOC content, and other environmental properties (Ladoni et al., 2015; Fine et al., 2017). Therefore, there is a need to scale plot-level relationships to landscape and regional environments (Andrews et al., 2004; Fine et al., 2017).

These challenges are acknowledged, and efforts to address them are underway through the Soil Management Assessment Framework (SMAF), Comprehensive Assessment of Soil Health (CASH), and Soil Sealth Assessment Protocol and Evaluation (SHAPE) tool (Andrews et al., 2004; Moebius-Clune et al., 2016; Nunes et al., 2021). These tools largely remain research based and have not been widely adopted at commercial soil testing laboratories (Wade et al., 2018). Consequently, without associated recommendations or interpretive frameworks, (Andrews et al., 2004; Fine et al., 2017; Nunes et al., 2021) reports are focused on raw values that do not account for specific soil or environmental conditions. Further, important factors that influence each of the seven soil health indicators are not well established. Soil formation factors and physical properties are already recognized (Zuber et al., 2020), but further work is needed to identify regional impacts upon regional efficacy of soil health metrics in Missouri.

Finally, there is no standardized universal sampling depth for soil health evaluations; which are encouraged for regionally relevant and reproducible recommendations. Rather, soil health sampling depths in are variable, and dependent upon sampling objectives. Some opt to sample surface soil depths (e.g., 0-5 cm) because of the increased sensitivity to management practices (Veum et al., 2015; Zuber et al., 2020) while other soil health evaluations replicate established sampling depths (Missouri; 0-15 cm). Both sampling depths provide benefits, with surface sampling providing increased sensitivity to management effects upon soil processes and functions (Veum et al., 2015), while deeper sampling streamlines on-farm implementation by eliminating an extra sampling depth. It remains undetermined whether one sampling depth is ideal for regional assessments. The associated objectives of this paper are to 1) identify important soil and environmental conditions to consider in regional assessments, 2) evaluate regional sensitivity of soil health indicators to manure application, tillage practice, cover crop implementation, and crop rotation, and 3) empirically evaluate two soil health sampling depth approaches.

4.3 Materials and Methods

4.3.1 Field Site Description and Data Collection

Soil samples were collected from commercial row crop systems in mid-Missouri between 2018-2020. Field sites were predominantly classified as Mollisols and Aflisols with smectite clay mineralogy (NRCS-SSURGO). Field sites were encompassed within six Major Land Resource Areas (MLRA) with variable soil conditions. Typical parent material for this area is a layer of loess over glacial till while alluvium dominates along the Missouri and Mississippi Rivers. Deep well drained soils were located in the Deep Loess Hills (107B) or Heavy Till Plain (109) while poorly drained soils were located in the Claypan (113) and Wooded Slopes MLRA's (115B).

Soil samples were collected in cooperation with a regional fertilizer response trial. Details of the fertilizer trials are described in detail in previous chapters. Bulk soil samples were collected in the spring (March through April) from 8-12 soil cores collected within a 148 m^2 area with. Three to five bulked samples were collected within a field with a total of 446 soil samples collected from 101 commercial row crop fields. Soil cores were sampled to a 15-cm depth and broken into two fixed depths; 0-5 cm and 5-15cm. These sampling depths were selected to reflect two approaches, 1) the 0-5 cm depth is more sensitive to management changes (Karlen et al., 2014), and 2) the 0-15 cm depth is the established Missouri soil sampling guideline (Nathan, et al., 2012). Sampled depths were individually mixed with 0.12 L and 0.25 L subsampled from the 0-5 cm and 5-15 cm depths for a soil fertility sample. In total, two soil health samples were collected, one at 0-5cm and one at 5-15cm depths. The third sample was the corresponding 0-15 cm soil fertility sample. All samples were transported in coolers to avoid major temperature fluctuations. Soil fertility samples were immediately air-dried and submitted for analysis, while soil health samples were stored in a cooler at 1.6° C and later homogenized by passing through a 1 cm screen, air-dried, and dry-sieved through a 2 mm screen. After analysis the soil health samples were standardized to reflect a 0-5 cm and 0-15 cm depth. The 0-15 cm depth was estimated by multiplying the top 0-5 analysis by 0.33 and the bottom 5-10cm by 0.66 to represent a 0-15 cm depth sample.

Soil fertility analysis were conducted at Ward Laboratories (Kearney, NE) with a standard suite of soil fertility analysis, including: loss on ignition organic matter (Nelson and Sommers, 1996), Bray extraction soil test phosphorus (STP) (Frank et al., 1998), soil test potassium (STK) from buffer pH 7.0 ammonium acetate extraction (Warncke , and Brown 1998), sum of base cations cation exchange capacity (CEC) (Burt and Soil Survey Staff, 2014), and water pH (1:1 w/w) (Coleman and Thomas, 1967). Soil texture was further analyzed to estimate sand, silt, and clay content. All soil biological analyses were completed through the USDA-ARS Soil Quality Lab in Columbia, MO.

Seven soil biological analyses were included in this investigation: SOC, soil respiration, soil autoclaved-citrate extractable protein (ACE Protein), potassium permanganate extractable carbon (POXC), β-glucosidase activity (Deng and Popova, 2011), arylsulfatase activity (Klose et al., 2011), and acid phosphatase activity (Acosta-Martínez and Tabatabai, 2011). The Cornell University CASH protocols were followed for soil respiration, ACE protein, and POXC (Moebius-Clune et al., 2016). Briefly, soil respiration was estimated by rewetting 20g of dry soil and capturing CO_2 release during a 4-day incubation period with a KOH alkali trap, ACE protein estimated by adding 20mM sodium citrate solution (pH 7) to 3.0 g of dry soil followed by autoclaving and mixing with 1mL of Pierce BCA protein reagent (Thermo Scientific). POXC was based on Weil et al., (2003) with 20mL of 0.02 mol L^{-1} $KMnO_4$ added to 2.5 g of dry soil, shaken for 2 minutes and allowed to settle for 10 minutes, after which 0.5 mL of supernatant was transferred to 49.5 mL of deionized water and sample absorbance read with a spectrophotometer (Cary 60 UV-Vis, Agilent Technologies, Santa Clara, CA, USA) at 550 nm (Moebius-Clune et al., 2016). All enzyme analyze were conducted by adding an enzyme specific substrate to

1 g of dry soil, incubating for 1 hr at 36° C, and measuring sample absorbance with a spectrophotometer at a enzyme specific wavelength (Cary 60 UV-Vis, Agilent Technologies). Soil organic carbon and total nitrogen were analyzed following (Nelson and Sommers (1996) on a LECO TRUMAC C/N combustion analyzer (LECO Corp., St. Joseph, MI, USA). For SOC, total nitrogen, and POXC soils were ground to a powder prior to analyze and decrease variability between replicates.

For soil profile characterization, a single 1.2 m deep and 4.086 cm diameter soil core sample was taken at the center of each monitoring site using a Giddings Model #5-UV / MGSRPSUV (Giddings Machine Company, Windsor, CO). Because these samples were collected in agricultural production fields, the first horizon was standardized to a 0-15 cm depth and assumed as the plough layer (Ap) horizon. Subsequent pedogenic horizons were characterized using visual and tactile clues with a maximum five horizons identified per soil core. Identified horizons were subsequently sampled and air-dried. Bulk density and soil moisture were collected for each sampled horizon, and the top three horizons analyzed for particle size (Burt and Soil Survey Staff, 2014) and the same soil fertility analysis previously listed.

Coordinates of each soil sample sites were collected using a Trimble GeoXT 6000 and Geo7x GPS device (Sunnyvale, CA, USA) with approximately 6-cm accuracy. Drainage class information, soil series, and MLRA were extracted from the NRCS Soil Survey Geographic (SSURGO) database (Soil Survey Staff, Natural Resources Conservation Service) from geospatial location. Drainage class was converted from a categorical to numeric ranking: 1) representing well drained soils, 2) moderately well drained, 3) somewhat poorly drained, and 4) poorly drained. Landscape positions for each sample site were identified during soil sample collection. Landscape positions included standard classifications of summit, backslope, and toe slope, with additional positions including terraced backslope, floodplain, and floodplain steppe. Previous five years of cropping system histories including tillage, cover crop implementation, manure application, and cash crop rotation were collected through communication with current farm managers. Management practices were indexed as to whether they were present or not present with management separated as follows: tillage (no-till or tillage); cover crops (yes or no), manure application (yes or no); and crop rotation (diverse or grower standard practice—GPS). To be noted, if any winter cover crop was grazed, it was indexed as yes to manure application. Cover crop indexing did not include stand density or biomass amount. Crop rotation only reflects cash crop diversity with diverse reflecting 3 or more cash crops or a perennial crop prior to row crop production, while GSP included corn-soybean and corn-corn rotations.

4.3.2 Data Processing, Analysis, and Statistics

Multiple statistical approaches were utilized to evaluate management impacts on soil health analysis within soil and environmental conditions. Dependent variables included each of the seven soil biological analyses at both 0-5 cm and 0-15 cm sampling depths. Independent factors included a combination of soil formation properties (drainage classification, landscape position, MLRA), edaphic soil properties (clay content, sand content, pH, CEC, SOC), and management factors (tillage, cover crop, crop rotation, and manure inputs). Soil organic carbon and CEC were removed as independent variables when predicting SOC as a dependent variable. Arylsulfatase and acid phosphatase facilitate the release of sulfate sulfur and phosphatase, and high concentrations of these nutrients can disincentivize plant and microbial populations production of these enzymes. To account for this effect, sulfate and soil test phosphorus concentrations were included when predicting these specific extracellular enzymes.

Two approaches were utilized to identify important co-factors to include when evaluating management impacts on soil health indicators, first a random forest algorithm and the least absolute shrinkage and selection operator (LASSO). All statistical analyses were conducted in R statistical software (R Core Team, 2016). Identification of important co-factors governing soil health analysis were identified through random forest and LASSO regression techniques. Random forest and LASSO regression models were fit using the 'caret', 'rpart', and 'glmnet' packages (Kuhn, 2017). The dataset was partitioned into calibration (70%) and validation (30%) datasets prior to analysis. Internal parameter tuning for both LASSO and random forest model development were conducted through tenfold cross validation repeated ten times. Internal model parameters were trained on nine of the ten folds and model accuracy assessed using RMSE between predicted and observed values in the tenth fold. Tuning parameters with the lowest RMSE across each of the 100 cross-validation folds were chosen for the final model (Breiman, 2001; Ransom et al., 2019). Final model performance was evaluated by calculating RMSE and R^2 for the partitioned validation dataset (Qin et al., 2018).

Nonparametric random forest (RF) algorithms were utilized for modeling each soil health analysis, to provide a robust means of investigating the complex interactions between soil, environmental, and management effects upon each soil health indicator without the same associated assumptions of linear regression (Breiman, 2001). The random forest control parameters were *ntree* = 501 (number of trees to grow) with *mtry* = 1:5 which is the optimum number of variables to consider for splitting at each node. For LASSO regression, lambda tuning hyperparameter was identified between 0.0001:1 with 100 randomly selected values to identify the optimal lambda hyperparameter. Variable importance evaluations were calculated for the random forest models using the 'randomForest' (Liaw & Wiener 2002; Hothorn et al., 2006; Hothorn & Zeileis, 2015.). Variable importance in the random forest model was estimated by the decay in mean standard error (MSE) as all other factors are held constant at their average value and the variable of interest is randomized. Highly correlated variables can interfere with variable importance evaluations. therefore, loss-on-ignition organic matter and total nitrogen were excluded because of a high correlation coefficient (R > 0.90) with SOC (Strobl et al., 2007; Elith et al., 2008).

Evaluation of important co-factors for interpreting soil health indicators were done based upon whether they were included in the final LASSO model. The top three largest coefficients in magnitude were identified as the "top tier" factors governing the prediction of a soil health indicator. Evaluation of sampling depth was based upon LASSO and random forest R^2 and RMSE values, with the model with the highest R^2 and lowest RMSE chosen as the optimal sampling depth.

4.4 Results and Discussion

The regional variety in soil conditions, environment, and management practices lead to a broad distribution of sampled soil health indicator values (Tables 4.1 and 4.2). Over 50% of sample sites were no-till management or cover crops with 38% of all sites were both no-till with implemented cover crops. Duration of cover crop and tillage practices implementation were variable, with cover crops planted at least three of the previous five seasons for 60% of the sites where cover crops were implemented. Only 18%

of the soil sample locations received manure application, and 14% of the sample sites were in a rotation that included a third cash crop (Table 4.1). For each soil health indicator, the 0-5 cm depth led to higher mean values than the 0-15 cm depth (Table 4.1). On average, surface sample indicators were 34-78% greater in the 5 cm depth (Table 4.1) which corresponds well with other observations (Veum et al., 2015). Limiting sampling depth to 5 cm emphasizes the dynamic soil interface with residue accumulation, water, atmospheric gas exchange, and temperature. As such, microbiological activity is generally higher in the soil surface relative to deeper soil samples. Conservation practices, especially no-till, facilitate this stratification, and depth ratios between biological measurement have been proposed as a soil quality indicator (Franzluebbers, 2002). Regarding on-farm recommendations, this stratification highlights the need for, and importance of, a standardized sampling protocol for regional soil health evaluations.

4.4.1 Random Forest and LASSO Results

Model accuracies varied by soil health analyses and sampling depth, but generally LASSO and random forest models produced similar RMSE per indicator and sampling depth (Table 4.3). Random forest models produced equal or better R^2 values than the LASSO approach, with four soil health indicators resulting in $R^2 > 0.50$ (POXC, SOC, acid phosphatase and arylsulfatase; Table 4.3). The model for soil respiration was the poorest ($R^2 = 0.22$). It is noteworthy that the R^2 for enzyme activity and ACE protein performed better on the 0-5 cm depth while POXC, soil respiration, and SOC R^2 were greater in the 0-15 cm depth.

Feature selection between the random forest and LASSO also yielded similar results, with the top indicators in random forest variable importance evaluations generally aligning with LASSO coefficients (Table 4.4, Appendix I). To eliminate redundancy, only the LASSO results are discussed further because of their advantage of eliminating non-significant factors and the benefits of their coefficients easily describing the relationship between independent variables and the selected soil health indicator. Figures depicting random forest variable importance for each soil health indicator by soil sampling depth are supplied in Appendix C.

4.4.1.1 Soil Organic Carbon

The final model SOC-5 cm included a diversity of environmental, soil properties, landscape, and management factors (Table 4.4). The only independent factors not included in the final model were crop rotation and sand content. The factors with the largest influence (i.e., highest coefficient) were MLRAs Deep Loess Hills and Heavy Till Plain, with the former having influence two times greater than any other factor. These factors too were positive, meaning compared to the population of samples, SOC-5 cm was greater for soils from these two MLRAs. Other strong and positive relationships were observed with manure application, no-till, and clay content. Others have also shown how manure and no-till have positive influence on SOC (citations). Other factors were significant, but their influence minor based on the magnitude of the model coefficients.

Results were similar for the SOC-15cm model, with the greatest effects deriving from MLRAs and clay content (Table 4.4). The relationship of other factors remained similar to the SOC-5cm in both magnitude and whether the effect was positive or negative. Of note, the influence of adding manure and tillage was half or less as important with the SOC-15cm sample as compared to the shallow sampling. These results reaffirm soil forming factors and processes effects on regional soil health assessments (Andrews et al., 2004; Nunes et al., 2021) and reaffirm reports that SOC varies significantly between MLRA's in Missouri (Zuber et al., 2020).

Sampling depth provided unique sensitivity of management practices on SOC. Both identified manure application as the management practice with the greatest impact. The SOC-5cm gave greater weight to tillage practices than SOC-15, which confirms other reports in Central Missouri that the effects of tillage practices are greater in surface samples (Veum et al., 2015).

Therefore, both sampling depths are appropriate for identifying regional effects of tillage, but the relative benefits of no-till on SOC will be more evident at a shallower sampling depth. This is because organic residue and nutrients become concentrated at the soil surface, which lead to greater differences in soil biological activity between tillage practices at this surface depth. These results were inadequate to fully investigate the potential regional impacts of management on SOC, but they were consistent with other soil and crop management studies. Further work with more observations within each MLRA may be necessary and beneficial for evaluating whether specific regions respond uniquely to conservation management practices.

4.4.1.2 Potassium Permanganate Oxidizable Carbon

Soil biological, chemical, and physical properties governed POXC-5cm rather than regional and landscape features (Table 4.4). Soil organic carbon and pH were positively related with POXC, while clay content was negatively associated. These top tier factor coefficients were over two times greater than the others. The second tier of factors included no-till and manure application, both positively related with greater POXC concentrations.

The final tier of factors included MLRA, landscape features, and crop rotation, all having similarly minimal impact on POXC-5cm.

Results for POXC-15cm were similar positive effects from SOC, pH, and negative effects from clay content (Table 4.4). Greater weight was given to manure applications in the POXC-15cm depth than the surface sample, with the manure effect being twice as large as no-till. The positive effects of tillage were minimized in POXC-15 with effects similar to landscape position and crop rotation. The effects of MLRA and drainage class were not included in the POXC-15cm model, which suggests their effects were not as important with deeper sampling.

POXC is one of the most recommended soil health indicators because of its advantages of readily field measurable and responsiveness to land management practices (Weil et al., 2003; Fine et al., 2017). Identifying SOC as the governing factor of regional POXC values is unsurprising considering POXC measures a specific SOC pool (Weil et al., 2003; Lucas and Weil, 2012; Culman et al., 2012). As SOC changes, it is reasonable that the other commensurately changes. Therefore, baseline SOC is critical for accurate interpretation of management effects upon POXC values. The positive relationship with pH is surprising considering the poor correlation in both sampling depths, and no reported links between POXC oxidation efficacy and soil pH. The POXC method is an unbuffered reaction (Weil et al., 2003) and pH variability between sites could potentially affect the thermodynamics of the $KMnO_4$ oxidation of the carbon (C) pool. Permanganate oxidation efficacy is reported to be pH-independent (Dombrowski et al., 2018), but decomposition rate of perfluoroctanesulfonate by permanganate in freshwater systems is optimized in low pH systems (Liu et al., 2012). Further investigation is necessary to confirm these observed effects of pH on the unbuffered POXC reaction, and whether pH specific adjustments are necessary in regional evaluations. There are already acknowledged modifications in POXC evaluations needed for textural variability, which are included in other soil health scoring calculations, and expanding this correction for pH may be necessary (Moebius-Clune et al., 2016; Nunes et al., 2021). It is noteworthy that MLRA impacts were limited to the POXC-5cm. I conclude this is because of the significant impact from SOC in the surface soil and therefore governing the POXC models. As mentioned previously SOC was highly impacted by MLRA, and I speculate that variability in POXC introduced by MLRA is already captured in the strong relationships between SOC and POXC. Extending the sampling depth to 15 cm appears to effectively remove any regional effect from MLRA.

In general, for both sampling depths, POXC values were greater in no-till systems, where manure was included with the cropping management, and in GSP crop rotations (Table 4.4). The effects of cover crops were not included and suggest POXC is not an effective regional indicator of the benefits from incorporating CC into a crop rotation, at least based on the amount of CC use on fields evaluated. The large impacts from tillage practice and manure inputs align with other work that has observed that POXC is an effective indicator of soil C stabilization and accumulation processes (Culman et al., 2012, 2013; Hurisso et al., 2016). This confirms that these are regionally robust relationships that can be measured and used to inform recommendations. Reducing sampling depth to 5 cm lends greater weight to tillage impacts on these processes, while the 15 cm depth highlights the impacts of manure inputs (Table 4.4).

4.4.1.3 Soil Respiration

Compared to the other indicator models, the model performance was poorest for soil respiration (Table 4.3), indicating the available independent features were insufficient to describe variability in this measurement at a regional scale. I conclude this is because the only biological factor provided was SOC, which only represents energy source for the microbial population. Soil respiration measures the release of CO_2, which further reflects microbial metabolic potential. I expect that expanding to include microbial biomass or communities would improve regional prediction of soil respiration. However, the practical feasibility (e.g., special sample handling and analysis costs) of using these evaluations to calibrate soil respiration rates is questionable.

Soil organic carbon, manure application, and MLRA Deep Loess Hills governed the Resp-5cm model (Table 4.4). The effect of SOC was two times greater than any other factor, with MLRA and manure application 30% greater than the other landscape, management, and soil chemical or physical properties. Soil texture properties, drainage classification, and landscape position effects were similar. Tillage practice was the only excluded management practice in the final model, with increased soil respiration rates observed in soils cover cropped and diversified crop systems. Model results for the Resp-15cm closely mimicked the Resp-5cm observations, with SOC and manure inputs providing the greatest relative impact (Table 4.4). Both Resp-5cm and Resp-15cm models included more environmental factors than any other evaluated soil health indicator (Table 4.4). This highlights this metric's unique sensitivity to site-specific environmental conditions, landscapes, soil properties, and management practice. This observation aligns with other reports that soil respiration is susceptible to site-specific characteristics interfering with scaling to regional implementation (Franzluebbers and Assmann, 2020; Wade et al., 2020).

