

Soil Health Assessment for the Agroecosystems of West Tennessee

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ABSTRACT

Soil health assessment is important for making informed sustainable management decisions in production systems. An established standardized method to quantify soil health is lacking and the validity of the existing methods across agroecoregions and cropping systems is not yet proven. Therefore, this study aims to evaluate the feasibility of widely discussed three soil health tests - Haney's Soil Health Test (HSHT), Comprehensive Assessment of Soil Health (CASH), and Alabama Soil Health Index (ASHI) to assess soil health in diverse cropping systems of Tennessee. Since these approaches were originally developed for specific agroecoregions, we hypothesized that these tests are not sensitive to detect soil health changes due to management differences for the cropping systems of Tennessee. Our feasibility criteria depended on the sensitivity of soil health scores and underlying indicators to management changes. To conduct this research, we leveraged three ongoing cropping system experiments in Tennessee that include a suite of tillage, cover crops, and N rate treatments. These experiments are: (i) continuous soybean (*Glycine max* L.) (SS), (ii) corn (*Zea mays* L.)-soybean rotation (CS), and (iii) continuous cotton (*Gossypium hirsutum* L.) (CC) systems. Our results showed that all three existing tests (HSHT, CASH, and ASHI) failed to differentiate soil health in long-term conventional systems from that in conservation systems. Therefore, a weighted soil health index (WSHI) was developed for a regionally-realistic soil health assessment of these cropping systems. The WSHI used soil health indicators sensitive to the tested cropping systems and scoring functions meaningful within the region. Finally, soil health gaps (SHG) between the regional benchmark soils (i.e., unmanaged grasslands and woodlots) and cropland soils were calculated. Results revealed that WSHI was more effective in discerning soil health differences between conservation and conventional managements of Tennessee cropping systems compared

to already existing tests. Additionally, SHG between unmanaged and intensively managed soils reiterated the degree of soil health degradation from cultivation. The SHG approach shows promise as a standardized method to compare soil health across agroecological regions.

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Chapter 1. General Introduction

Soil quality and soil health

Soil is a dynamic living system that supports agricultural productivity and ecosystem functions (Doran et al., 1996) and the degradation of soil resources can threaten land-based life on earth (Olson, 1981). Given the current concerns of feeding the rising population without compromising our environment, extensive research has been focused on sustainable agricultural production by building healthy soils (Janzen, 2006; Powlson et al., 2011). In general, soil health and soil quality are often used interchangeably (Bünemann et al., 2018). Soil quality is defined as “the capacity of a soil to function, within the ecosystem and land-use boundaries, to sustain productivity, maintain environmental quality, and promote plant and animal health” (Doran and Parkin, 1994; Doran et al., 1996). Thus, soil quality implies the soil’s fitness to support crop growth without resulting in the deterioration of soil and the environment (Oliver et al., 2013). Soil health, on the other hand, is defined as the “continued capacity of soil to function as a vital living system, by recognizing that it contains biological elements that are key to ecosystem function within land-use boundaries” (Doran and Zeiss, 2000; Karlen et al., 2001; Kinyangi, 2007). Although both terminologies are widely used, soil quality depicts a specific soil’s ability for specific use whereas soil health is a broader term which represents soil as a living system (Bünemann et al., 2018; Doran and Safley, 1997; Kibblewhite et al., 2008; Larkin, 2015). Farmers tend to prefer the term *soil health* while *soil quality* is still in use, primarily among scientific community (Bünemann et al., 2018; Idowu et al., 2008; Karlen et al., 1997). As *soil health* depicts a more holistic, stable, and living condition of the ecosystems, it is gaining more momentum than *soil quality*. Nonetheless, Bünemann et al. (2018) concluded that both terms are equivalent because the distinction between soil quality and soil health is mainly preference driven. It is also recognized that soil health encompasses a transition of focus from inherent to

dynamic soil properties (Bünemann et al., 2018; Moebius-Clune et al., 2016). Therefore, we will be using the term *soil health* throughout this dissertation.

Importance and history of soil health

In early times, soils were not considered as a part of management decisions despite being the backbone of agriculture (Herrick, 2000). The emphasis on the importance of soil health was started in the early 1990s by the recognition that soil is a key player in sustainable agriculture. This led to several publications linking soil health, ecosystem functions, and global environmental quality (Doran and Parkin, 1994; Doran et al., 1996; Karlen et al., 1997; Larkin, 2015; Papendick and Parr, 1992). Soil health assessment has been identified as one of the most important components when soil management options and priorities shifted the focus from only erosion control to overall sustainable agriculture (Wienhold et al., 2004). This change in outlook was fueled by the increased public interest in sustainability and environmental protection (Schoenholtz et al., 2000) by relying on technological improvement in agriculture (Lewandowski et al., 1999).