Soil respiration is typically correlated with SOC, microbial biomass, and is a sensitive and early indicator of management impacts on soil biological processes (Franzluebbers et al., 2000; Ladoni et al., 2015; Hurisso et al., 2016). In general, soil respiration rates were greater where organic inputs were greater (*e.g.*, manure) and where diversification of crop species occurred (cover crop implementation and diversification of cash crops; Table 4). The larger soil respiration rates observed in manure incorporated cropping systems confirms other observations that integrated livestock systems have significant positive impacts on soil respiration metrics (Franzluebbers et al., 2020). Though tillage was included as a factor in the final 15 cm depth model, its relative impact was minor. This too confirms other research that rather than soil stabilization processes, soil respiration is sensitive to the benefits of organic amendments, C inputs, and practices that stimulate mineralization (Culman et al., 2013; Hurisso et al., 2016).

4.4.1.4 ACE Protein

Modeled ACE protein yielded no notable difference in important parameters between the two sampling depths, with both sampling depths identifying the same major and minor factors (Table 4.4). Though similar between soil depths, overall model performance was better for the ACE-5cm model (Table 4.3). Consequently, while the relevant factors were identified regardless of sampling depth, the features were more pronounced with the shallower sampling. These results support the 5 cm sampling depth is preferred for regional evaluations of ACE protein.

Since important factors were similar by depth and to avoid redundancy, discussion on factors governing ACE protein will focus on the 5 cm depth. The top tier factors included SOC, pH, and the Heavy Till Plain MLRA (Table 4.4). These effects were 50-300% greater than the other environmental, management, and soil properties. Greater SOC and soils from the Heavy Till Plain led to ACE Protein, while a negative relationship was found with pH. ACE protein samples the organic nitrogen pool which explains the high impact of SOC (Geisseler et al., 2019). The inclusion of soil pH as a strong predictor of ACE protein was unexpected, a point not previously reported in the literature. Further research is needed to shed light upon the mechanisms behind this indicated relationship. ACE protein measurements appear to be robust against variability introduced in landscape and MLRA features. The only MLRA impact was the Heavy Till Plain, and minor sensitivity to landscape position and drainage class. The minor effect from clay content does not align with other regional ACE protein evaluations, where textural classification was a major contributor in ACE protein measurements (Fine et al., 2017). I conclude that this discrepancy is because the results reported by Fine et. al. (2017) represented three U.S. regions, which contain substantially greater textural and soil formation variability that the Mid-Missouri region in which these data were collected.

Though the magnitude effect was minor, the greater concentrations of ACE protein found in the diversified crop rotations aligns with other work that diversified crop rotations and perennial systems facilitate ACE protein and aggregate stability (Wright et al., 1999; Wright and Anderson, 2000). ACE protein is typically connected to aggregation processes, such as manure and tillage inputs (Wright and Upadhyaya, 1998; Geisseler et al., 2019). It has been noted that ACE protein sensitivity to management effects vary regionally with conservation management practices increasing ACE protein at only one of seven research sites (Liebig et al., 2006). Further regional work in Missouri is necessary to validate tillage and manure impacts on ACE protein. Currently, these results suggest connections between ACE protein and diversified cropping systems are regionally robust.

4.4.1.5 β-Glucosidase Activity

The most influential governing factors impacting β-glucosidase at both sampling depths were SOC, pH, and manure, effects at least three times greater than other environmental, soil, and management practices (Table 4.4). Generally, no-till also promoted β-glucosidase activity, but the effect was minor. β-glucosidase catalyzes the hydrolysis of β-D-glucopyranosides in the final, rate-limiting step in the degradation of cellulose (Stott et al., 2010). Therefore, it's activity is directly connected to organic C inputs. The greater activity rates observed in manure applied fields correlates well with the improved C cycling that occurs when organic amendments are utilized (Bandick and Dick, 1999). Sensitivity to pH is not typically reported in soil health assessments. Analytical analysis of β-glucosidase activity utilizes a buffered solution to maintain pH during enzyme activity analysis (Deng and Popova, 2011); however, there is evidence that universal buffering can deviate from target pH as much as 1.6 units depending on soil pH and clay content (Li et al., 2021). Future research is necessary to identify whether the role of pH is from interactions with laboratory methods, or the facilitation of *in situ* stabilization of β-glucosidase.

Also, as mentioned with previous indicators, further work is needed for verifying whether inherent soil pH conditions are necessary for regional soil health assessments. Sensitivity to management practices were similar between the two sampling depths, with positive effects found with manure additions, diversified crops, and no-till. These results align with other observations that β-glucosidase is sensitive to tillage, organic amendments, and residue management (de la Horra et al., 2003; Roldán et al., 2005). Since overall model performance was greater in the 5 cm sampling depth, these results support the conclusion that the shallower sampling is preferred for regional evaluations of β-glucosidase.

4.4.1.6 ArylSulfatase

Arylsulfatase activity in the 5 cm depth was largely governed by three positive relationships: SOC, MLRA Wooded Slopes, and no-till management (Table 4.4). These effects were at least two times greater than the other factors. The next tier of factors included pH, clay content, MLRA Heavy Till Plain, and diversified crop rotation. Results were similar for the 15 cm sampling depth, but the effect of no-till was not as prominent in the 15 cm depth. Generally, measured arylsulfatase activity was greater where SOC and pH was high, and where no-till and diversified crop rotations were also implemented. Further work is necessary regarding the strong effect from pH for the same reasons discussed in the previous section (Tabatabai and Bremner, 1970). The strong effect of no-till practices aligns with plot level observations of greater arylsulfatse activity in long-term no-till systems (Dick, 1984) and reduced activity in tilled systems (García-Ruiz et al., 2008). These results confirm tillage relationship impacts are regionally observable and are especially highlighted in the 5 cm sampling depth. Arylsulfatase has also been reported to have greater sensitivity to organic amendments rather than tillage management (Deng and Tabatabai, 1997; Klose et al., 1999); however this relationship was not found with this investigation.

4.4.1.7 Acid Phosphatase

Important positive relationships for acid phosphatase activity at both sampling depths were SOC and crop rotation. Cation exchange capacity, clay, and landscape position were likewise positively related, but with less influence. Inclusion of SOC and pH were unsurprising, with strong links between acid phosphatase and SOC well established in this dataset and other published reports (Dick, 1984). Further, it is well established that pH influences the activity and abundance of acid phosphatase (Eivazi and Tabatabai, 1977). For both depths greater acid phosphatase activity was observed in diversified crop rotations, not unlike what others have shown (Eivazi and Tabatabai, 1977; Dick, 1984) The unique sensitivity to crop rotation for both sampling depths highlights the potential sensitivity of this soil health indicator to benefits from a diversified crop rotation. The reason manure application and cover crops inhibited acid phosphatase activity is unknown but deserves additional study. I recommend a 5 cm sampling depth because of the improved overall model performance (Table 4.3) and similar sensitivity to management practices between sampling depths.

4.4.2 Soil and Environmental Conditions Effect Upon Soil Health Factors

Each soil health indicator was affected by regional and landscape properties, highlighting the need to develop regionally specific frameworks for interpretation (Fine et al., 2017; Nunes et al., 2021). While not significant at each sampling depth, landscape position and MLRA were significant drivers of variability for each soil health indicator. ACE protein appears to be the least affected by these environmental conditions, but as described previously, I expect that these effects were likely incorporated through the strong relationship with SOC. Further work verifying this observation is needed, and if true, could indicate that standardizing soil health indicators by SOC could overcome challenges in providing robust regional recommendations. The effects of landscape position and MLRA affirm other regional soil health evaluation conclusions that MLRA is a significant driver in determining baseline soil health indicator values (Zuber et al., 2020). These results further align with conclusions regarding the implementation of the CASH soil health assessment, that regionalized parameterization is likely necessary for correct interpretation of soil health metrics (Fine et al., 2017).

Soil organic carbon and pH were included as significant factors for each soil health metric and sampling depth. The inclusion of SOC was expected, with commonly reported connections between each soil health indictor and baseline SOC values (Eivazi and Tabatabai, 1977; Lucas and Weil, 2012; Moebius-Clune et al., 2016). Soil texture is also commonly reported and incorporated into regional soil health evaluations, but this study did not find texture to be as relevant, likely because most of the sampled production fields are of soils with loess parent material. This research suggests the role of soil pH is an underrealized and underreported controlling factor in soil health evaluations. While pH is commonly incorporated into holistic soil health evaluations (Andrews et al., 2004; Fine et al., 2017), these data suggests there are direct impacts from pH on specific biological indicator values. Soil pH plays an integral part in regulating microbial processes, such as in nitrogen fixation and denitrification. Further, it regulates solubility of essential nutrients for microbial and plant communities. Inclusion of pH in soil respiration was unsurprising considering pH's governing role in microbial processes, its effect on microbial composition and diversity and equilibrium effects nutrient solubility. Specifically, to enzyme activity, pH can affect the concentration of inhibitors or activators in the soil solution and variability in clay and pH in soils can cause challenges in universal buffer evaluations in extracellular enzyme evaluations (Wade et al., 2021). This study further highlights that while biology assessments are sensitive to management practices, pH evaluations remain a vital part of a soil health assessment and regional adjustments for interpreting soil health indicators. Adjustments for these indicators based on soil pH could be warranted, and further research is required to confirm the magnitude and effect of pH in soil health indicator measurements to go along with sensitivity assessment to management practices.

Overall, these results confirm recommendations by Stewart et al., (2018) that baseline chemical and physical soil properties are essential for proper interpretation of regional health assessments. Adjustments for soil texture and soil formation factors are well acknowledged; at the same time these results highlight the underrealized potential effect of soil chemical properties on soil biological assessments used in soil health evaluations. These findings support that soil biological tests examined for sensitivity to management practices need to include baseline soil properties for proper interpretation.

4.4.3 Regional Evaluation Soil Health Indicators Sensitivity to Management Practices

Each soil health indicator demonstrated distinctive regional sensitivity to the evaluated management practices. Effects of manure were evident with over half of the soil health indicators responding to it being incorporated into the cropping system management practices (Table 4.5). Soil organic carbon, POXC, soil respiration, and β-glucosidase were each highly sensitive to manure application. These data confirms that previous plot level relationships between manure application and soil health indicators are regional applicable (Deng and Tabatabai, 1997; Hurisso et al., 2016; Hargreaves et al., 2019). Manure has been identified as an effective management practices to facilitate C sequestration, which aligns with the SOC and POXC sensitivities which are reported to reflect soil C stabilization

(Culman et al., 2012; Hurisso et al., 2016). The sensitivity of soil respiration and β-glucosidase highlight this practice impact on nutrient cycling (Du et al., 2020) which highlights the regional robust sensitivity of these indicators to the benefits of manure inputs on soil health. Finally, the sensitivity to a robust set of soil health indicators highlights the importance and positive benefits on soil processes and function from manure application in row crop systems.

Soil organic carbon, POXC, and arylsulfatase were all uniquely sensitive to tillage practices, with greater activity and concentrations observed in no-till management (Table 4.4 & 4.5). These results confirm that plot-level connections between POXC and SOC and soil stabilization processes facilitated from reduced tillage are regionally robust (Culman et al., 2012; Hurisso et al., 2016). The inclusion of arylsulfatase confirms that no-till practices facilitate nutrient cycling (specifically sulfur) and can be regionally employed to evaluate nutrient cycling benefits from no-till practices (Tabatabai and Bremner, 1970). Arylsulfatase is uniquely qualified for evaluating no-till benefits, with tillage practice being the only regionally robust sensitivity for that indicator. Further, the effect of tillage practice was relatively greater in the 5-cm sampling depth for SOC and POXC, which corresponds with other reports that reduced tillage effects are more prominent in surface soil samples (Veum et al., 2015; Nunes et al., 2018).

Despite plot level reports that cover crops promote many of these soil health indicators, these data suggests these reported links do not scale regionally. The insensitivity of the evaluated soil health indicators to cover crop implementation is likely because of the variability in cover crop species, establishment, and specific soil interactions in this dataset (Poffenbarger et al., 2015). This variability is common in cover crop management systems and highlights the challenges of observing regional impacts of cover crops on soil biological properties. Despite these challenges soil respiration is well suited as an effective regional measurement for cover crop benefits upon soil health. This derives from soil respirations sensitivity to recent residue and organic inputs that promote mineralization processes (Culman et al., 2013; Hurisso et al., 2016; Franzluebbers and Assmann, 2020).

These results offer a unique opportunity to identify soil health indicators that are regionally sensitive to benefits from conservation management practice. Specifically, these results lay the groundwork for targeting soil health assessments for specific management practices. For example, acid phosphatase was uniquely sensitive to the diversification of cash crops while, arylsulfatase, SOC, and POXC were ideal candidates to demonstrate improvements from no-till implementation. Soil respiration was uniquely sensitive to cover crop implementation whilst SOC, POXC, soil respiration, and β-glucosidase were ideal soil health indicators for measuring changes in soil processes from manure management.

From this work, a potential framework for identifying which practices are ideal for identifying benefits from soil health management practices are summarized in Table 4.5. Benefits of this framework could potentially reduce the number of required soil health samples required to empirically measure benefits from soil health practices. For example, if a practitioner desired to evaluate the benefits of cover crops, acid phosphatase is a soil health indicator that is regionally sensitive to cover crop benefits upon soil health processes. Further, this table summarizes recommendations that proper interpretation requires SOC and pH assessments and a knowledge of soil formation factors and processes (MLRA). Finally, for optimal sensitivity, a sampling depth of 5-cm is recommended for that indicator (Table 4.5). Further work is necessary to confirm these observations, with the next steps being to develop empirical thresholds where agronomic benefits occur. These will provide an interpretive framework that can be leveraged to inform on-farm management decisions and facilitate the improvement of soil health assessments and management adoption.

4.5 Conclusions

This paper reports a regional evaluation of the sensitivity of seven soil biological metrics to soil formation factors and processes, variability in intrinsic soil properties, and management practices. For each soil health indicator, MLRA, SOC, and pH were major factors governing soil health measurements. Effects of MLRA and SOC are widely recognized, however, the impacts of pH on baseline soil health metrics are commonly underreported. Further work is necessary to identify the magnitude of the effect of pH on these soil biological factors and whether parameterization based on this soil characteristic is necessary for regional interpretation of these indicators. These results emphasize current challenges in developing regional interpretations of soil health metrics that operate across natural variability in Missouri cropping systems. Despite these challenges, sensitivities to conservation management practices remained evident, justifying regional employment of these soil biological indicators to assess management impacts upon soil health. In general, optimal sampling depths were specific to each soil health indicator, with effects from specific management practices generally observed regardless of sampling depth. Soil organic carbon and POXC were an exception, with greater effects of no-till practices observed in the 0-5 cm sampling depth. Finally, a framework is provided for each soil health indicator identifying optimal sampling depths, important soil factors to consider, and regional management sensitivity. Overall, these results provide a needed framework for practitioners and soil test laboratories to consider when providing regional soil health evaluations.

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4.7 Tables and Figures

Table 4.1 Summary statistics for soil biological analysis broken apart by depth. Summary statistics include mean ± standard error, minimum, and maximum observed values.

		0-5 cm			0-15 cm		
Soil Health Indicator	**Units**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
SOC	g 100 g soil^{-1}	2.15 ± 0.01	1.0	4.7	1.61± 0.01	0.85	2.98
POXC	mg POXC kg soil^{-1}	648 ± 3.25	253	1040	440 ± 2.41	198	836
ACE Protein	mg protein g soil^{-1}	4.86 ± 0.04	1.8	15.5	3.63 ± 0.02	1.5	8.8
Soil Respiration	mg C-CO2 kg soil^{-1}	219 ± 1.82	35.5	612	140 ± 1.16	22	345
Acid Phosphatase	µg p-nitrophenol g^{-1} soil h^{-1}	241 ± 1.42	62.4	344	174 ± 1.19	66	298
ArylSulfatase	µg p-nitrophenol g^{-1} soil h^{-1}	72.2 ± 0.639	18.7	256	46.0 ± 0.41	16	154
β-Glucosidase	µg p-nitrophenol g^{-1} soil h^{-1}	139 ± 0.968	4.5	310	78.1 ± 0.49	4.0	163

Table 4.2 Number of soil samples for each management practice.

	2018	2019	2020	Total
Management Practice	Number of Observations			
Tillage Practices				
No-Till	13	101	137	251
Tillage		94	101	195
Cover Crop Incorporation				
No	9	109	83	201
Yes	4	86	155	245
Manure Application				
Yes	-	22	58	80
No	13	173	180	366
Crop Rotation				
C-SB	11	166	207	384
Diverse	2	29	31	62

Table 4.3 Results for random forest and least absolute shrinkage selection operator (LASSO) statistics. Final model correction factors are included for random forest (mtry) and LASSO (lambda). Models were trained on 80% of the dataset with reported R^2 and RMSE from final model predictions upon the validation dataset. The highest R^2 are bolded to identify the best model performance for each soil health indicator.

		0-5 cm					**0-15 cm**				
		LASSO		**Random Forest**			**LASSO**		**Random Forest**		
Soil Health Indicator	**Units**	**R^2**	**RMSE**	**mtry**	**R^2**	**RMSE**	**R^2**	**RMSE**	**mtry**	**R^2**	**RMSE**
Soil Organic Carbon	g 100 g soil^{-1}	0.39	0.36	4	0.43	0.33	0.39	0.26	4	**0.50**	0.23
POXC	mg kg soil^{-1}	0.39	99.5	5	0.29	106	0.55	70	5	**0.56**	70.1
Soil Respiration	mg C kg soil^{-1}	0.11	67.4	5	0.09	62.5	0.09	46.2	5	**0.22**	41.0
ACE Protein	mg g soil^{-1}	0.32	1.19	5	**0.43**	1.10	0.21	0.85	5	0.29	0.81
β-Glucosidase	µg p-nitrophenol g^{-1} soil h^{-1}	0.43	33.2	5	**0.49**	31.2	0.3	18.1	5	0.37	16.9
ArylSulfatase	µg p-nitrophenol g^{-1} soil h^{-1}	0.52	18.7	5	**0.53**	19.0	0.46	12.3	5	0.48	12.4
Acid Phosphatase	µg p-nitrophenol g^{-1} soil h^{-1}	0.49	47.00	5	**0.52**	46.5	0.37	41.9	5	0.40	41.2

Table 4.4 Coefficients for final LASSO regression models used to predict each soil health indicator and separated by sampling depth. The top three coefficients with the largest magnitude are bolded for each model. Coefficients are scaled, but the relative magnitude within a model represent the relative impact that indicator presents on the specific soil health metric.

	SOC		**POXC**		**Soil Resp.**		**ACE Protein**		**β-Gluc.**		**Arylsulf.**		**Acid Phos.**	
Sample Depth (cm)	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**	**5**	**15**
lambda	0.10	0.01	1.52	2.02	1.01	0.505	0.035	0.027	1.52	0.51	0.51	0.76	1.01	0.61
Intercept	2.1	1.6	649	439	218	140	4.8	3.6	138	78	71	46	239	173
Soil Physical, Chemical, and Biological Properties														
SOC	NA	NA	**102**	**69.9**	**24.6**	**16.7**	**0.79**	**0.42**	**23.1**	**11.2**	**18.5**	**9.88**	**25.0**	**23.3**
pH	0.03	0.02	**42.0**	**28.8**	-2.25	1.15	**-0.17**	**-0.10**	**12.9**	**8.00**	2.83	**2.59**	**-13.8**	-4.00
CEC	NA	NA			-0.01						-2.86		9.14	7.69
Clay	0.06	**0.07**	**-19.3**	-13.0	-7.67	**-6.02**	-0.04	-0.003					4.49	3.56
Sand				0.81	-6.67	-4.59	-0.01	-0.01	-1.37	-0.97	-0.39	-0.21		0.22
Environmental Conditions														
Drainage Class	-0.05	-0.03	-1.34		5.79	2.62	-0.11	-0.08				-0.10	2.70	
MLRA : Deep Loess Hills	**0.16**	**0.11**	1.84		**10.0**	4.96				-1.68	0.32		-4.13	**-11.1**
MLRA : Heavy Till Plain	**0.08**	**0.06**			-4.81	-3.78	**0.27**	**0.15**		-1.09	-2.13	-0.62	-7.08	-7.61
MLRA : Wooded Slopes	-0.02	-0.03	3.83		2.90	2.29					**5.07**	**4.25**		
LP : Floodplain	-0.04	-0.04	4.76	7.99				0.01	-1.03					
LP : Summit			-3.39	-3.31	-2.67	-2.05								
LP : Backslope	-0.01	-0.02	-4.51	-6.24	-5.29	-3.93			1.64	0.94	1.24	0.26	2.84	4.07
LP : Toeslope				-1.11	-5.54	-3.65	0.11	0.05	0.25		0.76		1.07	1.25
Management Practices														
No-Till	0.06	0.01	9.90	5.29		1.16	-0.06	-0.06	2.04	1.30	**4.89**	2.52		0.55
Cover Crop: Yes	-0.0004	-0.01			7.57	5.55							-0.88	-3.55
Manure Application	**0.08**	0.04	8.43	**13.1**	**12.9**	**10.6**	-0.15	-0.08	**6.89**	**4.07**	-0.44		-2.56	-3.89
Diversified Crop Rotation		-0.01	-4.60	-4.10	5.47	2.40	0.10	0.06	0.43	0.49	2.03	1.22	**13.4**	**10.1**
S-SO_4											-0.04			
Soil Test Phosphorus														2.20

Table 4.5 On-farm recommendations for regional soil health assessments. Recommended sampling depths were determined by choosing the lowest RMSE and highest R^2 of the models. Soil and environmental co-factors were included if their coefficient magnitude was one of the top three in magnitude in the LASSO models (Table 3). Checkmarks reflect regional sensitivities with ✓✓ identifying the effect was one of the top three factors governing that soil health indicator while a single ✓ represents the effect was meaningful but not one of the governing factors identified in Table 4.4.

Soil Health Indicator	Recommended Sampling Depth (cm)	Important Environmental Factors	No-Till	Cover Crops	Manure Application	Diverse Crop Rotation
SOC	15	MLRA	✓		✓	
POXC	15	SOC; pH; Clay Content	✓		✓✓	
Soil Respiration	15	SOC; MLRA; Clay		✓	✓✓	✓
ACE Protein	5	SOC; MLRA; pH				✓
β-Glucosidase	5	SOC; MLRA; pH			✓✓	
Arylsulfatase	5	SOC; MLRA; pH	✓✓			
Acid Phosphatase	5	SOC; pH; MLRA				✓✓

DISSERTATION CONCLUSIONS

The objectives of this dissertation were to address current questions and critiques regarding on-farm soil health utilization and interpretation. The presented results outline benefits and limitations of regional on-farm soil health assessments. These results confirm the efficacy of current University of Missouri fertilizer recommendations and highlight existing limitations in P and K recommendations. Soil-test estimation of yield response to fertilization was most accurate at low nutrient levels and exhibited diminished precision at or above established critical concentrations. Variable importance analysis confirmed the effectiveness of current soil-tests, and indicated CEC is potentially an underutilized tool in P fertilizer recommendations. These results reflect challenges in developing regional recommendations that effectively operate across natural variability among a wide range of soil types, environmental conditions, and management practices. Integrating soil health indicators failed to improve current model identification of yield response to P and K fertilization. These finding found little to support using soil health metrics to identify crop P and K fertilization needs. Further work is necessary to evaluate whether other soil health indicators are effective indicators of P and K fertilization needs, or potential impacts upon other fertilizer nutrients whose plant availability are largely governed by biological processes (e.g. nitrogen). Although soil health metrics offer insight into environmental or agronomic benefits, established soil fertility analysis remains the most effective tool to guide P and K fertilizer decisions in Missouri corn production.

Further investigations into grain productivity resulted with a plausible framework for interpreting soil health indicators in Missouri corn cropping systems. These results report POXC as a more sensitive predictor of corn grain yield than traditional measures of soil organic matter and soil fertility analyses. Further, a clear POXC benchmark is reported whereupon corn grain yield was maximized. Further evidence is required to validate this benchmark within and outside of Missouri corn cropping systems across soil and weather conditions. This study found little to support the use of soil respiration as an indicator of corn grain yield under these conditions and only identified a weak relationship with ACE protein. Future work is needed to explore the specific soil properties and processes represented by POXC and how POXC contributes to grain yield productivity. This will inform whether yield directly responds to improvements in POXC, or whether POXC serves as a proxy or indirect measure of other soil properties that enhance the growing environment. These results also demonstrate the advantages of using statistical non-parametric approaches to understand relationships between soil health indicators and productivity. Overall, this provides the first empirical relationship between POXC and corn grain yield that is uniquely designed to inform on-farm decision support systems. Identifying and leveraging similar quantitative soil health relationships with economic incentives will aid in incentivizing and spurring on-farm adoption of conservation management practices.

Finally, random forest and LASSO regression models were developed to determine whether soil health indicators are viable for on-farm implementation and associated recommendations. The summarization of important observations include, 1) current assessments of soil health do not improve current P and K fertilizer recommendations for corn in Missouri, 2) POXC is an effective indicator of corn grain productivity with a reported framework to guide Missouri POXC interpretations, 3) robust regional soil health recommendations would benefit from incorporating site-specific SOC, pH, and soil texture, and 4) a guide for optimal sampling depths and sensitivities to seven soil health indicators is provided. Validation of these results are encouraged through further research efforts to improve on-farm implementation and application of soil health indicators.

APPENDIX

APPENDIX A:

Supplementary Materials for Chapter 2

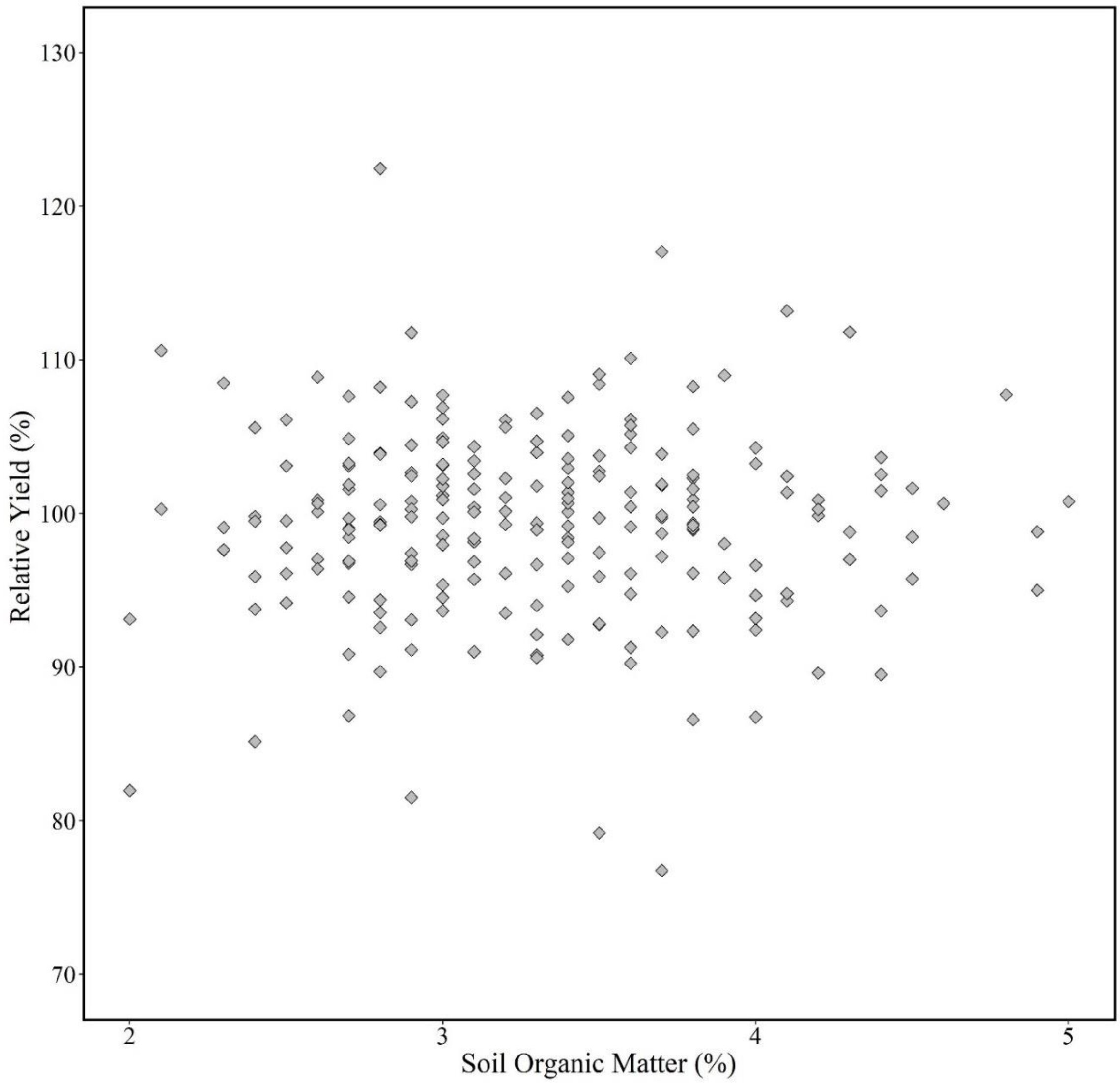

Figure A.1 Relationships between soil organic matter and relative corn yield response from sulfur fertilization across all experimental years. Generally, soil organic matter was a poor indicator of yields response to sulfur fertilizer application.

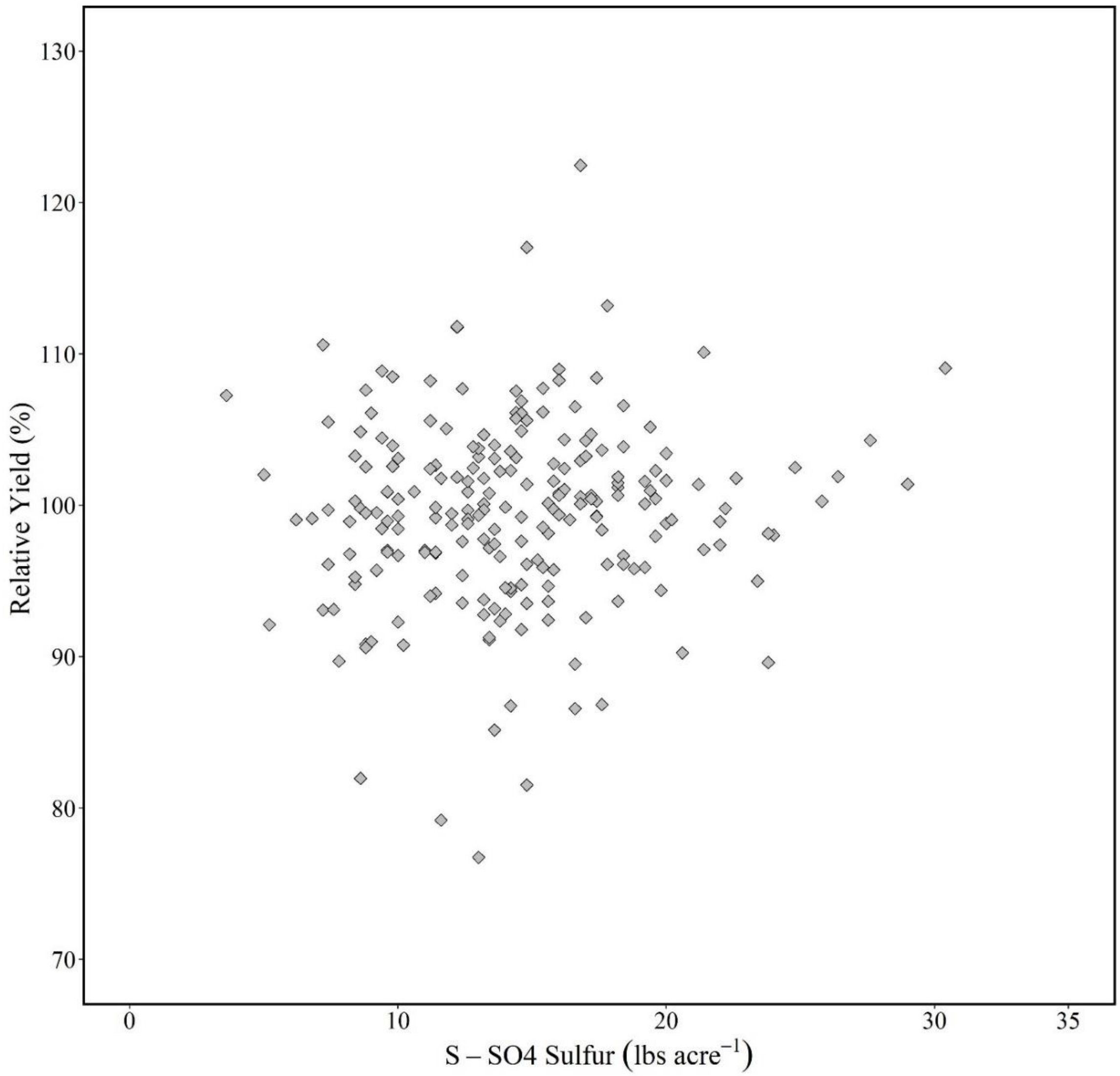

Figure A.2 Relationships between the University of Missouri recommended sulfate-sulfur test and relative corn yield response from sulfur fertilization across all experimental years. No significant trends were identified, indicating that the current recommended sulfur soil analysis is an ineffective indicator of sulfur nutrient status.

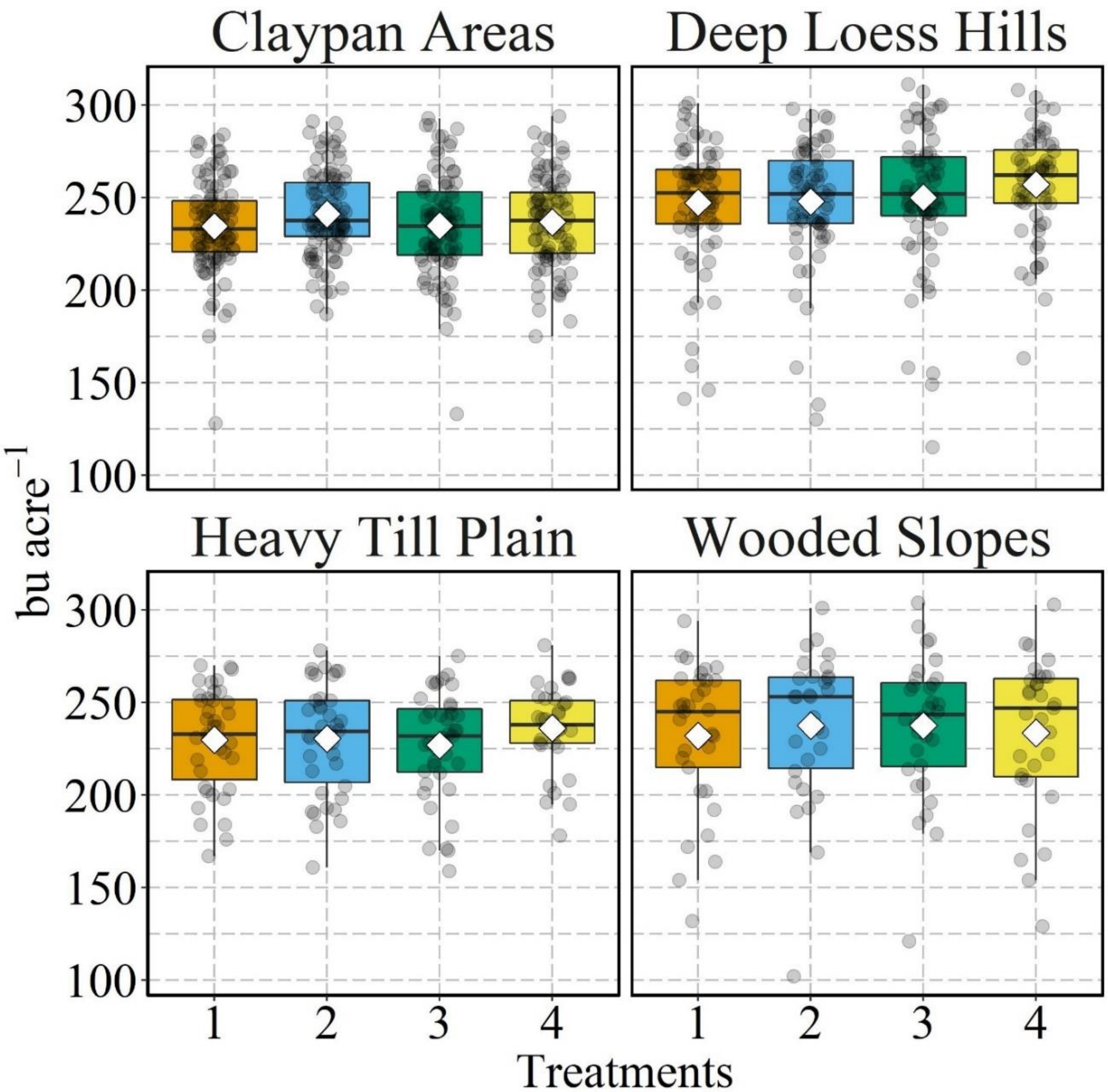

Figure A.3 Observed yields over all experimental sites for each fertilizer treatment divided by major land resource area (MLRA). Treatments included 1) unfertilized control; 2) 112 kg ha^{-1} K_2O; and 3) 112 kg ha^{-1} P_2O_5 and 4) 28 kg ha^{-1} of sulfate-sulfur. White diamonds represent average for the treatment and colored boxplots correlate with fertilizer treatments.

APPENDIX B:

Supplemental Materials for Chapter 3

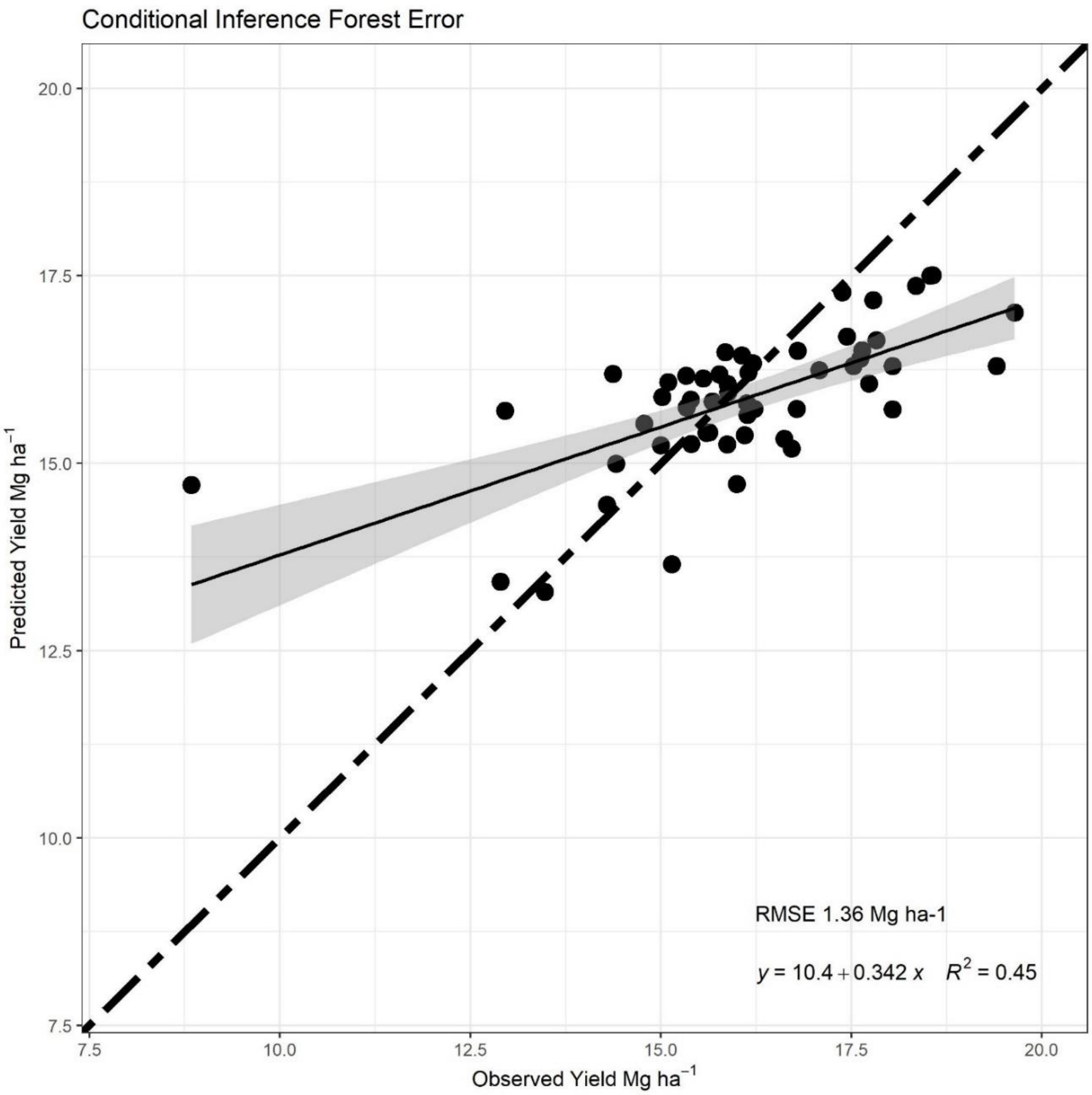

Figure B.1 Grain productivity prediction error on the validation set for the conditional inference forest method.