Soil physical, chemical, and biological properties undergo inevitable changes due to agricultural management. In some cases, these changes in soil properties may be beneficial to agricultural production but detrimental to other soil ecosystem functions (e.g., water quality, microbial activity) (Powlson et al., 2011). Additionally, soil faces degradation threats due to disturbances, land-use changes, and intensive agricultural managements leading to soil nutrient loss, soil carbon loss, metal contamination, soil erosion, and acid deposition (Smith et al., 2016). In agricultural systems, soil health monitoring is needed to assess soil's continued functional capacity. This functional capacity may be degraded over time if the main focus is on increasing

productivity through external inputs of fertilizers and plant protection chemicals (Powlson et al., 2011). Degraded agricultural soils are regarded as the major constraints to sustainability.

A recent shift in focus from improving only soil fertility to improving overall soil health entailed consideration of soil physical, chemical, and biological attributes (Doran and Parkin, 1994; Doran et al., 1996; Larkin, 2015). Consequently, soil health assessments that integrate soil physical, chemical, and biological components become necessary. These assessment approaches attempted to develop an overall soil health index by integrating multiple soil properties or soil health indicators to measure soil health and to provide meaningful information to growers and other stakeholders (Lima et al., 2013; Mohanty et al., 2007). It has been generally considered that healthy soils contain high organic matter, high water holding capacity and drainage, adequate and accessible nutrient supply, sufficient depth for root growth, diverse microbial community, low pathogen pressure, and resistance and resilience to soil degradation under stress (Gugino et al., 2009; Larkin, 2015; Magdoff, 2001).

The first comprehensive soil health assessment approach known as ‘Soil Management Assessment Framework (SMAF)’ was developed in the 1990s, which integrates soil physical, chemical, and biological parameters representing critical soil properties and processes into a minimum data set that responds to a soil function (Andrews et al., 2004). The SMAF provides site-specific interpretations for soil health indicators and derives a soil health index. This framework involves three main steps: i) selecting soil properties as soil health indicators, ii) interpreting soil health indicators, and iii) combining indicator scores into a soil health score (Andrews et al., 2002; Andrews et al., 2004). Several recent soil health assessment methods including Comprehensive Assessment of Soil Health (CASH) (Idowu et al., 2009; Moebius-Clune et al., 2016; Schindelbeck et al., 2008), Ontario soil health assessment (OSHA)

(Congreves et al., 2014; Eerd et al., 2014), and Alabama Soil Health Index (ASHI) (Bosarge, 2015; Mitchell et al., 2015) were evolved based on the SMAF framework.

Indicators of soil health

Since soil health has been defined in terms of soil functions (e.g., nutrient cycling, water and solute transport, contaminant removal) and these functions are tightly linked to soil properties, in order to assess soil health, appropriate soil physical, chemical and biological properties need to be selected (Larson and Pierce, 1994). These selected soil properties that represent the soil function in question are known as soil health indicators (Vasconcellos et al., 2013). Each selected soil health indicator is given a unitless score (ranging from 0-1 or 1-100, based on the assessment method) using a non-linear curve called “scoring function” based on the measured value of the indicator and the relationship of each indicator with the selected soil function. For some soil properties, a higher scoring function is desirable (e.g., soil organic matter), for some other properties, a low value is better (e.g., soil compaction) and for yet other properties optimum range is better (e.g., pH) (Table 1.1). It is necessary to utilize scoring functions for each soil health indicator so that the measured values of different soil properties with different units can be combined into a unitless value (Andrews et al., 2002; Doran and Safley, 1997; Doran and Zeiss, 2000).

Physical indicators of soil health

Physical indicators of soil health mainly refer to the physical condition of the soil to aid the storage and transmission of air and water in the soil, and that guide the process of developing best land management practices (Iovino et al., 2016; Reynolds and Topp, 2008). Most widely included physical soil health indicators in different soil health assessment methods are bulk density, infiltration, water holding capacity, penetration resistance, soil texture, and aggregate

stability (Doran and Safley, 1997; Doran et al., 1996; Moebius-Clune et al., 2016). These indicators impact several processes in the soil such as seed germination, root elongation, soil erosion, crust formation, aggregation, and run-off (Topp et al., 1997).

Chemical indicators of soil health

Chemical indicators mostly reflect nutrient and carbon (C) cycling in soil. Most commonly used chemical soil health indicators are soil pH, electrical conductivity (EC), cation exchange capacity (CEC), percent base saturation, exchangeable sodium percent (ESP), total nitrogen (N), soil nitrate, and extractable macro- and micro-nutrients (P, K, Mg, Fe, Mn, and Zn) (Doran and Safley, 1997; Doran et al., 1996; Gugino et al., 2009; USDA-NRCS, 2015b).

Biological indicators of soil health

Biological soil health indicators represent the living components of soil. These indicators are most sensitive to management practices, contamination, disturbance, or any change in the physical and chemical properties of soil. Also, these indicators can be related to soil structure, nutrient storage, and microbial activities (Knoepp et al., 2000). The biological components have been neglected in the earlier attempts to quantify soil health due to the lack of awareness on the importance of biological soil properties and the difficulty in measurements. The biological components in soil include bacteria, fungi, actinomycetes, protozoa, earthworms, mites, insects, nematodes, algae, and belowground plant parts. All these components take part in various soil functions and affect numerous soil properties (Larkin, 2015; Njira and Nabwami, 2013). The most widely used biological indicators of soil health include microbial biomass C and N, soil respiration, potentially mineralizable N (PMN), total organic carbon (TOC), particulate organic matter (POM), and earthworm population. Some other biological indicators used in limited extent are soil microbial community structure, soil protein, enzymatic activity, soil biodiversity,

and micro-and macro-fauna population (Larkin, 2015; Martinez-Salgado et al., 2010; Njira and Nabwami, 2013; Van Bruggen and Semenov, 2000; Vasconcellos et al., 2013).