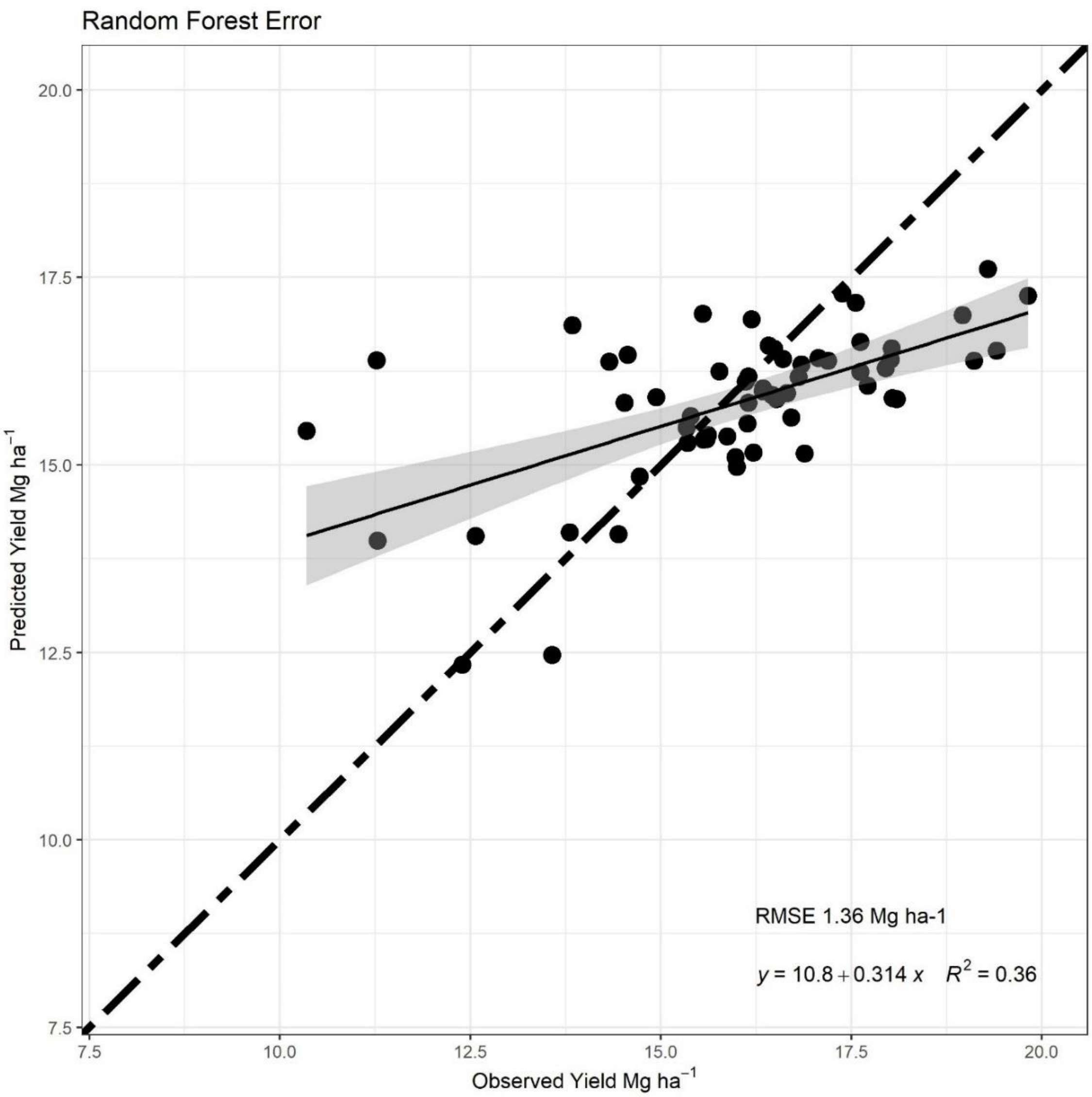

Figure B.2 Grain productivity prediction error on the validation set for the random forest regression method.

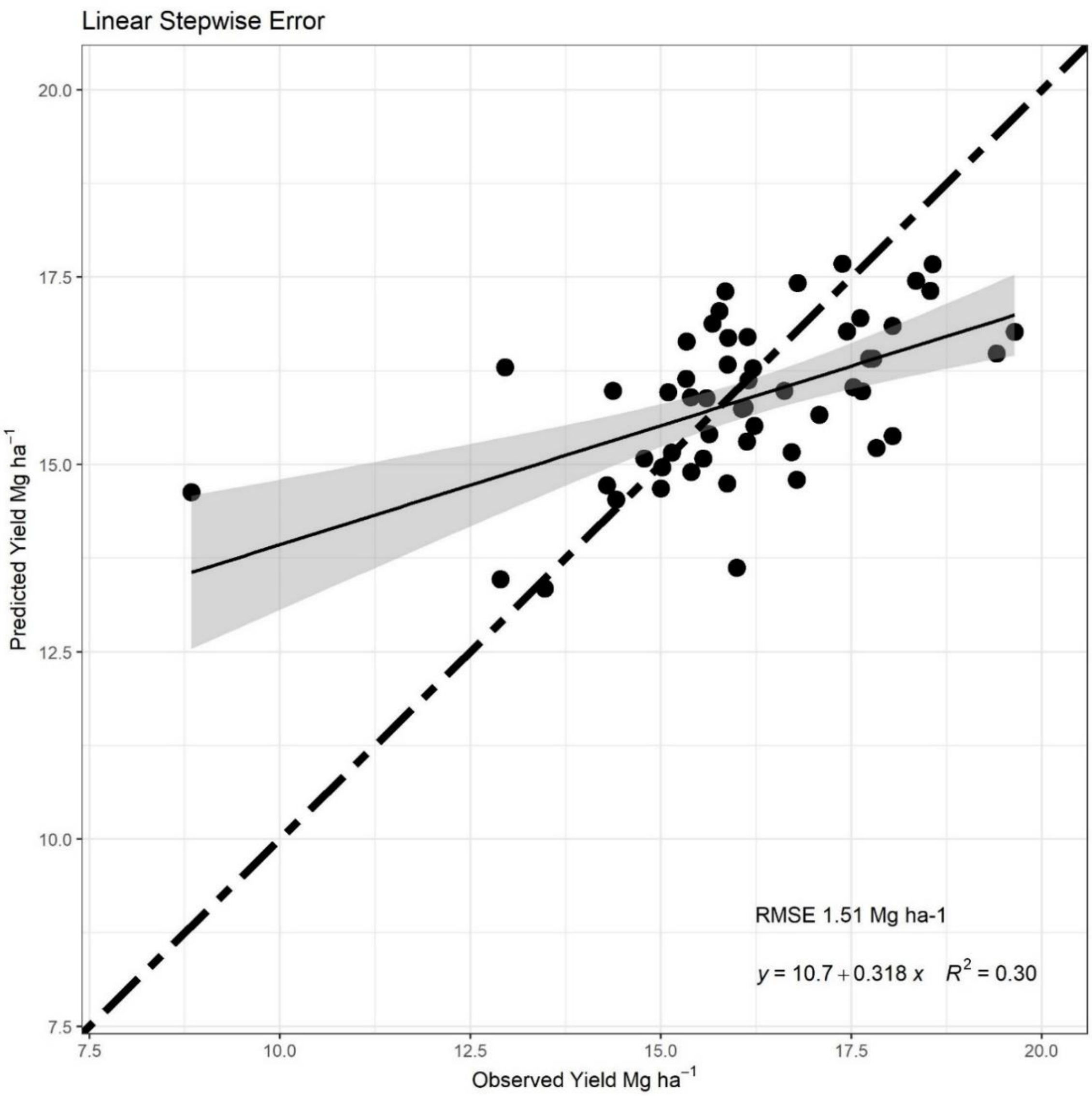

Figure B.3 Grain productivity prediction error on the validation set for the best subset linear regression method.

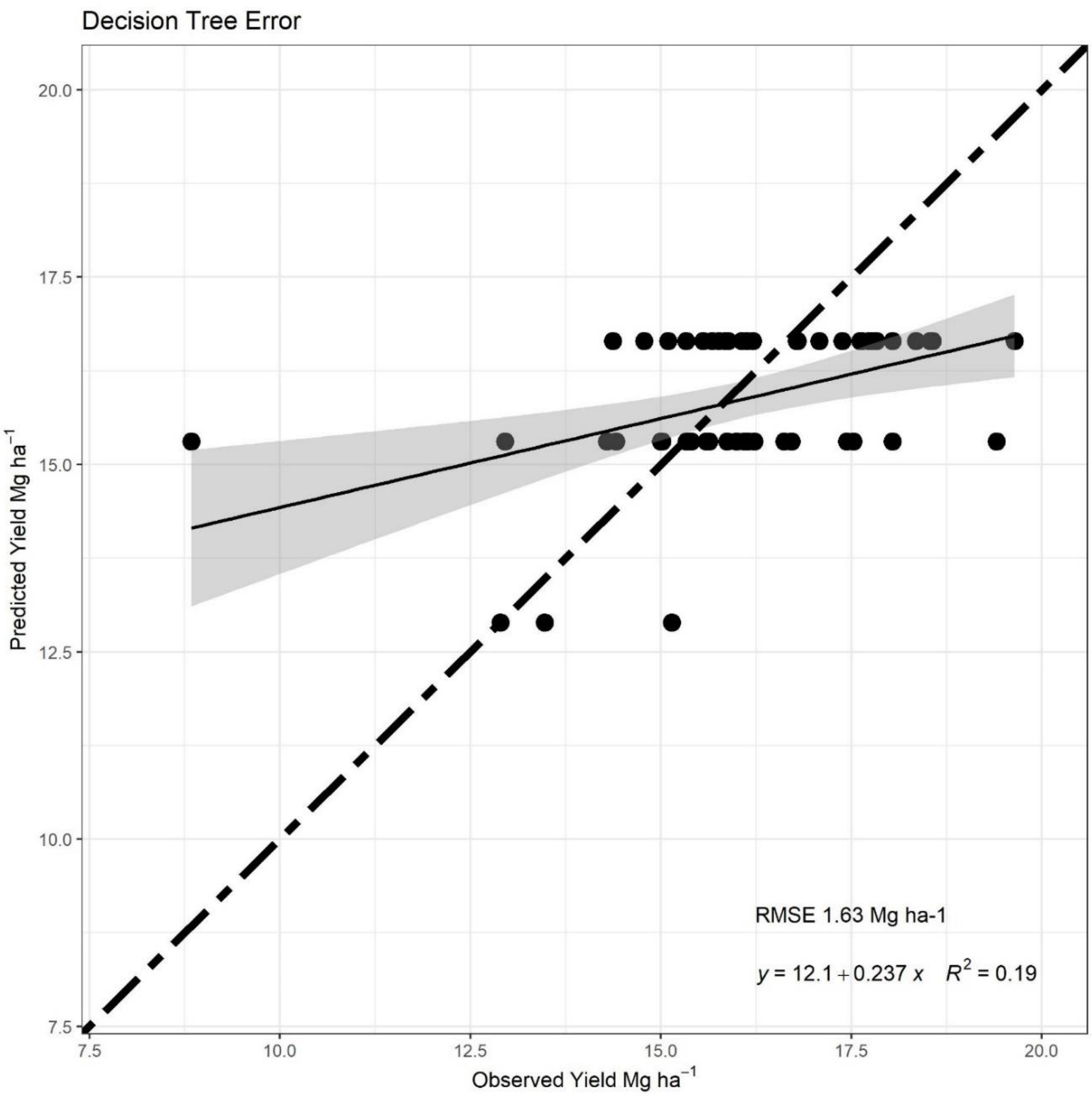

Figure B.4 Grain productivity prediction error on the validation set for the decision tree method.

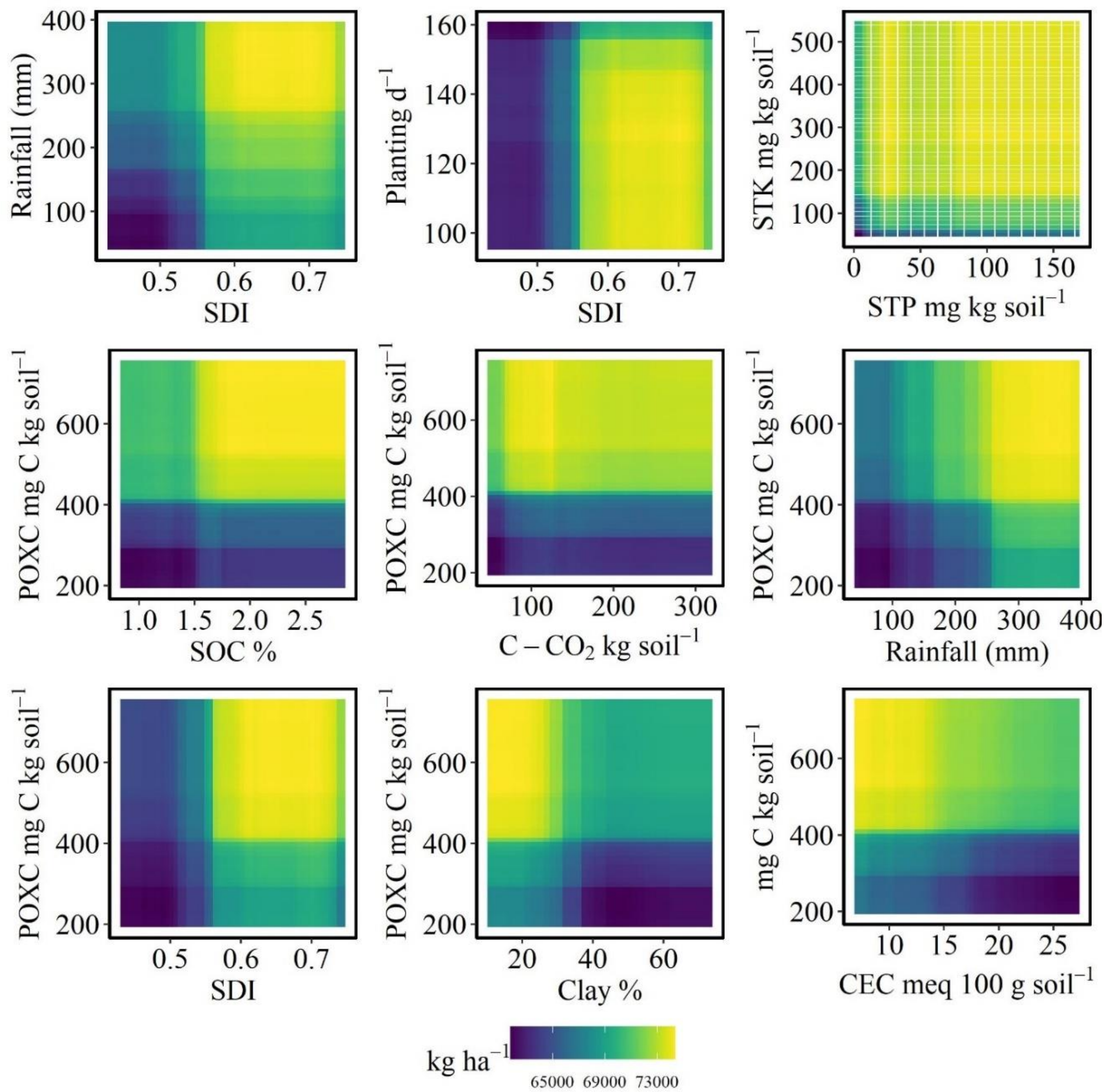

Figure B.5 Interaction plot for weather, soil health, and soil chemical factors based on the random forest results predicting grain yield kg ha^{-1}. These results highlight that yield increases from soil health and soil fertility were first governed by weather parameters (SDI and Seasonal rainfall).

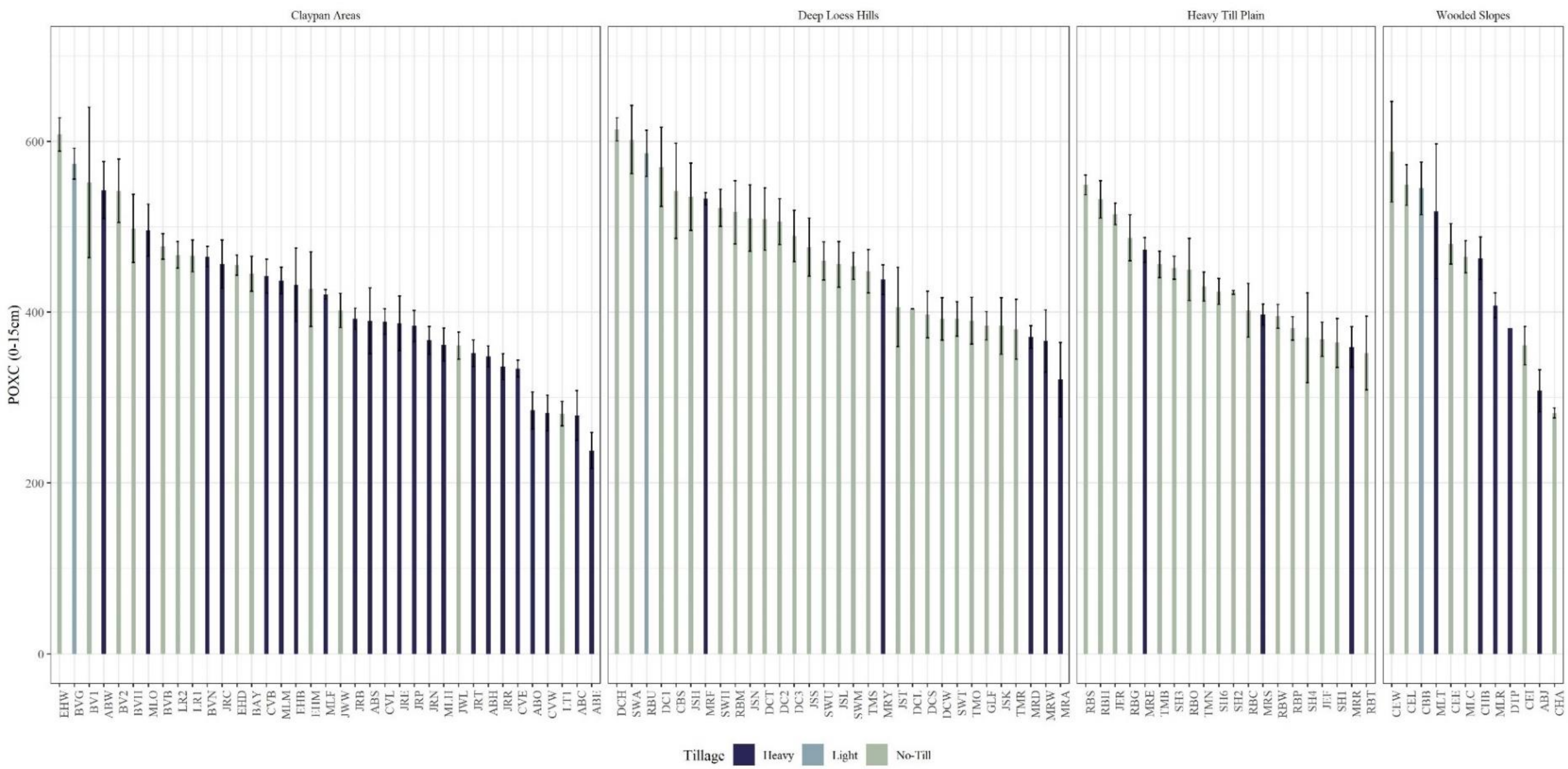

Figure B.6 Average POXC values for each field included in this dataset divided by major land resource area (MLRA). Colors represent the tillage practice, with Heavy indicating more than three tillage events in five years, light less than three tillage events, and no-till meaning no tillage occurred in the previous five years. Error bars are the standard error of the mean.