Current soil health assessment methods

Several methods have been developed for assessing soil health with varying numbers of indicators and different indicator integration approaches. These methods include CASH (Moebius-Clune et al., 2016; Schindelbeck et al., 2008), ASHI (Bosarge, 2015; Mitchell et al., 2015), Haney's Soil Health Test (HSHT) (Haney et al., 2008), and Biological Quality of Soil (QBS) (Parisi et al., 2005). The CASH was developed by Cornell University in 2007 for assessing soil health in the New York region (Gugino et al., 2007; Moebius-Clune et al., 2016; Schindelbeck et al., 2008). The HSHT, developed by Haney et al. (2008), is exclusively a soil biology-based method, originally developed for the Texas region. The ASHI is developed by Auburn University (AU) by adapting SMAF and CASH frameworks in 2015 for the cropping conditions of Alabama (Bosarge, 2015; Mitchell et al., 2015).

Comprehensive Assessment of Soil Health (CASH)

The CASH is an integrative soil health assessment tool comprised of various physical, chemical, and biological soil properties as soil health indicators (Table 1.2). CASH was developed based on the three-step process outlined in the SMAF framework, which includes, (i) selection of soil health indicators and development of minimum dataset, (ii) measurement and interpretation of soil health indicators, and (iii) integration of soil health indicators into a soil health index (Andrews et al., 2004; Idowu et al., 2009). CASH indicators were selected from 39 soil properties (Gugino et al., 2009; Idowu et al., 2008). The scoring function used in CASH is adjusted for soil texture with the assumption that a significant portion of the variability can be explained by the study location with characteristic climate, and soil textural variation (Congreves

et al., 2014; Gugino and 2007). Using individual indicator scores, an unweighted average is derived to calculate an overall soil health score. Based on the soil health scores, farmers are given soil health management guidelines and suggestions (Moebius-Clune et al., 2016).

Although CASH is a useful method for integrating soil properties into an assessment approach to measure soil health, the integration process is based on an un-weighted averaging that does not account for the relationships among soil attributes (Congreves et al., 2015). As an effort to overcome this limitation of un-weighted averaging, a weighted score integration system has been proposed that is more sensitive than the CASH to tillage and crop rotation treatments (e.g., OSHA) (Congreves et al., 2015). Similar weighted scoring approaches were also used by many other studies to address the unweighted integration of soil health indicator scores into a final soil health score (Chahal and Van Eerd, 2019). The CASH, when applied to the long-term agronomic systems in North Carolina, showed a lack of sensitivity to management practices (Roper et al., 2017). So it appears that though CASH is intended as a standardized soil health assessment tool and worked well in Northeast and portions of Midwest US regions (Moebius-Clune et al., 2016), its cross-regional applicability is still questionable (Congreves et al., 2015; Roper et al., 2017). In addition, Fine et al. (2017) and Moebius-Clune et al. (2011) also reported that the CASH approach can be adapted to different regions but modifications in scoring functions, based on soil and climatic conditions, is required for more appropriate interpretations of indicators and overall CASH scores.

Alabama Soil Health Index (ASHI)

The ASHI method, specifically developed for the agricultural soils of Alabama, includes soil parameters from both routine and non-routine tests that are pertinent to soil health estimation (Mitchell and Huluka, 2012) (Table 1.3). The ASHI was introduced in 2015 based on data from

soil samples collected from Alabama (247 samples) and Georgia (47 samples) (Bosarge, 2015; Mitchell et al., 2015). This test is intended to be suitable for the soils of Alabama with the objective of making growers aware of the health status of their soils and cost-effective improvement strategies (Mitchell and Huluka, 2012). This index can be used to assess soil health in the same piece of land over a period of time to assess how production practices are changing the overall soil health. The first iteration of ASHI was developed on the basis of CASH and then a weight was assigned to each soil parameter based on the best judgment of several experienced extension and research scientists to generate a final soil health index, which ranged from 0 and 100. According to Bosarge (2015), the SHI of most Alabama soils ranges from 40 to 80. The ASHI's soil health score calculation includes higher weightage assignment (relative importance) to soil chemical indicators (50 out of 100) as compared to soil physical (10 out of 100) and biological indicators (40 out of 100) (Bosarge, 2015). Soil chemical indicators (e.g., pH, P, K, BS, and ECEC) can be easily altered by fertilizer and/or lime applications. However, these improvements in soil health that strongly dependent on soil fertility changes, may not reflect long-term management-induced changes in soil health.

Since this index is recently been developed, it has not been tested outside of Alabama. More research is needed to assess the applicability of ASHI beyond the state of Alabama. We are particularly interested in testing it for Tennessee agroecosystems because it was originated from another southeastern region.

Haney's Soil Health Test (HSHT)

The HSHT, developed by Haney et al. (2008), has emerged as a new approach in recent years by exclusively focusing on soil biological properties to quantify soil health (Strauss et al., 2015). In HSHT, the soil parameters included are 1-day CO₂-C emission, water-

extractable organic C (WEOC), and water-extractable organic N (WEON). The 1-day CO₂-C represents cumulative CO₂ emission measured using the Solvita[®] gel system after a 24-hr incubation of re-wetted dry soil. This method of soil respiration measurement showed a correlation with traditional methods of soil respiration measurements including the acid-base titration and the infrared gas analysis (Haney et al., 2008). The 1-day CO₂-C also showed a relationship with biologically active soil organic matter pools, soil microbial activity, and soil fertility (Haney and Haney, 2010). In addition, HSHT includes WEOC and WEON concentrations as proxies for bioavailable C and N. Since WEOC and WEON contents are closely associated with the soil microbial activity, HSHT approach considers WEOC/WEON ratio as a more sensitive indicator of soil microbial activity than the traditional total C:N ratio (Haney et al., 2012). Ultimately, the HSHT score is calculated from Solvita[®] 1-day CO₂-C, WEOC, and WEON (eq. 1).

$$\text{HSHT Score} = \frac{\text{1-day CO}_2\text{-C}}{\text{WEOC/WEON}} + \frac{\text{WEOC}}{100} + \frac{\text{WEON}}{10} \quad (1)$$

Depending on the soil type and management, the reported HSHT score varied from 0 to 50, and a score >7 is considered acceptable for many agricultural soils (Presley, 2016). A health score as high as 100 was reported recently from native grasslands systems (Haney et al., 2018). The major caveat of this method is that only biological parameters are used to calculate the overall soil health score, ignoring the importance of soil chemical and physical parameters for building a healthy soil (Haney, 2018). Measuring soil respiration immediately after re-wetting dried soil could also be problematic. Re-wetting can liberate a large amount of CO₂, causing over-estimation of microbial activity. Higher soil respiration or biological activity is not always a good indicator of healthy soil because it may indicate an unstable system (e.g., soil respiration increases after an intensive tillage operation). Accordingly, more release of CO₂ may indicate

loss of carbon pool from the soil that may be undesirable for soil health. According to Xiang et al. (2008), the wetting phase of drying-wetting cycles leads to the loss of extractable C as well as increased growth of biomass leading to enhanced mineralization of otherwise unavailable C and N. In addition, amount of water needed for moisture optimization varies with soil type. Clearly, soil respiration measurement needs more robust optimization of moisture content and temperature for CO₂-C evolution (Sullivan and Granatstein, 2015). Furthermore, HSHT scores calculated by different laboratories varied widely, warranting cross-laboratory validation (Sullivan and Granatstein, 2015). A study conducted by Morrow et al. (2016) found that 1-day CO₂-C and HSHT scores did not differentiate among long-term agricultural management treatments in Washington, Oregon, and Idaho. Another recent study conducted in North Carolina also showed that HSHT scores were not statistically different among long-term tillage treatments (Roper et al., 2017). Thus, it is imperative that extensive testing and/or modification of the HSHT indicators or algorithm across soils, cropping systems, managements, and geographic locations are required (Sullivan and Granatstein, 2015). Further, different published studies evaluating HSHT (Bavougian et al., 2019; Chu et al., 2019; Haney et al., 2018; Mitchell et al., 2017) and commercial laboratories that offer HSHT (e.g., Midwest Laboratories and Ward Laboratories) utilized many different modified forms of calculating HSHT soil health scores. This complicates comparative evaluation of current and past test results as well as monitoring soil health using HSHT (Bavougian et al., 2019).

Research gap and objectives

Most of the currently available approaches for soil health assessment were developed or standardized for specific regions, and they may or may not be suitable for evaluating different agroecosystems in other regions, particularly in the humid southeastern US. A comparative

evaluation of current assessment methods is required to determine their feasibility in other regions. Additionally, in order to ensure a realistic assessment of soil health for specific cropping systems and agroecological regions, it is essential to develop a robust and comprehensive method that takes into account realistic upper and lower bounds of soil health that is specific to regions. In this dissertation, I plan to compare the existing soil health assessment methods for the cropping systems of Tennessee. So far, only a very few studies have been conducted to assess the soil health changes from the adoption of conservation management practices in Tennessee. Those studies had tested either a single soil health assessment method on a single cropping system or individual soil properties (Chu et al., 2017; Chu et al., 2019; Mbuthia et al., 2015; Nouri et al., 2018; Nouri et al., 2019; Seybold et al., 2002). Thus, my dissertation research aims to, (i) evaluate the suitability of several commonly used assessment methods (HSHT, CASH, and ASHI) for a suite of agricultural practices and cropping systems in West Tennessee, and (ii) develop a Tennessee-centric approach for a soil health assessment to understand the effectiveness of agromanagement practices in the region.