APPENDIX C:

Supplemental Materials for Chapter 4

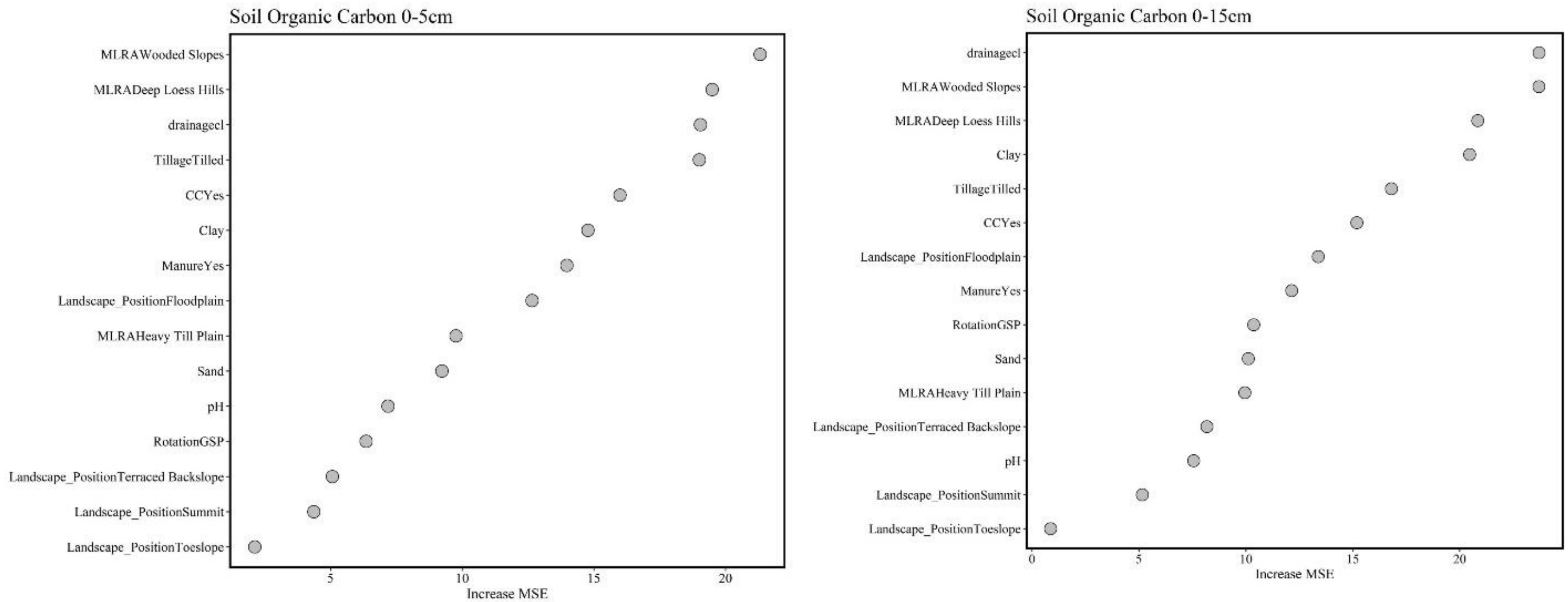

Figure C.1 Variable importance rankings from random forest model results predicting soil organic carbon (SOC) sampled at two depths (0-5 cm; 0-15 cm) at 446 sites across 101 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting SOC.

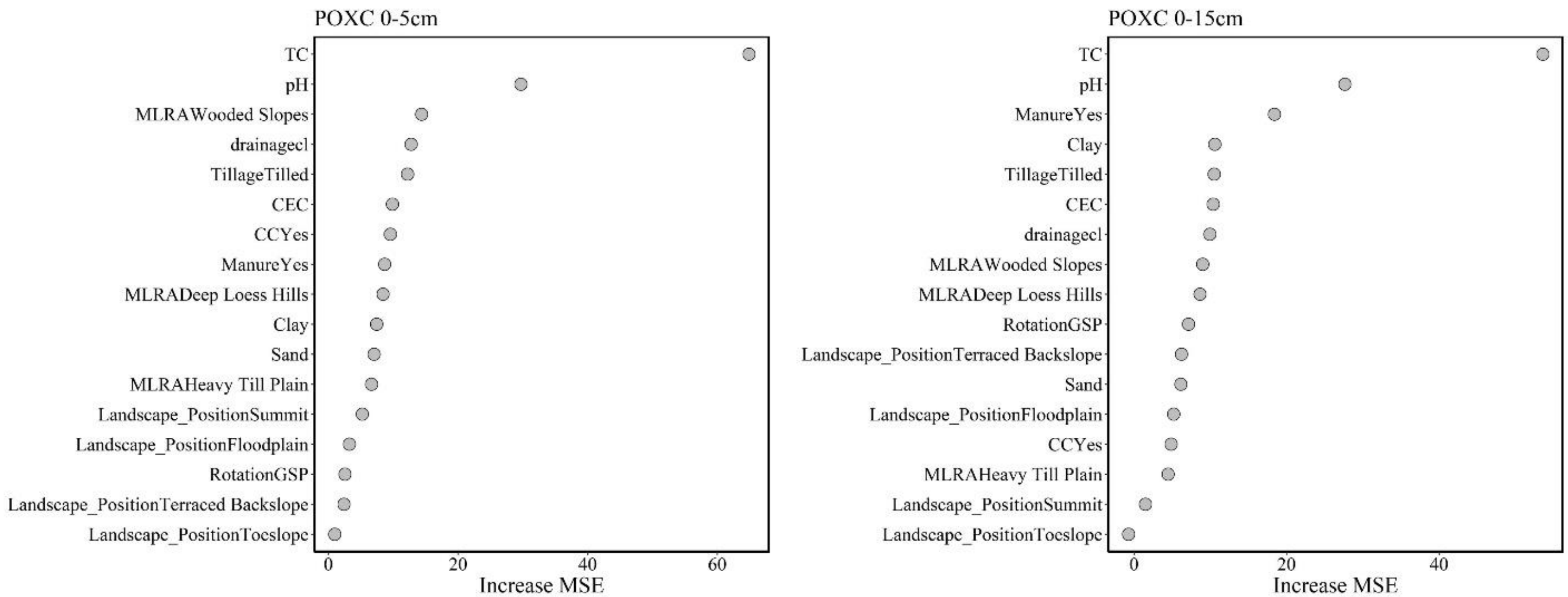

Figure C.2 Variable importance rankings from random forest model results predicting potassium permanganate oxidizable carbon (POXC) sampled at two depths (0-5 cm; 0-15 cm) at 446 sites across 101 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting POXC.

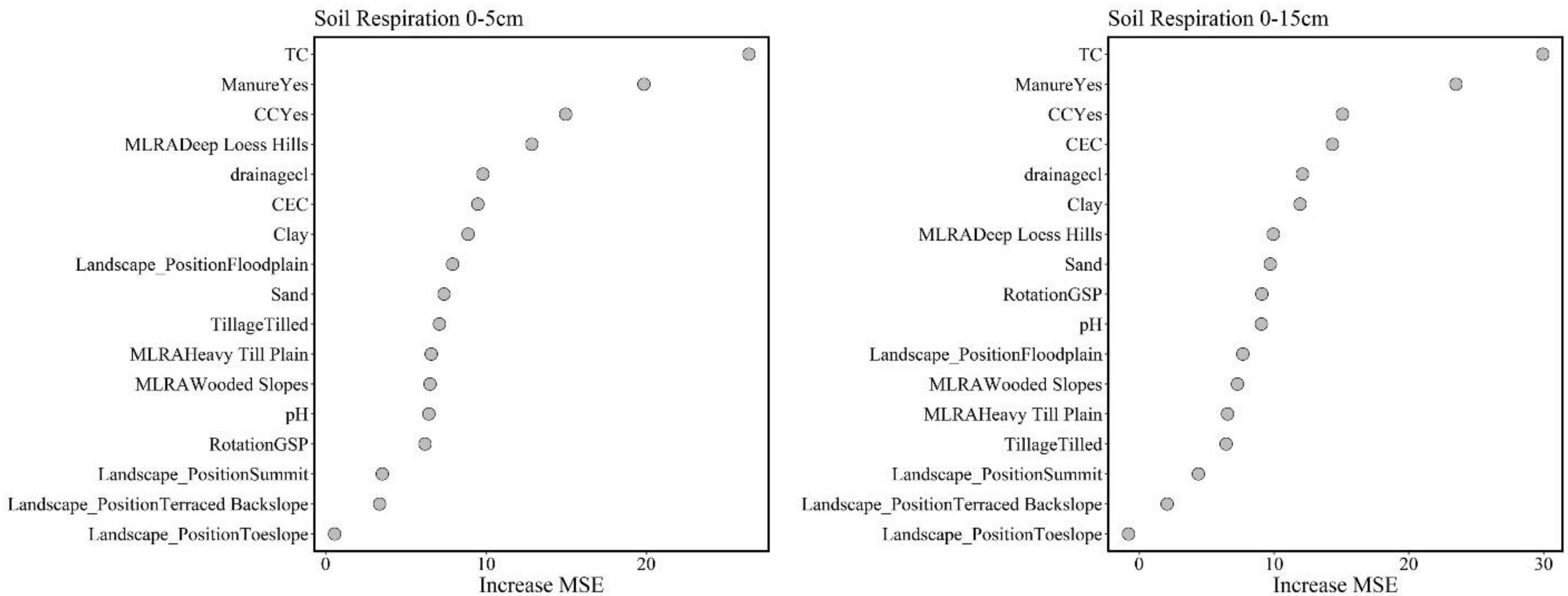

Figure C.3 Variable importance rankings from random forest result model results predicting soil respiration sampled at two depths (0-5 cm; 0-15 cm) at 446 sites across 101 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting soil respiration rates.

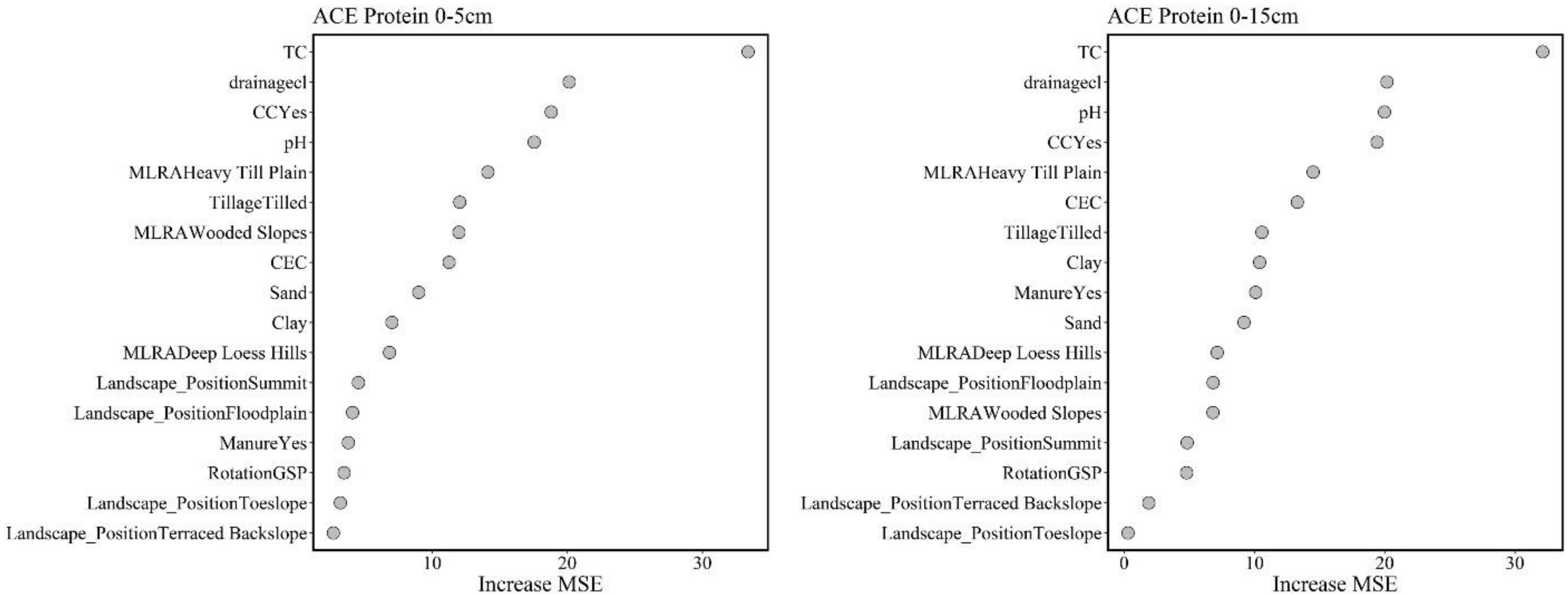

Figure C.4 Variable importance rankings from random forest result models predicting autoclaved citrate extractable soil protein (ACEp) sampled at two depths (0-5 cm; 0-15 cm) at 446 sites across 101 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting ACE protein.

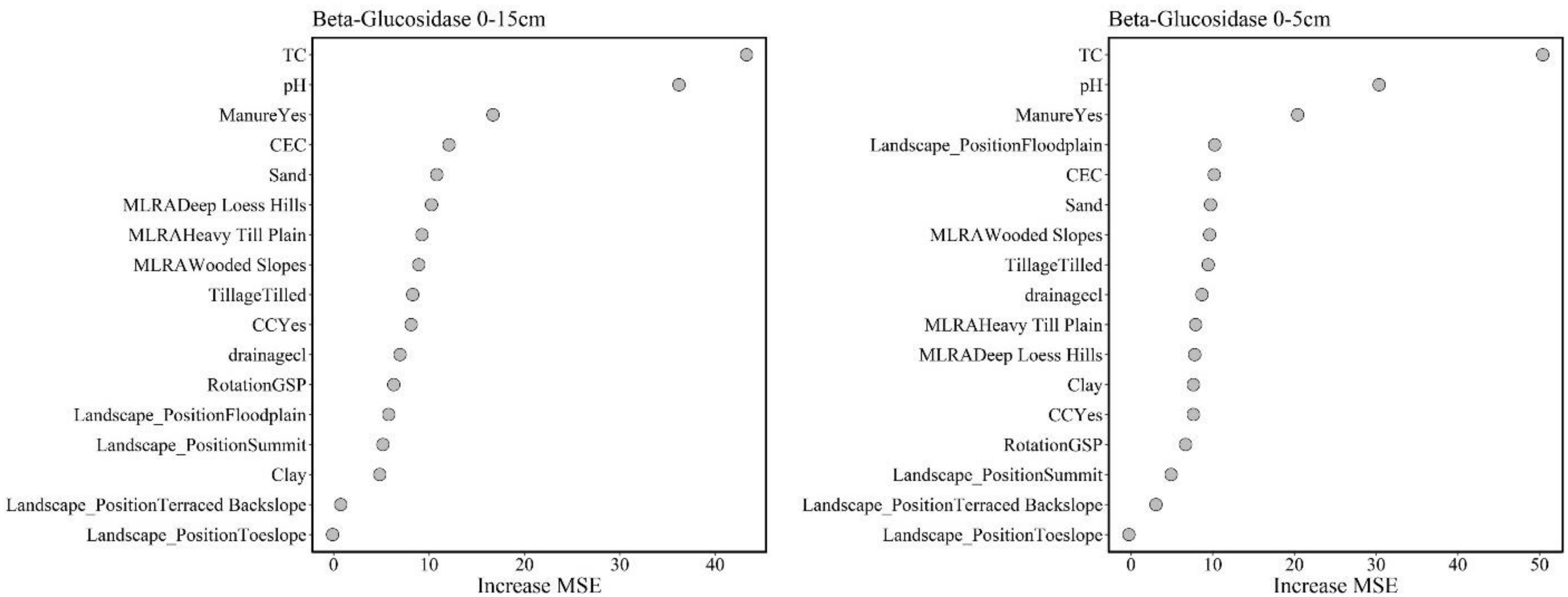

Figure C.5 Variable importance rankings from random forest model results predicting β-glucosidase activity sampled at two depths (0-5 cm; 0-15 cm) at 446 sites across 101 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting β-glucosidase activity.

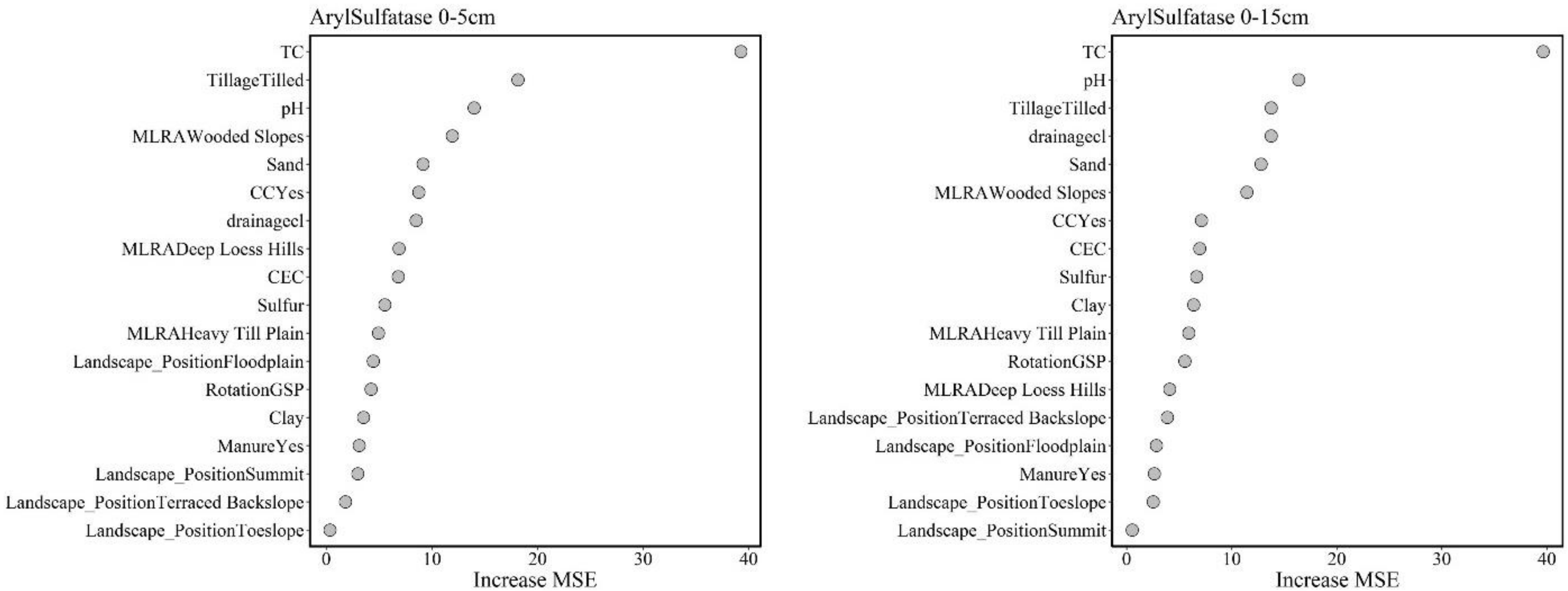

Figure C.6 Variable importance rankings from random forest results models predicting arylsulfatase activity sampled at two depths (0-5 cm; 0-15 cm) at 446 sites across 101 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting arylsulfatase activity.

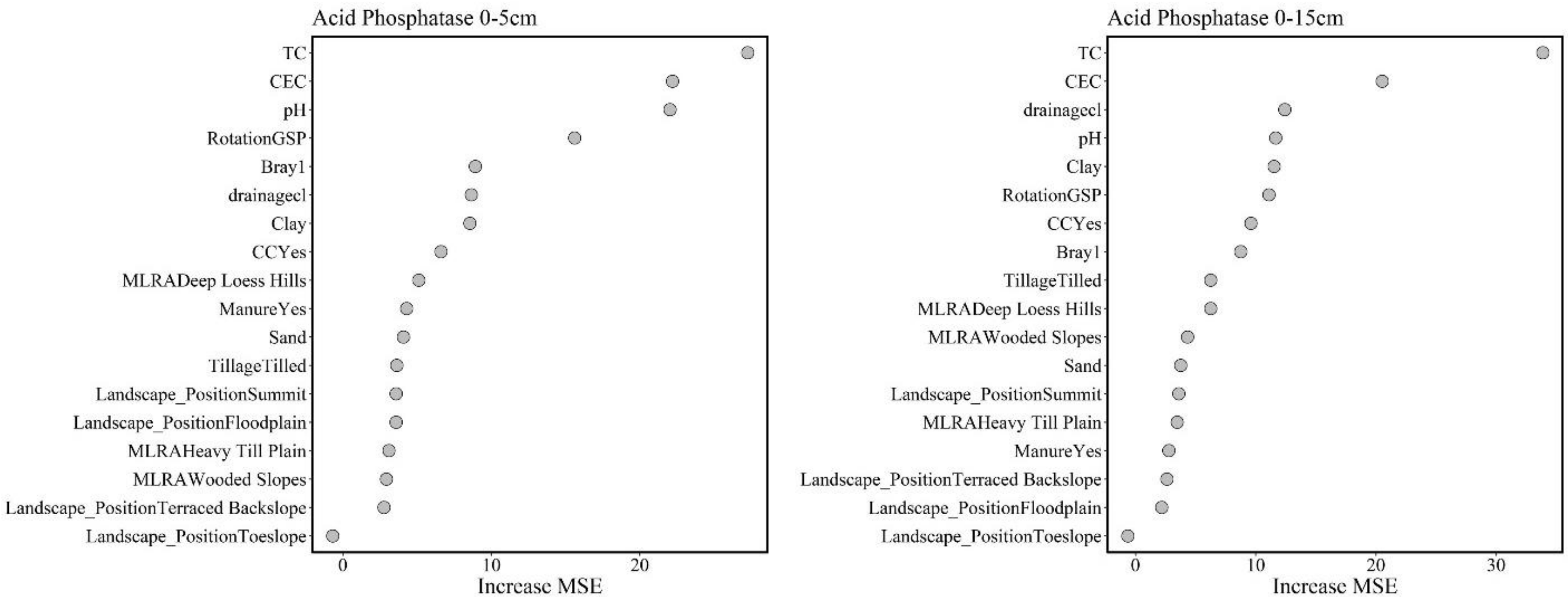

Figure C.7 Variable importance rankings from random forest models predicting acid phosphatase activity sampled at two depths (0-5 cm; 0-15 cm) at 446 sites across 101 fields in Missouri. Variable importance is calculated by measuring the mean decrease in accuracy (MSE) as an explanatory variable is randomly permutated. The greater the number and ranking, the more important the variable in predicting acid phosphatase activity.