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Appendix 1

Table 1.1 Selected soil health indicators, their relationship with soil functions, types of scoring curves, and the reasons for the relationship (modified from Andrews et al., 2004)

Indicators	Relation to soil function	Types of curves	Reasons
Total Organic Carbon (TOC)	More is better/ascending logistics	Upper asymptotic sigmoid curve	Role in soil fertility, water partitioning, and structural stability
Water Stable Aggregation (AGG)	More is better/ascending logistics	Upper asymptotic sigmoid curve	Role in soil fertility, water partitioning, and structural stability
Available Water Capacity (AWC)	More is better/ascending logistics	Upper asymptotic sigmoid curve	Role in crop productivity and other biological activity
Potential Mineralizable Nitrogen (PMN)	More is better/ascending logistics	Upper asymptotic sigmoid curve	Nutrient availability, microbial activity, and plant productivity
Microbial Biomass Carbon (MBC)	More is better/ascending logistics	Upper asymptotic sigmoid curve	Role as readily available C and N, and relation to improved soil structural functioning
Bulk Density (Db)	Less is better	Lower asymptote	Adverse effect of high Db on root growth and soil porosity
Soil pH	Midpoint optima	Gaussian function	Crop sensitivity and effects on nutrient availability
Electrical Conductivity (EC)	Midpoint optima	Gaussian function	Crop sensitivity and effects on nutrient availability
Sodium Adsorption Ratio (SAR)	Midpoint optima	Gaussian function	Potential for soil dispersion, environmental (water quality) risk, and associated EC levels
Phosphorus (P)	Midpoint optima	Gaussian function	Based on crop response and environmental risk

Table 1.2: Soil parameters used as indicators in CASH as listed in the standard package (adapted from Moebius-Clune et al., 2016)

Indicator types	Indicators
Physical	Soil Texture Available Water Capacity (AWC) Surface and Subsurface Hardness Wet Aggregate Stability (WAS)
Biological	Organic Matter (OM) Soil Protein Index (SPI) Soil Respiration Active Carbon
Chemical	Soil pH Extractable Phosphorus (P) Extractable Potassium (K) Minor Elements (Mg, Fe, Mn, Zn)

Table 1.3: Soil parameters used in Alabama soil health index (adapted from Mitchell et al., 2015).

Indicator type	Parameters
Physical	Wet Aggregate Stability (WAS)
Biological	Soil OM (SOM) Potential mineralized N (PMN) Solvita [®] based Soil respiration (1d CO ₂)
Chemical	Soil estimated cation exchange capacity (ECEC) Soil pH Mehlich-1 extractable P Mehlich-1 extractable K Base saturation

Chapter 2. Agroecosystem management responses to Haney soil health test in the southeastern United States

Abstract

Quantifying the impacts of agricultural managements on soil health is critical for making informed sustainable management decisions as soil resources inevitably undergoes alterations due to managements. One recently popular and soil biology-based method of soil health assessment is the Haney Soil Health Test (HSHT), whose most recent version is known as the *soil health tool*, attempts to integrate soil health and fertility. A comprehensive evaluation of HSHT and its underlying indicators in different agroecological regions is currently lacking. This study, therefore, evaluates the HSHT on three ongoing field experiments in the southeast US, (i) 39-year of continuous-soybean (*Glycine max* L.) with different tillage treatments, (ii) 4-year of corn (*Zea mays* L.)-soybean rotation with different cover crop treatments, and (iii) 37-year of continuous-cotton (*Gossypium hirsutum* L.) with tillage, cover crops, and nitrogen (N) rates. Soil samples (0-15 cm depth) were analyzed for HSHT indicators (i.e., Solvita CO₂-C, water-extractable organic C (WEOC), water-extractable organic N (WEON), and WEOC:WEON), two versions of soil health score (SHS) calculations (SHS2015 and SHS2018), and potential N mineralization rates (N_{min}). Additionally, H3A- and Mehlich-1-based extractable soil nutrients were determined to test the fertility component of HSHT. The individual HSHT indicators, SHS2015, SHS2018, and N_{min} showed inconsistent responses to managements, where most variation in SHS was driven by WEON ($0.68 < R^2 > 0.86$; $p < 0.001$). Additionally, H3A solution extracted nutrients with high variability ($18.5 < CV\% > 76$) than Mehlich-1. This lack of consistent response management in southeastern US croplands implies that comprehensive evaluation and/or modification of HSHT is required for broader applicability.

Introduction

As a dynamic ecosystem, soil supports agricultural productivity and ecosystem functions (Doran and Parkin, 1996), but intensive monoculture cropping has often led to soil degradation (Zornoza et al., 2015). Given the current need for producing more food to feed a growing population under changing climatic conditions, extensive research is focused on building sustainable production systems by improving soil health (Janzen, 2006; Powlson et al., 2011). Soil health reflects the “continued capacity of soil to function as a vital living system, within the ecosystem and land-use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal, and human health” (Doran and Safley, 1997), thus recognizing both the physico-chemical and biological components of soil that are key to ecosystem functions (Doran and Zeiss, 2000; Karlen et al., 2003; Andrews et al., 2004).

The interaction of intrinsic soil properties, climate, and soil management dictates the health of a given soil at any particular time. This health can be reflected in the soil physical, chemical, and biological properties that undergo inevitable changes due to agricultural management impacts (Giller et al., 1997). In some cases, the changes in soil properties that are beneficial for agricultural production can be detrimental to other ecosystem services (Powlson et al., 2011). However, agricultural practices that can build and maintain healthy soils are expected to increase productivity without an associated cost to environmental quality. It is thus essential to quantify the impacts of agricultural practices on soil health in order to make informed sustainable management decisions (Karlen, 1999; Lewandowski et al., 1999; Schoenholtz et al., 2000; Wienhold et al., 2004).

Soil health measurement is not straightforward due to the multiple soil physical, chemical, and biological properties contributing to soil health, and their dynamic interactions

(Arias et al., 2005; Van Eerd et al., 2014). Several soil health assessment approaches exist, such as the Soil Management Assessment Framework (SMAF) (Andrews et al., 2004), the Comprehensive Assessment of Soil Health (CASH) (Gugino et al., 2007; Fine et al., 2017), the Haney Soil Health Test (HSHT) (Haney et al., 2008a), and more recently the *Soil Health Tool* (Haney et al., 2018). Different sets of physical, chemical, and biological properties are used as soil health indicators in these various approaches (Cardoso et al., 2013). The earlier approaches emphasized soil physical and chemical indicators, but soil biological indicators have been given more importance in recent years (Strauss et al., 2015).

The HSHT was originally developed by Haney et al. (2008a), and focused primarily on testing soil biological activity for a soil health assessment (Strauss et al., 2015). Accordingly, an overall soil health score (hereafter designated as SHS2015) was calculated, which integrates 1-day CO₂-C production, water-extractable organic C (WEOC), and water-extractable organic N (WEON) following equation 1 (Haney, 2015):

$$SHS2015 = \frac{\text{Solvita CO}_2 - \text{C}}{\text{WEOC: WEON}} + \frac{\text{WEOC}}{100} + \frac{\text{WEON}}{10} \quad (1)$$

The Solvita CO₂-C represents cumulative CO₂ production measured using a Solvita[®] gel system after a 24-hr incubation of re-wetted air-dried soil. This method of soil respiration measurement showed a positive correlation with traditional methods of soil respiration measurement, including the acid-base titration and the infrared gas analysis (Haney et al., 2008a; McGowen et al., 2018). The Solvita CO₂-C also showed a positive relationship with biologically active soil organic matter pools, soil microbial respiration, and soil fertility (Haney and Haney, 2010). Solvita CO₂-C is also considered to predict plant N availability (Franzluebbers, 2018; Franzluebbers and Haney, 2018; Franzluebbers et al., 2018) and inorganic C (Rogers et al., 2019), but high inter-laboratory variability raised concerns about the result reproducibility (Sullivan and Granatstein,

2015), which is vital for a robust soil health assessment (Wade et al., 2018). Since WEOC and WEON contents represent the bioavailable portions of total C and N, respectively, the HSHT considers the WEOC:WEON ratio as a more sensitive indicator of soil microbial activity than the traditional total C:N ratio (Haney et al., 2012). The HSHT includes a new extractant termed H3A (Haney, Haney, Hossner, and Arnold), which was designed to mimic the chemical composition of plant root exudates (Haney et al., 2006; 2010; 2017). This extractant is a combination of three organic acids (citric acid, oxalic acid, and acetic acid), and is used to extract inorganic N (ammonium-N and nitrate-N) and other nutrients from the soil. The H3A extracted inorganic N is also used to determine WEON by subtracting H3A extracted inorganic N from the water-extractable total N.

The SHS2015 was intended to serve as a benchmark for monitoring management strategy effectiveness. Depending on soil type and management, the SHS2015 generally varies from 0 to 50, and a score >7 is considered ‘good’ for many agricultural soils (Presley, 2016) but under pastures or native grasslands, scores could be as high as 100 (Haney et al., 2018). A new version of HSHT, widely termed the *soil health tool*, has been developed recently by combining the soil health and fertility components (Haney et al., 2018). The goal behind the *soil health tool* was still to address limitations of other widely adopted soil health assessment approaches (e.g., SMAF and CASH) and soil fertility tests (e.g., using Mehlich-1 and Mehlich-3 extractant). Accordingly, a revised equation was proposed to calculate the soil health score (SHS2018) following equation 2:

$$SHS2018 = \frac{\text{Solvita CO}_2 - C}{10} \times \frac{WEOC}{100} \times \frac{WEON}{10} \quad (2)$$

Hereafter, HSHT will refer to the general approach, while SHS2015 and SHS2018 will refer to the specific soil health scores calculated by equations (1) and (2). These different versions of the

soil health score calculation have been used in different published studies. For example, SHS2015 was used in Chu et al. (2019), Mitchell et al. (2017), and Roper et al. (2017), while SHS2018 was used in Bavougian et al. (2019). Additionally, different commercial laboratories that offer HSHT also use different versions of the equations: Midwest Laboratories uses equation 1 (Midwest Laboratories, 2016) and Ward Laboratories uses a completely different version, equation 3 (Ward Laboratories, 2019).