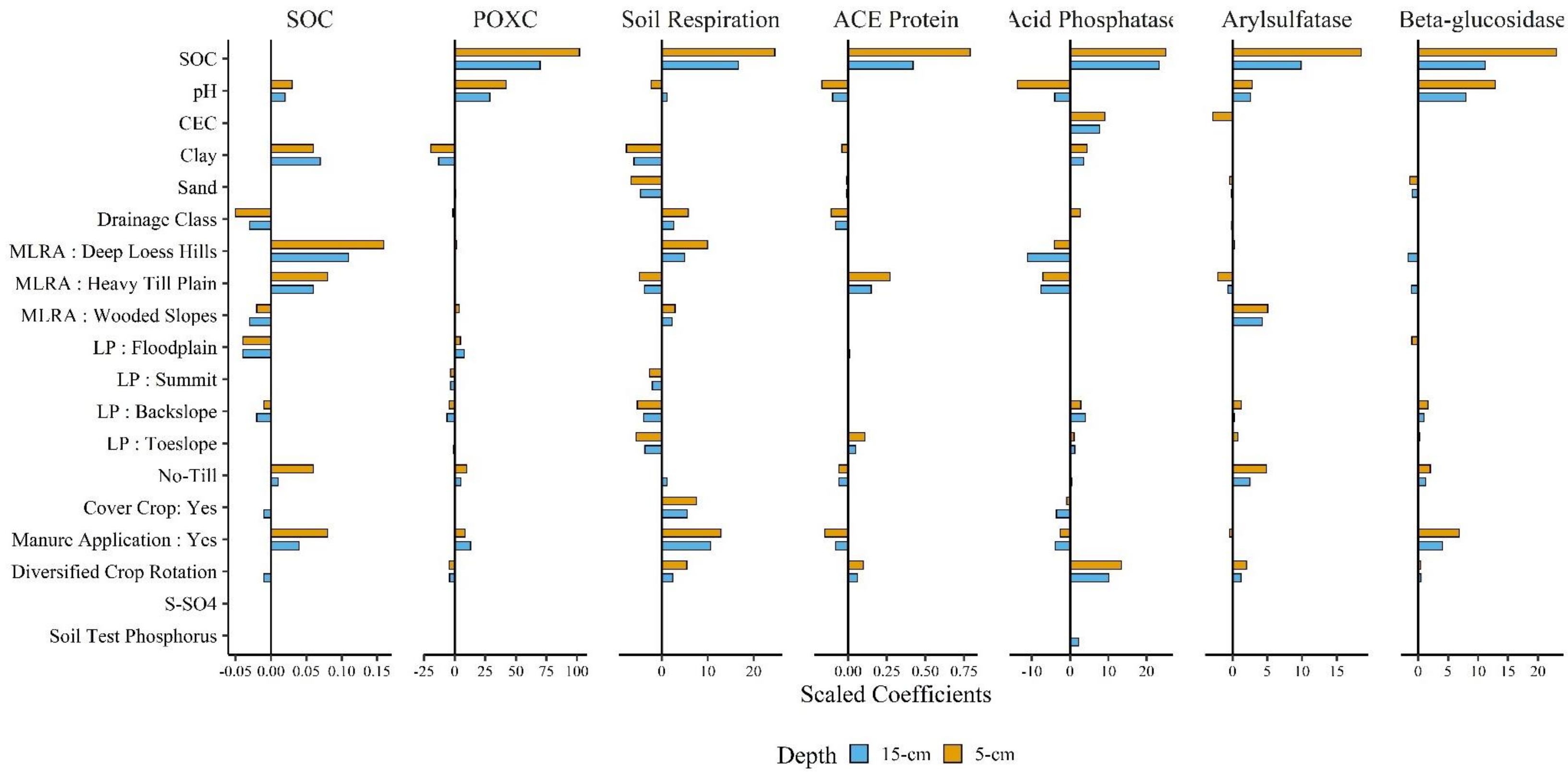

Figure C.8 Least absolute shrinkage selection operator (LASSO) coefficients for each dependent variable for all soil health indicators. Coefficients are separated by selected soil health indicator and colored bars refer to unique coefficients used for the 15 cm and 5 cm sampling depths. The larger the magnitude of the dependent variable coefficient indicates a strong relationship with that soil health indicator.

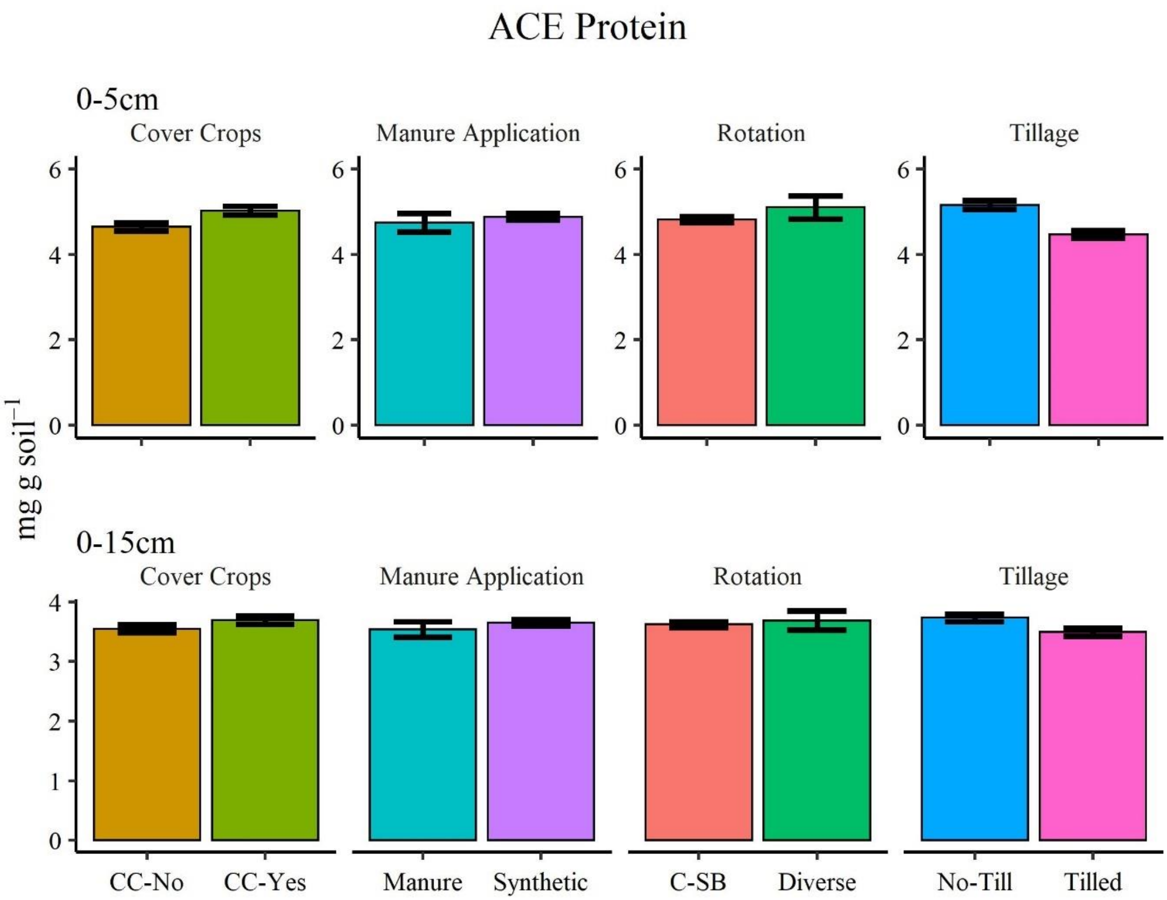

Figure C.9 Average observed Autoclaved citrate extractable (ACE) protein values separated by management practices and sampling depth. Error bars are the standard error of the mean.

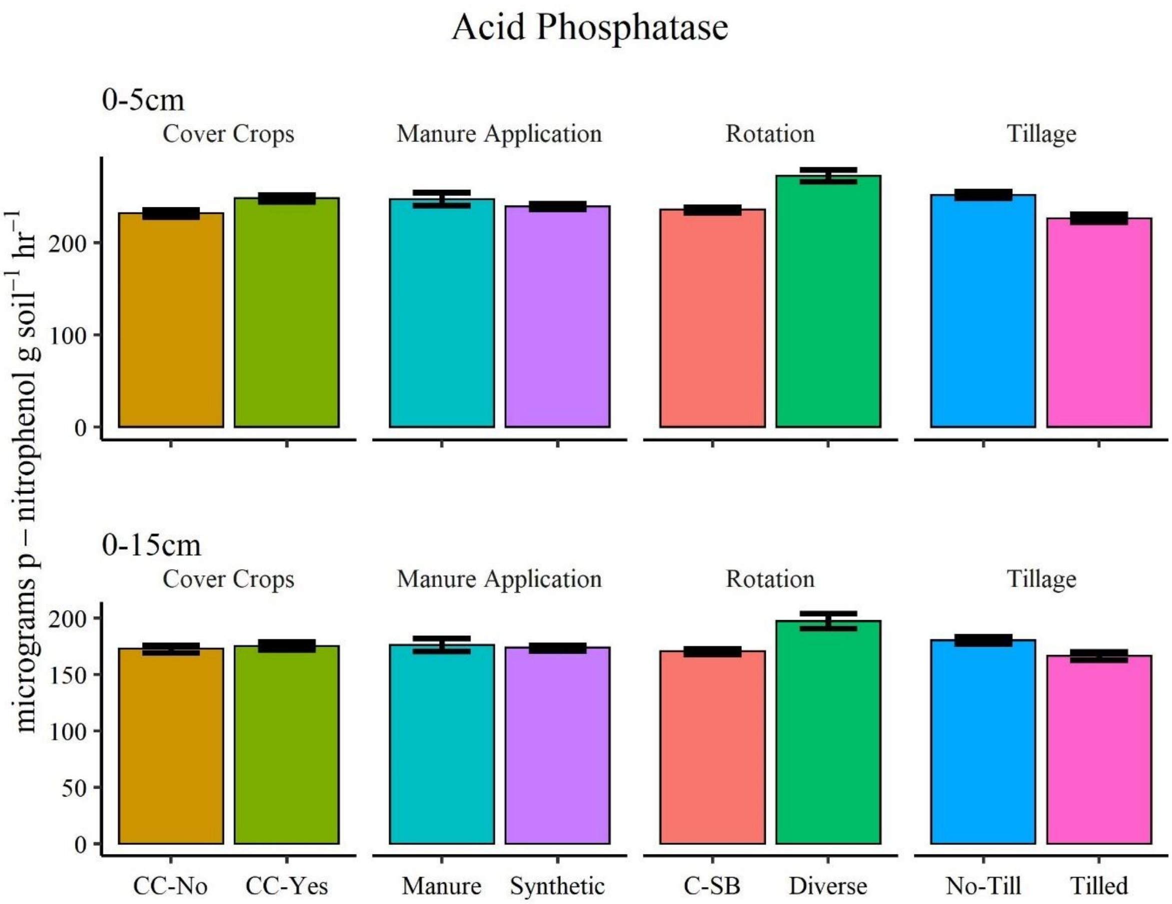

Figure C.10 Average observed Acid Phosphatase values separated by management practices and sampling depth. Error bars are the standard error of the mean.

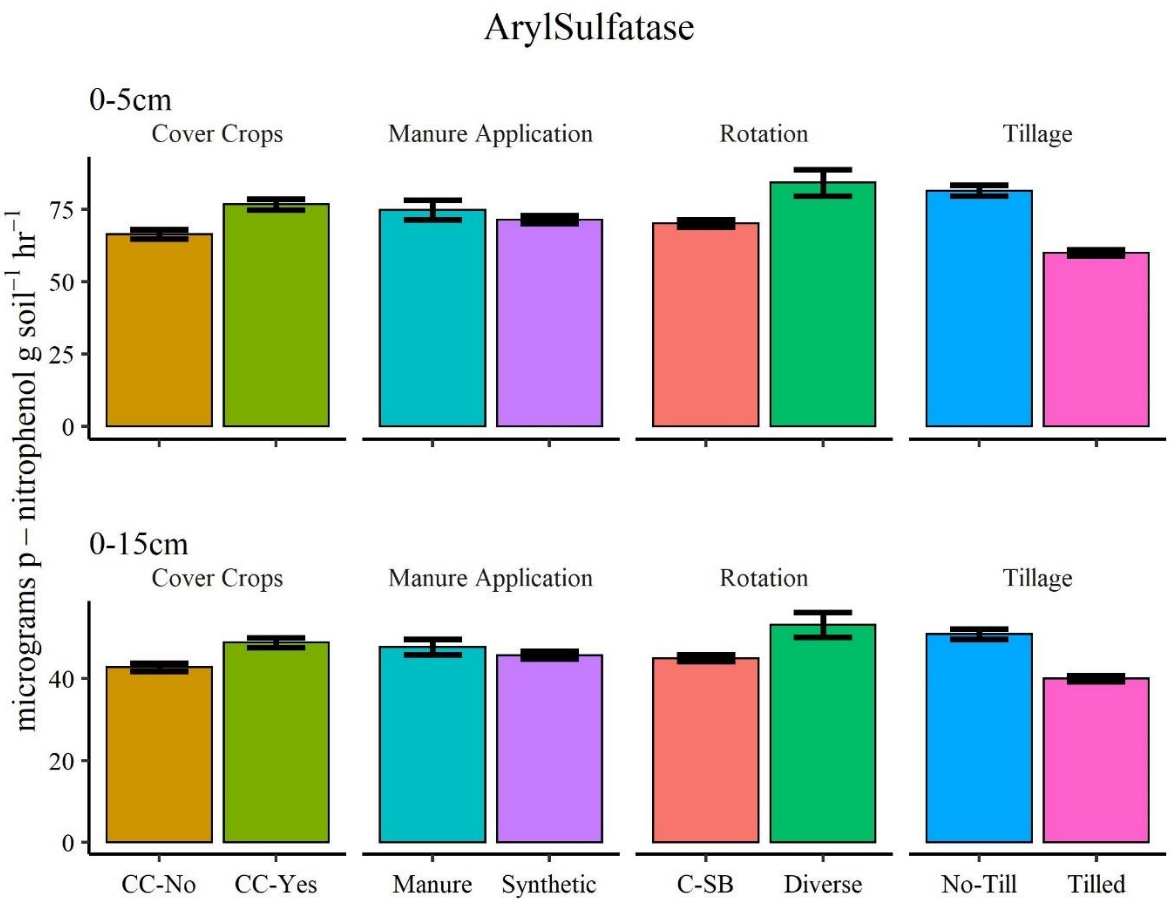

Figure C.11 Average observed Arylsulfatase values separated by management practices and sampling depth. Error bars are the standard error of the mean.

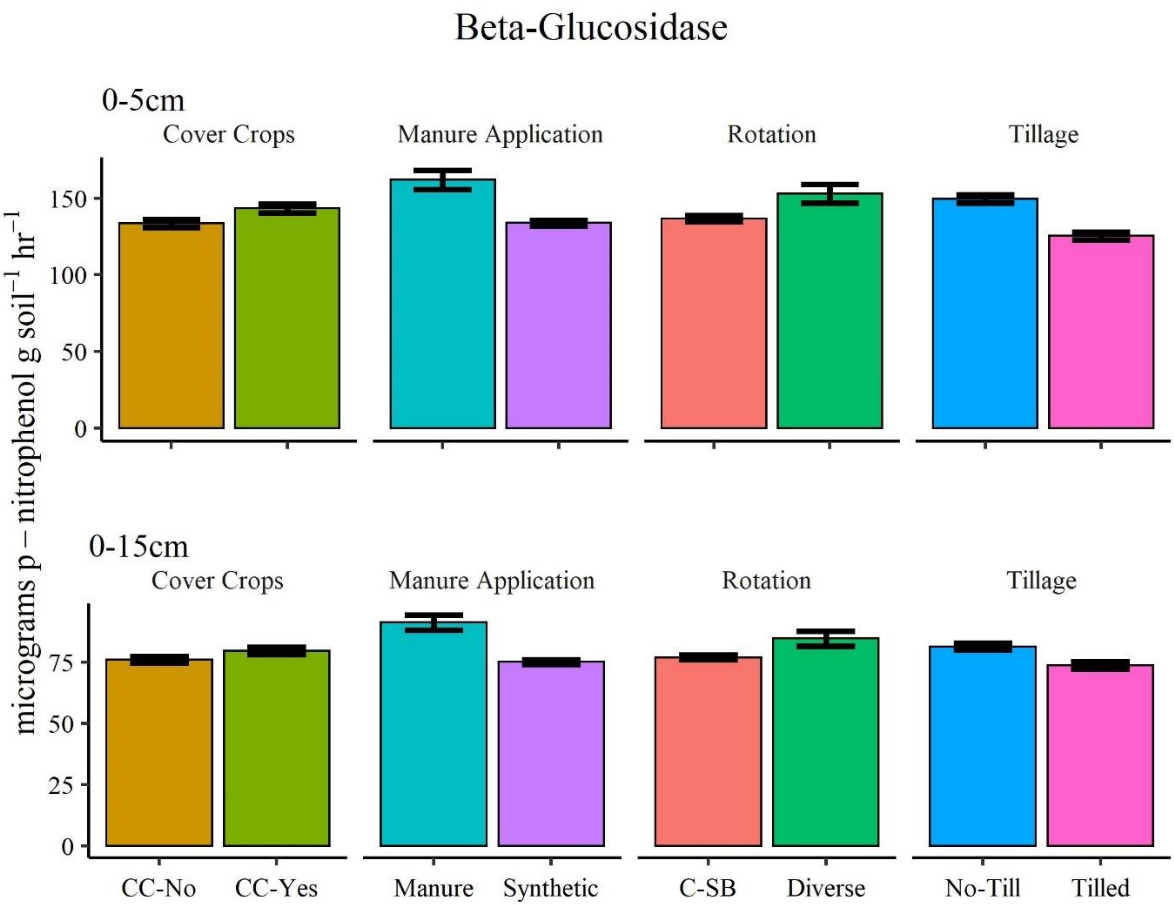

Figure C.12 Average observed β-glucosidase values separated by management practices and sampling depth. Error bars are the standard error of the mean.