$$SHS = \frac{\text{Solvita CO}_2 - C}{10} + \frac{WEOC}{50} + \frac{WEON}{10} \quad (3)$$

As is the case with all soil health models, the applicability of HSHT can be limited by indicator and site-specificity. The HSHT was first developed for the agricultural soils of Texas, with limited calibration and validation across the other soils/regions (Kusi, 2019; Strauss et al., 2015). The use of HSHT in assessing soil health in other regions, therefore, provided inconsistent results. Some studies (e.g., Harmel and Haney, 2013; Mitchell et al., 2017; Culman et al., 2013) showed that HSHT scores responded to management changes, whereas other studies (e.g., Chu et al., 2019; Roper et al., 2017; Chahal and Van Eerd, 2018; Bavougian et al., 2019) showed that the scores were not sensitive to management changes. Because of these inconsistencies and the limited number of published studies, the effectiveness of HSHT in reflecting management-driven changes in soil health in different regions is unclear. The present study was therefore conducted with the following specific objectives: (i) to determine the sensitivity of SHS2015 and SHS2018 in reflecting soil health changes measured on several short- and long-term soil management practices in croplands of Tennessee, (ii) to evaluate the potential applicability of HSHT for estimating N mineralization rates, and (iii) to understand the efficiency of H3A compared to Mehlich-1 solution for extracting soil nutrients.

Materials and methods

Study sites and treatments

This study was conducted on three ongoing agricultural experiments located in West Tennessee on silt loam soils. The 30-year mean annual temperature of the region is 15.6 °C and the mean annual rainfall is 1375 mm. Detailed descriptions of the field experiment treatments are shown in Table 2.1. All the three field experiments include four replications of each treatment.

Agronomic managements

Continuous soybean (SS) study

This study was started in 1979 (39-year old at the time of soil sampling) and each experimental unit was 18 m by 6 m with four rows of soybean. In tilled treatments (moldboard [MP], disc [DP], and chisel plow [CP]), tillage and harrowing operations were generally performed in late May before soybean planting (Table 2.1). In the no-till with wheat cover crop (NTW) system, a wheat cover crop was seeded after harvesting soybean grain in October-November, and the cover crop was chemically terminated using 0.71 kg ha⁻¹ paraquat (1-methyl-4-(1-methylpyridin-1-ium-4-yl) pyridin-1-ium) two to three weeks prior to planting soybeans. In all the plots, soybeans were planted in late May of each year but in the no-till wheat-soybean double crop (NTWD) system, wheat was grown for grain from mid-October to mid-June, followed by soybean each year. All plots were treated with 3.36 kg ha⁻¹ alachlor (2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide) and 0.42 kg ha⁻¹ metribuzin (4-Amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one) for pre-emergence weed suppression and 0.13 kg ha⁻¹ clethodim (2-[1-[[[(E)-3-chloroprop-2-enoxy] amino] propylidene]-5-(2-ethylsulfanylpropyl) cyclohexane-1,3-dione) for post-emergence weed control.

Corn-soybean no-till (CS) study

This study was started in 2013 (4-year old at the time of soil sampling) and each experimental unit was 9 m by 4 m with five crop rows. A no-till planter was used to plant corn and soybean in April-May. Winter cover crops were seeded using a 1.83 m Tye drill in October-November after the harvest of corn or soybean and chemically terminated in April-May. All fertilizers were applied based on the University of Tennessee's (UT) recommendations for no-till corn and soybean. Cover crop seeding rates were based on the recommendations of United States Department of Agriculture-Natural Resources Conservation Service (USDA-NRCS) for Tennessee, which were 84 kg ha⁻¹ for wheat (W), 84 kg ha⁻¹ for cereal rye (CR), 26 kg ha⁻¹ CR and 24 kg ha⁻¹ hairy vetch (HV) for the CR/HV treatment, and a mixture of 17 kg ha⁻¹ CR, 7 kg ha⁻¹ HV, 2.3 kg ha⁻¹ Daikon Radish (DR), 4.5 kg ha⁻¹ crimson clover (CRM) and 23 kg ha⁻¹ Oats (O) for the soil health mix (SHM) treatment (Table 2.1). Prior to row-crop planting, Gramoxone[®] SL 2.0 (N, N'-dimethyl-4, 4'-bipyridinium dichloride) was applied to terminate cover crops and weeds. Roundup PowerMax[®] (Glyphosate) was used as a post-emergence herbicide to control weeds during the row-crop growing season.

Continuous cotton (CC) study

This study was started in 1981 (37-year old at the time of soil sampling) and each experimental unit was 12 m by 8 m with eight rows of cotton. Following UT recommendation, P fertilizer was applied as triple superphosphate at 101 kg P₂O₅ ha⁻¹ and K fertilizer was applied as muriate of potash (KCl) at 134 kg K₂O ha⁻¹. The main plot treatment was N rates, the source of which was ammonium nitrate (NH₄NO₃), applied a week after planting at 0 or 67 kg ha⁻¹ based on treatments (Table 2.1). The sub-plots are three cover crop treatments (winter wheat, hairy vetch, and no cover crop) and the cover crops were chemically terminated in late April to early

May of each year using 3.51 L ha⁻¹ of Gramoxone® SL. The sub-sub plots are conventional tillage and no-till treatments. The conventional tillage was done by disking twice to 10 cm deep using a standard disc harrow, followed by roller harrow leveling to smooth the land before planting in May.