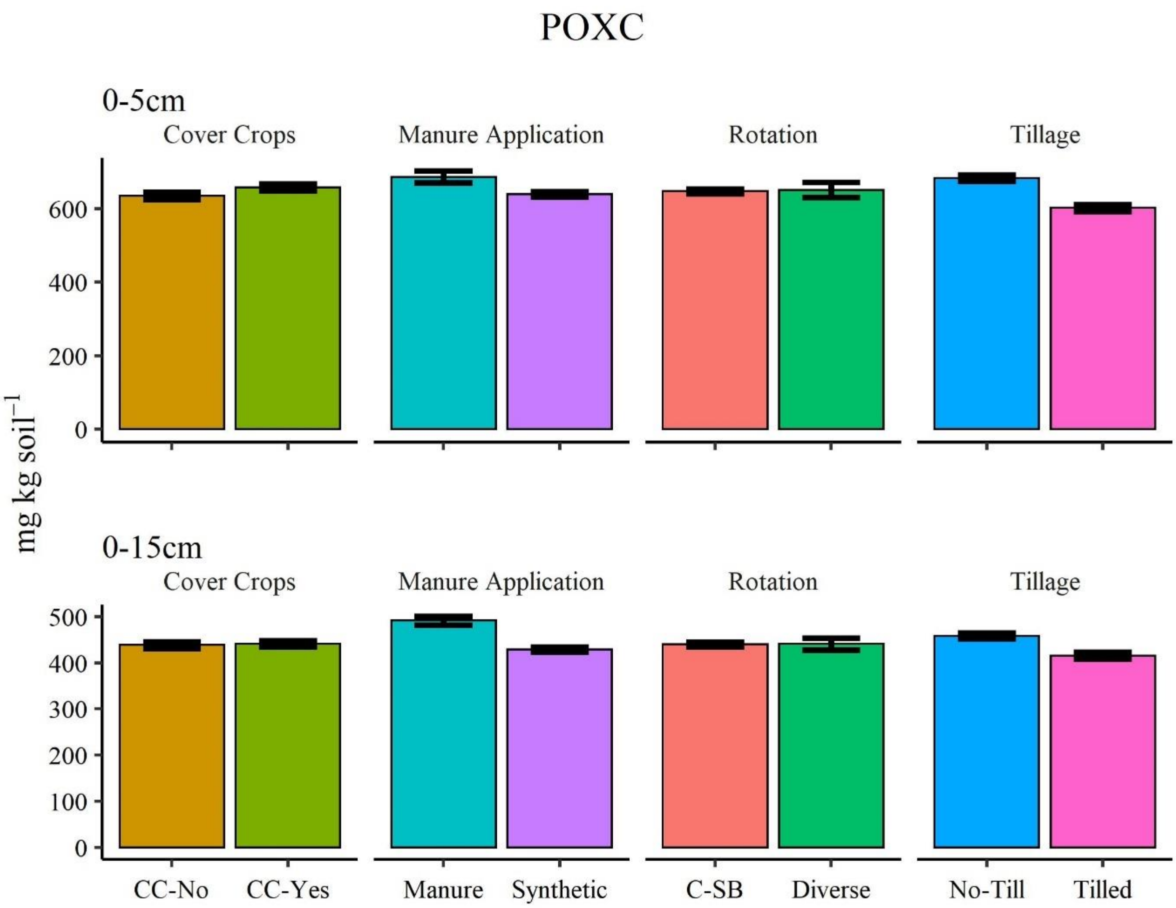

Figure C.13 Average observed potassium permanganate oxidizable carbon (POXC) values separated by management practices and sampling depth. Error bars are the standard error of the mean.

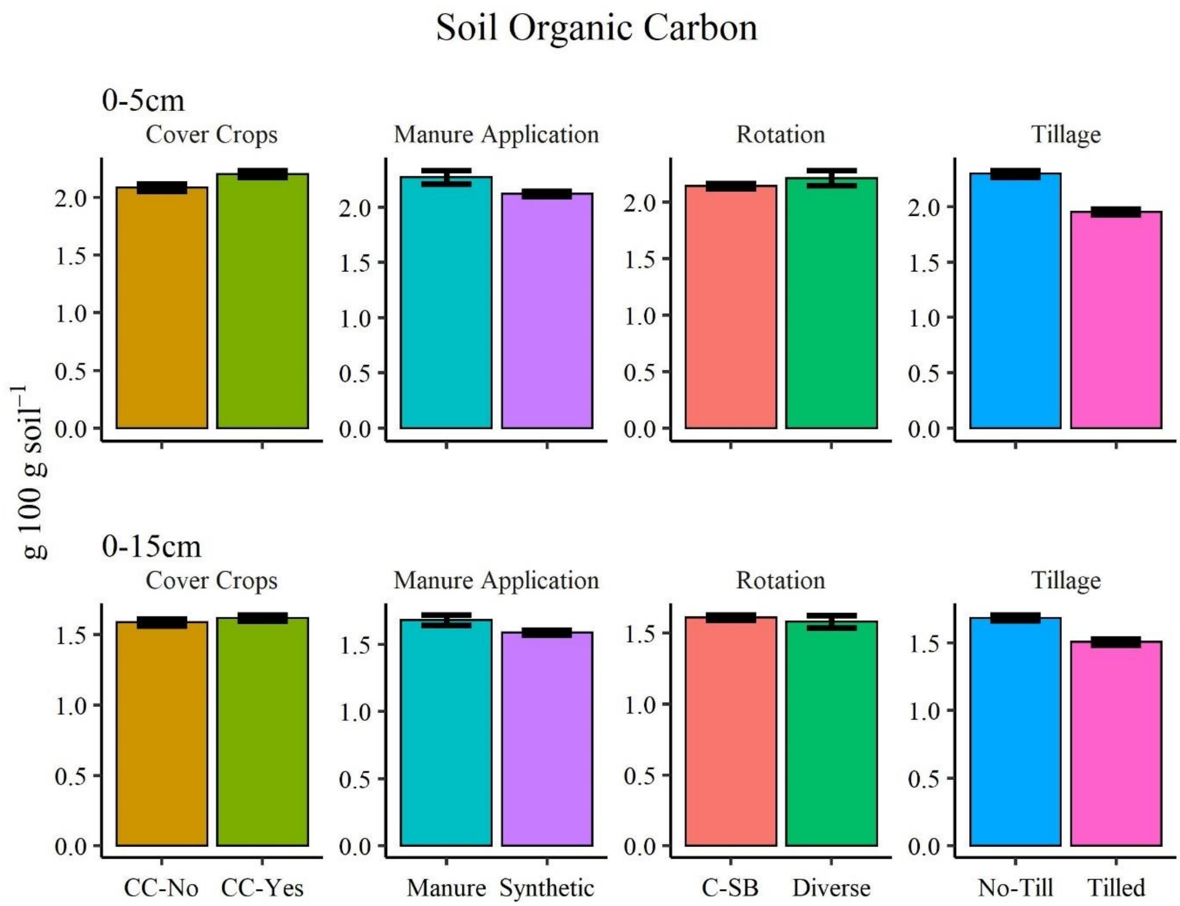

Figure C.14 Average observed soil organic carbon (SOC) values separated by management practices and sampling depth. Error bars are the standard error of the mean.

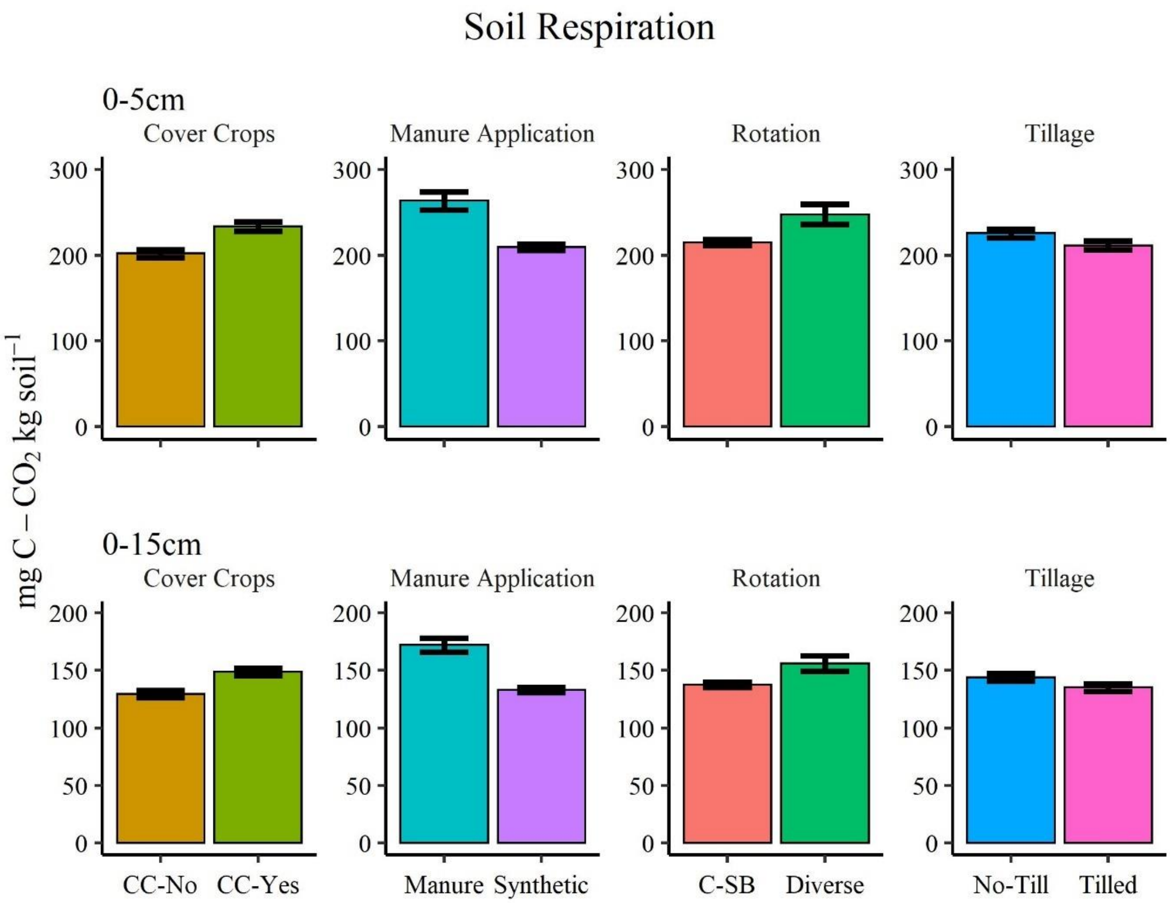

Figure C. 15 Average observed soil respiration values separated by management practices and sampling depth. Error bars are the standard error of the mean.

APPENDIX D:

Legacy Benefits of Pasture Systems on Soil Health and Productivity Remain 10 Years after Row Crop Production

D.1 Abstract

The absence of clear empirical relationships between soil health and crucial agronomic outcomes remains an obstacle for implementing soil health recommendations in commercial row crop systems. Two adjacent commercial fields presented an excellent opportunity to empirically evaluate the benefits of soil health on corn (*Z. mays)* and soybean (*Glycine max* L. Merr.*)* productivity. Prior to 2011, the north field (historical pasture) was a perennial pasture while the other south field (long-term grain field) was a corn-soybean rotation with annual tillage and history of erosion. After 2011, both fields were converted to no-till corn soybean crop rotation. The objectives of this research included 1) measure soil health status in both the historical pasture and long-term grain fields, 2) evaluate whether variation in soil health is an effective indicator of productivity. Soil health assessments (0-15 cm depth) collected in 2021 were 62% greater in the North field. Likewise, the North field yield averages from 2011-2021 were 51% greater for corn and 42% for soybean. Soil health tests were significantly related to the ten-year average yields, with the strongest links with citrate extractable protein ($r^2 > 0.70$) and soil organic carbon ($r^2 = 0.77$). However, these strong relationships deteriorated when evaluated on a per field basis rather than between fields. Thus, yield benefits from soil health are generally not relevant for within field evaluations, but rather between fields with diverse management histories. These results demonstrate significant agronomic benefits from soil

health management adoption and the lasting impact of poor soil management on soil health and productivity.

D.2 Materials and Methods

Three distinct field sites within a 0.65 km^2 area near Clifton Hills Missouri, USA were selected because of their distinct cropping system histories. Prior to 2011, the North (historical pasture) and West (on-going pasture) fields were in perennial pastures for 30+ years (per cooperating farmer's memory). During the same 30+ year period, the South field (long-term grain field) was in a corn-soybean rotation with annual tillage. In 2011, no-till was implemented in the long-term grain field while the historical pasture was converted to a no-till corn soybean rotation while the on-going pasture remained as perennial pasture. Both the historical pasture and long-term grain fields have remained in a no-till corn soybean rotation since 2011, with terraces implemented in the long-term grain field in 2012. In summarization, three systems represent three cropping systems 1) perennial system (on-going pasture), 2) aspirational soil health management system (historical pasture), 3) restoration system (long-term grain field). The fields are a complex of Grundy and Lagonda soil series (NRCS-SSURGO) formed from loess upland prairies. The Lagonda is a somewhat poorly drained Aquertic Hapludalf located on hillslopes and shoulders while the Grundy is a somewhat poorly drained Aquertic Argiudoll located on summits and interfluves.

Soil samples were collected on a single sample date in March, 2021 on 30-m grids with one third of the samples collected randomly to measure small-scale variability. Thirty-two soil samples were collected across 2.67 ha in the west field, thirty-three samples sampled across 2.54 ha in the north field, and 74 samples collected across 5.02 ha for the south field. The south field was further divided based on anthropogenic impact with 39 samples collected across 2.41 ha of the terraces, and 35 samples collected across 2.61 ha of summit landscape position. Coordinates of each soil sample sites were collected using a Trimble GeoXT 6000 and Geo7x GPS device (Sunnyvale, CA, USA) with approximately 15 cm accuracy. Soil samples were collected from eight cores to a 15 cm depth. After sampling, soils were stored in re-sealable zipper storage bags and transported in coolers. Soil samples were stored in a cooler a 1.6° C and later homogenized by passing through a 1cm screen, air dried, then dry-sieved through a 2 mm screen. Further preparation was made by grinding a subsample to a powder for potassium permanganate oxidizable carbon (POXC) and soil organic carbon (SOC) analysis.

Four soil biological indicators of soil health were evaluated at each sample location, including soil respiration, POXC, autoclave citrate extractable protein (ACE Protein), and SOC analysis were completed through the USDA-ARS Soil Quality Lab in Columbia, MO. The Cornell Soil Health Assessment protocols were followed for soil respiration, ACE Protein, and POXC. Soil organic carbon and total nitrogen were analyzed following on a LECO TRUMAC C/N combustion analyzer (LECO Corp., St. Joseph, MI, USA). Soil test phosphorus (STP) was conducted at the soil health assessment center (University of Missouri, USA) following the Bray 1 soil text extraction method (Bray and Kurtz, 1945). Yield data for the North and South fields were collected from 2011-2021 using a commercial combine equipped with a calibrated yield monitor (John Deer, Moline, IL, USA) with an attached mass flow sensor. Calculated means for each sample site are the mean of observed datapoints from a 0.05 ha (circle around geospatial location with 12.5 m radius) surrounding each sample site geospatial location.

For soil profile characterization, a single 1.2 m deep and 4.086 cm diameter soil core sample was taken at the center of each monitoring site using a Giddings Model #5-UV / MGSRPSUV (Giddings Machine Company, Windsor, CO). Because these samples were collected in agricultural production fields, the first horizon was standardized to a 0-15 cm depth and assumed as the plough layer (Ap) horizon. Subsequent pedogenic horizons were characterized using visual and tactile clues with a maximum five horizons identified per soil core. Identified horizons were subsequently sampled and air-dried. Bulk density and soil moisture were collected for each sampled horizon, and the top three horizons analyzed for particle size and the same soil fertility analysis previously listed.

D.3 Results and Discussion

Table D.1 Annual yield from two adjacent fields with similar soil and topographies. Both fields are currently corn-soybean rotation with no-till soil management. Before 2011, the North field was a perennial pasture for 30+ years while the south was in a corn-soybean rotation with annual tillage. Positive effects from the perennial system are evident, with greater yield observed in the North field in every season. Further, effects from the perennial system remain after 10 years of row cropping, with 2021 corn yield in 130% greater in the North field. Perennial systems effects disproportionately benefited corn yield with a 10% greater average yield increase than soybean over the 10-year period. Further work is necessary to identify why corn grain yield is more sensitive to soil health systems, but I hypothesize that at this field site differences occur because of challenges in planting and emergence in the south field. The impacts from historical erosion processes on the hydrologic cycle and surface topsoil in the South field provide challenges in planting with effects on seedling emergence, which disproportionately effects corn grain yield. Therefore, effects of improved soil health are not limited to soil biological, physical, and chemical processes, but can expand to effect agronomic practices that facilitate productive and sustainable cropping systems.

Year	**2011**	**2012**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**Average**	
Crop	Corn	SB	SB	Corn	SB	Corn	SB	Corn	SB	Corn	Corn	SB
						Mg ha^{-1}						
Historical Pasture*	11.0	1.92	5.40	12.3	5.05	14.2	5.37	9.32	4.59	11.7	11.7	5.7
Long-term Grain**	7.2	1.34	3.79	10.5	4.47	10.9	3.46	4.98	3.24	5.07	7.74	4.0
						%						
Percent Difference	53.3	42.9	42.4	16.4	13.1	30.0	55.0	87.4	41.9	130	51.1	41.5

*North Field; **South Field

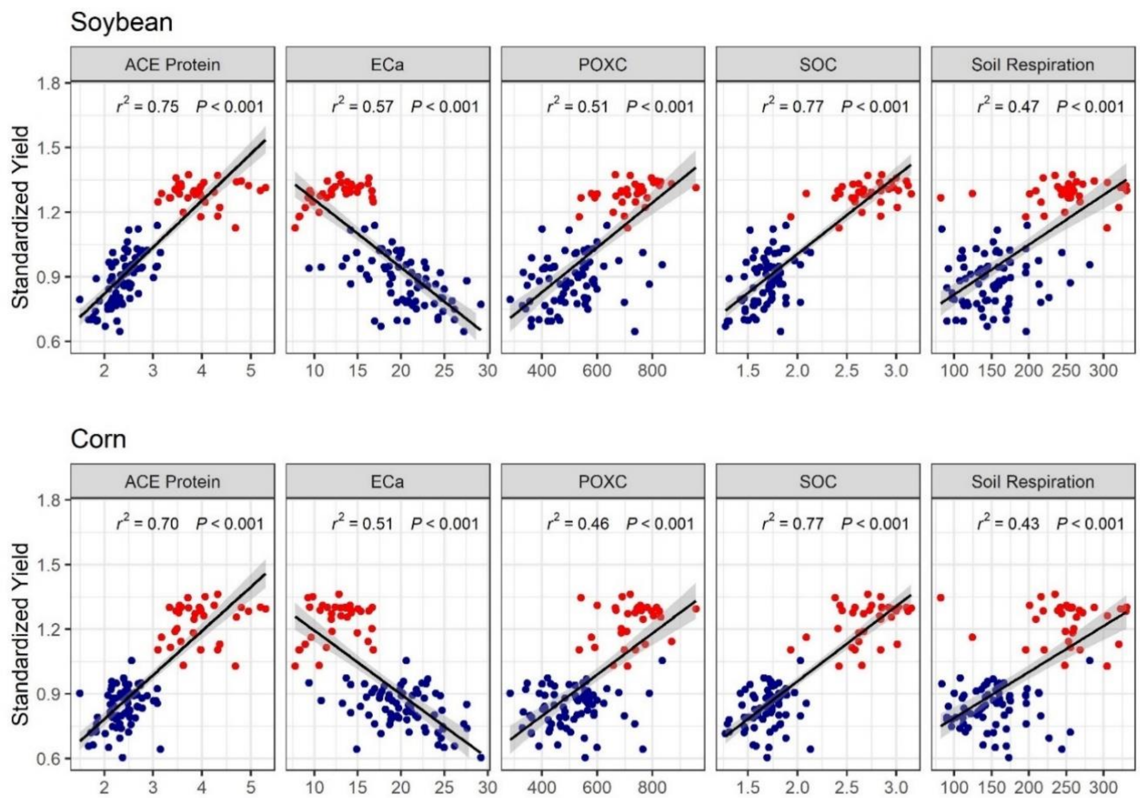

Figure D.1 Linear relationships between common soil health metrics and standardized yield between 2011-2021 between the North (Red) and South (Blue) fields. Since 2011 both fields soil and crop management are no-till and a corn-soybean rotation. Prior to 2011 the North field was in a perennial hay system for 30+ years while the south field remained in corn soybean rotation with annual tillage. Universal improvements in soil health indicators on the North field remain evident 10 years after row crop production. Further, improvements in soil health were strongly related with average grain production, with soil organic carbon and autoclaved citrate extractable protein displayed the strongest relationship with yield (r^2> 0.70). Sensitivity to grain productivity was unique to each soil health indicator, with soil respiration displaying the poorest (r^2 < 0.43). These results demonstrate positive agronomic outcomes by fostering soil health through conservation management practices.

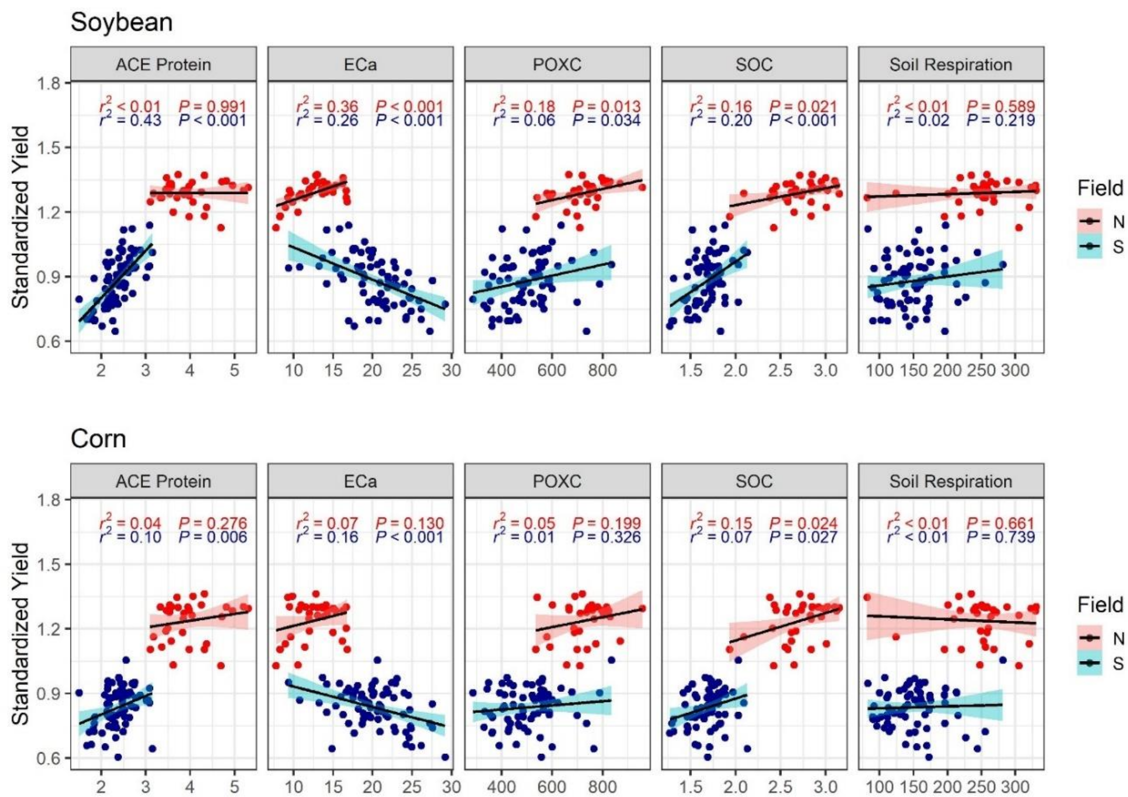

Figure D.2 Linear relationships between soil biological indicators of soil health and average yield from 2011-2021. Results treat each field as unique populations with results reporting *within field* relationships. Relationships between yield and soil health metrics were universally poor ($r^2 < 0.43$). Soybean yield appeared to be more correlated than corn yield with generally higher r^2, but these relationships remain poor. I conclude that within these field sites, impacts from topsoil depth, water dynamics, weed pressure, etc. have greater impacts on productivity than soil health. Consequently, benefits from soil health are best observed when evaluated between fields with divergent management histories.

APPENDIX E:

Anthropogenic Management Practices Effect Spatial Variability of Common Soil Health Indicators

E.1 Abstract

Spatial characteristics of soil health assessments are underrepresented in the literature. Further, it remains unknown whether different conservation management practices have unique effects upon the spatial structure of these measurements. Field based research was conducted in 2021 to explore the spatial characteristics of soil health practices in three fields with distinct management histories. The objectives of this research included 1) the quantification of soil health spatial autocorrelation between management systems and 2) comparison of these trends with established soil fertility tests. Soil samples (0-15 cm depth) were collected Spring 2021 from three fields with unique management histories. The North field was a perennial system for 30+ years until 2011 when it was transferred to a no-till corn-soybean rotation. Finally, the south field has been in corn-soybean rotation for the past 40+ years, with no-till implementation beginning in 2010. Terraces were built in 2011 to assist in erosion control. As such, the fields were evaluated with ascending levels of anthropogenic influence (Pasture < North < S-Summit < S-Terraces). One hundred and forty-three soil samples were collected on 30-m grid split between the three field locations. Spatial trends were calculated with fitted semi-variograms. Soil health measurements were 10, 41, and 46% less on the North, S-Summit, and S-Terraced fields relative to the perennial systems. In general, the soil health indicators reflected landscape positions in the perennial system, while ACE Protein failed to provide adequate information to identify spatial structure at all but the terraced field site. In general soil health assessments were

similar in spatial structure to other soil fertility evaluations in the row-crop systems. These results justify that, excluding autoclaved citrate extractable protein, common soil health indicators display similar spatial structure to soil fertility assessments in row-crop systems. While soil heath sampling could be reduced to sampling unique landscape positions in perennial systems.

E.2 Materials and Methods

See Materials and Methods within APPENDIX D for a description of data collection and cropping system management histories.

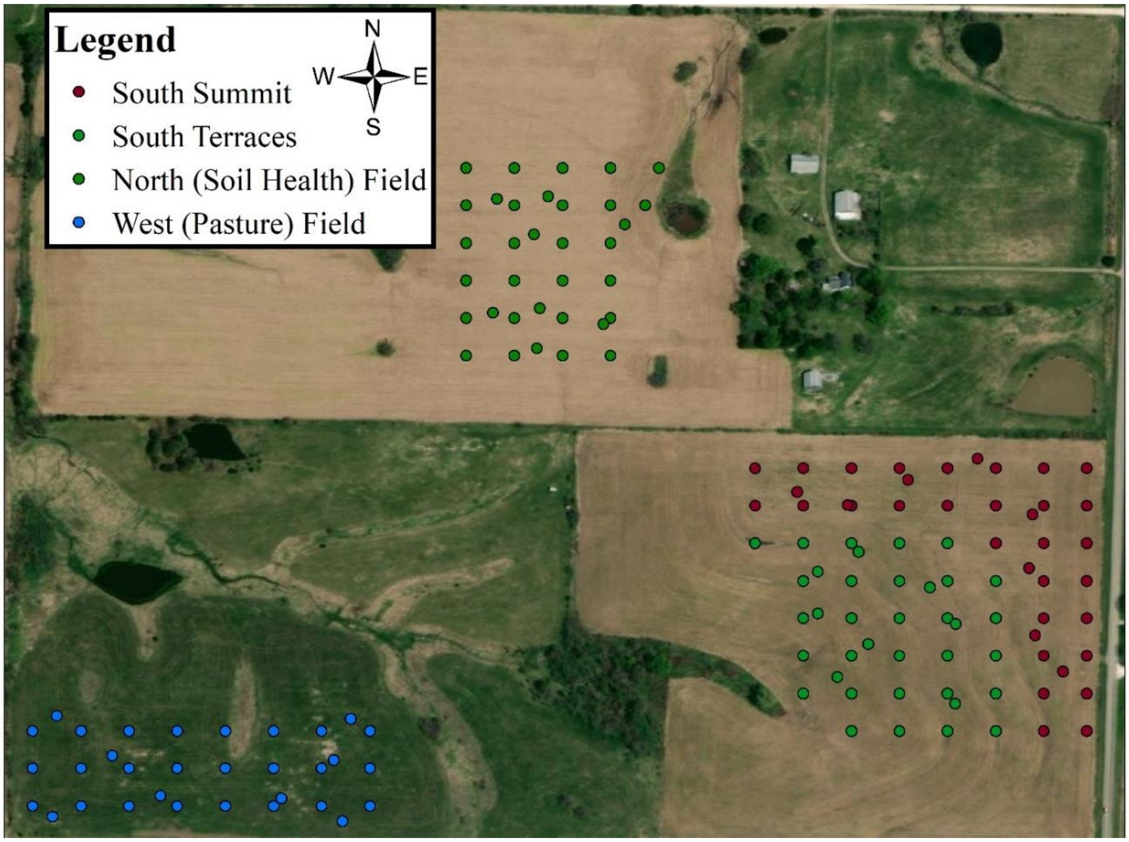

Figure E.1 Field sample sites for the North (aspirational soil health field), South (business as usual), and West (perennial pasture) fields. The South field sample sites were separated by management effects. With half of the sites overlaying recently built terraces (2011) and the other South field sampling reflecting the summit landscape position.

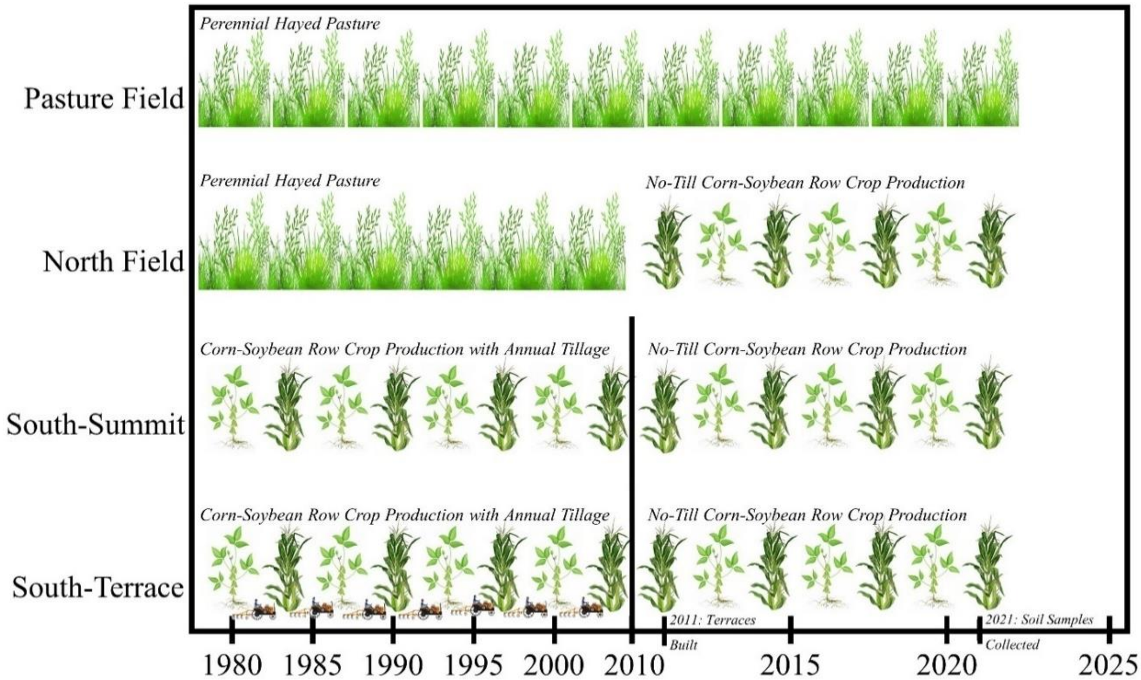

Figure E.2 Graphical description of historic management practices for each field prior to soil sampling in March 2021.

E.3 Results and Discussion

Table E.1 Mean statistics and results for selected soil health indicators and corresponding soil fertility tests. Erosion impacts are evident in the clay content, with clay content being the greatest where the terraces were recently implemented and the least in the perennial pasture. The greater clay content in the S-Terraces reflects the impact of topsoil erosion and subsequent exposure of subsoil clay content.

Field	**POXC**	**Resp**	**TP**	**SOC**	**pH**	**STP**	**STK**	**Clay**
	mg kg^{-1}	mg kg^{-1}	g mg^{-1}	%		mg kg^{-1}	mg kg^{-1}	%
Pasture	818	301	4.18	3.01	6.24	12.0	72	22.6
North	725	250	3.97	2.82	5.75	17.8	135	25.2
S-Summit	541	150	2.5	1.81	6.16	36.3	104	25.3
S-Terraces	470	152	2.23	1.66	6.26	26.3	103	29.7

Potassium permanganate oxidizable carbon, POXC; Soil Respiration, Resp; autoclaves citrate extractable protein, TP; soil organic carbon, SOC; soil test phosphorus, STP; soil test potassium, STK

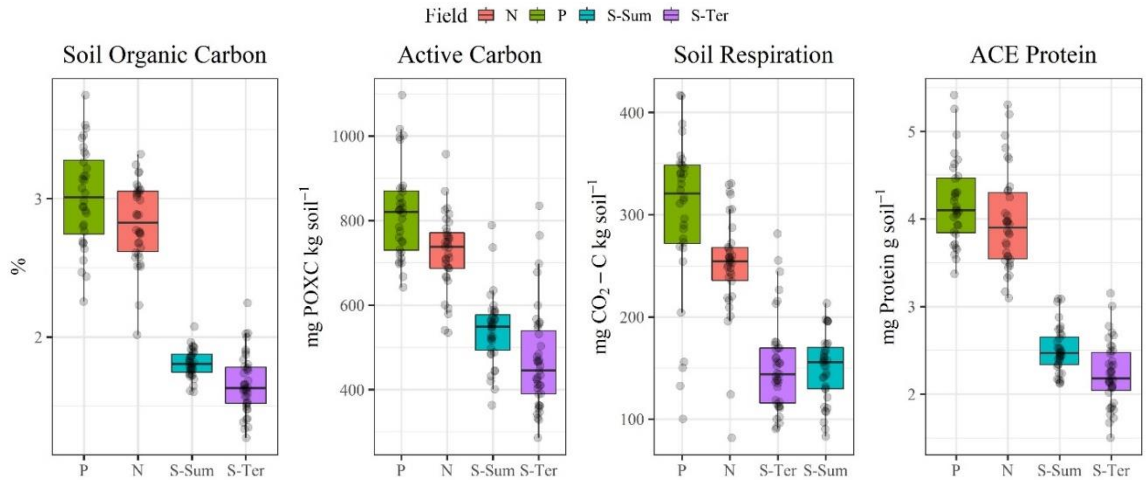

Figure E.3 Box and whisker plots of selected soil health analysis separated by anthropogenic influence. The Pasture is treated as a reference state for the North and South Field sites to qualify soil health in the two row crop systems. Positive effects upon the North Field soil health from the previous perennial system remain ten years after implementing no-till corn-soybean crop rotation. Soil health decreased with greater anthropogenic impact (Perennial > North > South Summit > South Terrace) for nearly every soil health indicator (exception soil respiration). This dataset does not provide information regarding the trajectory of the soil health status of each system. Future soil health assessments would provide information whether the North field has reached a new equilibrium or further decay is possible and whether recently implemented regenerative practices in the South field will facilitate further increases in soil health.

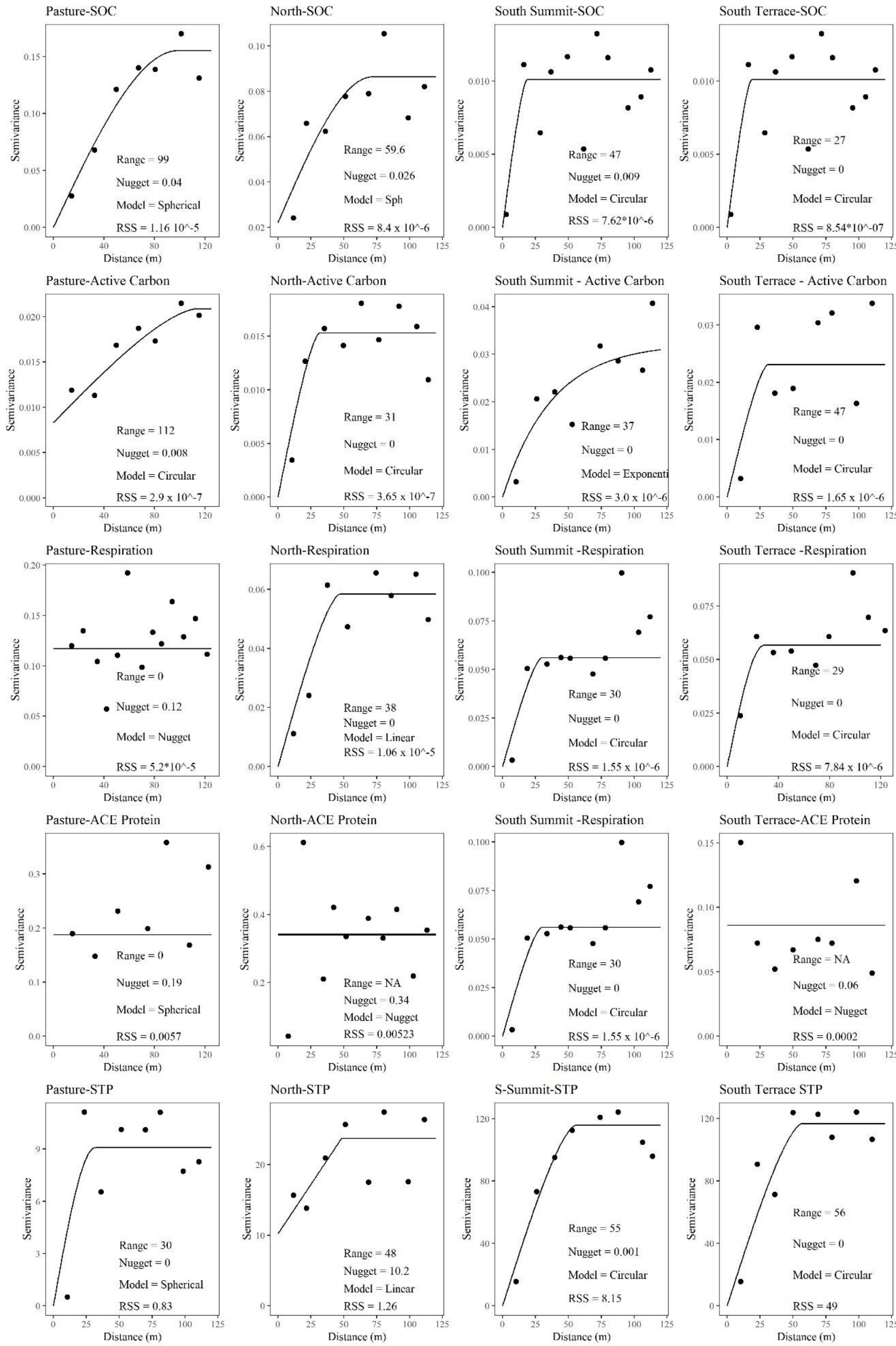
Pasture-SOC
Range = 99
Nugget = 0.04
Model = Spherical
RSS = 1.16 10^-5
North-SOC
Range = 59.6
Nugget = 0.026
Model = Sph
RSS = 8.4 x 10^-6
South Summit-SOC
Range = 47
Nugget = 0.009
Model = Circular
RSS = 7.62*10^-6
South Terrace-SOC
Range = 27
Nugget = 0
Model = Circular
RSS = 8.54*10^-07
Pasture-Active Carbon
Range = 112
Nugget = 0.008
Model = Circular
RSS = 2.9 x 10^-7
North-Active Carbon
Range = 31
Nugget = 0
Model = Circular
RSS = 3.65 x 10^-7
South Summit - Active Carbon
Range = 37
Nugget = 0
Model = Exponenti
RSS = 3.0 x 10^-6
South Terrace - Active Carbon
Range = 47
Nugget = 0
Model = Circular
RSS = 1.65 x 10^-6
Pasture-Respiration
Range = 0
Nugget = 0.12
Model = Nugget
RSS = 5.2*10^-5
North-Respiration
Range = 38
Nugget = 0
Model = Linear
RSS = 1.06 x 10^-5
South Summit -Respiration
Range = 30
Nugget = 0
Model = Circular
RSS = 1.55 x 10^-6
South Terrace -Respiration
Range = 29
Nugget = 0
Model = Circular
RSS = 7.84 x 10^-6
Pasture-ACE Protein
Range = 0
Nugget = 0.19
Model = Spherical
RSS = 0.0057
North-ACE Protein
Range = NA
Nugget = 0.34
Model = Nugget
RSS = 0.00523
South Summit -Respiration
Range = 30
Nugget = 0
Model = Circular
RSS = 1.55 x 10^-6
South Terrace-ACE Protein
Range = NA
Nugget = 0.06
Model = Nugget
RSS = 0.0002
Pasture-STP
Range = 30
Nugget = 0
Model = Spherical
RSS = 0.83
North-STP
Range = 48
Nugget = 10.2
Model = Linear
RSS = 1.26
S-Summit-STP
Range = 55
Nugget = 0.001
Model = Circular
RSS = 8.15
South Terrace STP
Range = 56
Nugget = 0
Model = Circular
RSS = 49
Semivariance
Distance (m)

Figure E.4 Semi variograms with fitted functions and reported values for range, nugget, and model error (residual sum of squares), and fitted model function. In general, spatial trends in soil health indicators varied between management systems. As demonstrated by SOC and POXC, the large range to reach spatial independence suggest that these features are inherently governed by landscape position. Then, once management impacts are imposed, the spatial structure of these indicators begin to decrease and reflect erosion processes or disruptions to soil processes. Therefore, the stronger the anthropogenic influence (S-Terraced Field) the shorter the spatial scale. This was further highlighted by the results that no spatially measurable structure was observed for ACE protein and soil respiration. Measuring spatial structure was most difficult with ACE protein. I expect this is because of insufficient sample collection, or too greater of a sampling grid (30-m). These results highlight that ACE protein could require greater sampling intensity than the other soil health indicators. Soil test phosphorus spatial structure was generally similar to other soil health indicator ranges—with the notable exception being in the perennial pasture system. Finally, these results confirm that integrating soil health and soil fertility in spatial assessments are justified in row crop systems, while soil heath sampling could be reduced to sampling unique landscape positions in perennial systems.

VITA

Jeffrey D. Svedin was born November 9, 1991, in Idaho, USA, where he grew up on a family row crop farm. He received his Bachelor of Science in Environmental Science in 2016 from Brigham Young University where he later obtained his Master of Science in Environmental Science in 2018. He then moved to Missouri to earn his Doctorate of Philosophy in Soil Science under the advisement of Drs. Newell R. Kitchen, Kristen S. Veum, and Stephen H. Anderson.