Soil sampling and processing

In July 2017, soil samples were collected from each of the four replicated experimental plots for all treatment combinations in the three field experiments. Six random cores were collected from the inter-row area from 0-15 cm depth using a bucket-type auger of 5 cm diameter. Samples from each plot were combined and homogenized to make a composite soil sample. The composite samples were air-dried and passed through a 2 mm sieve for the measurement of Solvita® based CO₂-C, WEOC, WEON, and H3A as well as Mehlich-1 extractable soil nutrients.

Laboratory analyses

Solvita CO₂-C measurement

The flush of CO₂ released in 24 h after re-wetting the air-dried soil was measured using a pH-sensitive Solvita® gel paddle and a diode array detection-based digital color reader as described by Haney et al. (2008a). Briefly, 40 g of air-dried soil (sieved to <2 mm) was wetted to reach field capacity through capillary action and incubated in a closed lid jar containing a Solvita® gel paddle for 24 h at 25 °C. After incubation, the paddle was removed and the CO₂ reading was recorded using the digital color reader (Haney et al., 2008a).

Extractable organic C and nutrients

The WEOC and WEON were measured as described in Haney et al. (2008a). Briefly, a 4 g sample of air-dried soil was mixed with 40 mL deionized (DI) water and shaken for 10 minutes.

After shaking, the solution was centrifuged and filtered using Whatman® No. 42 filter paper, and the extracts were analyzed for total C and N using the CN analyzer (Elementar Vario TOC cube in liquid mode, Hanau, Germany). Inorganic N was extracted by mixing a 4 g sample of air-dried soil with 40 mL of H3A extractant followed by shaking for 10 minutes, centrifuging, and filtering. The extracts were analyzed for ammonium-N (NH₄-N) and nitrate-N (NO₃-N) using a Continuous Flow Analyzer (FIA-Skalar, Breda, Netherlands). Water extractable organic N (WEON) was calculated by subtracting inorganic N (NH₄-N and NO₃-N) from water-extractable total N. Other soil nutrients including P, K, Mg, Fe, Mn, Na, Ca, and Al were determined from H3A extracts and Mehlich-1 extracts (1:4 soil to solution ratio) using inductively coupled plasma-optical emission spectrometry (ICP-OES) (Haney et al., 2010; Sikora and Moore, 2014).

Calculations and statistical analyses

Haney soil health score and potential N mineralization calculations

The Solvita CO₂-C, WEOC, WEON, and WEOC:WEON data described above were used to calculate the HSHT scores using both the SHS2015 and SHS2018 versions of the HSHT. The N mineralization (Nmin) rate, based on HSHT, was calculated using equation 5 (Haney et al., 2018)

$$N_{min} \text{ (kg/ha)} = MAC \times WEON \times 2.24 \times n \quad (5)$$

where MAC (microbially active C) is the amount of Solvita CO₂-C in relation to WEOC (MAC = Solvita CO₂-C/WEOC), 2.24 is the factor to convert Nmin from mg kg⁻¹ to kg ha⁻¹, and n is the number of rainfall/irrigation events (>2.5 cm) during the growing period.

Statistical analysis

For Solvita CO₂-C, WEOC, WEON, WEOC:WEON, SHS2015, SHS2018, as well as H3A and Mehlich-1 extractable soil nutrients, an analysis of variance (ANOVA) was conducted using the PROC GLIMMIX procedure in SAS v9.4 statistical package (SAS, 2012). Separate ANOVA were conducted for each field experiment based on Completely Randomized Design, Randomized Complete Block Design (RCBD), and RCBD with split-split arrangement for SS, CS, and CC experiments, respectively. Least square means were separated using the Least Significant Difference (LSD) at $p \leq 0.05$. The least square means of SHS2015 and SHS2018 were also compared within each treatment using the LSD values at $p \leq 0.05$. Separate simple linear regression analyses of Solvita CO₂-C, WEOC, WEON, and WEOC:WEON against SHS2015 and SHS2018 were also conducted using PROC REG in SAS v9.4. Coefficients of variation (CV) for extractable nutrients was calculated separately for the H3A and Mehlich-1 extraction methods to compare the effect of extractants on nutrient concentrations across the treatments in each study. The PROC CORR procedure in SAS was used to conduct canonical correlation analyses and the strengths of covariance between H3A and Mehlich-1 extractable nutrients were expressed as Pearson's correlation coefficients (R).

Results

Haney soil health test (HSHT) scores (SHS2015 and SHS2018)

In the SS study, both SHS2015 and SHS2018 showed only MP as differentiating itself from the other treatments (Fig. 2.1). Scores based on both SHS2015 and SHS2018 were significantly lower under MP, while the scores for the other treatments were similar. Overall, SHS2018 showed greater soil health scores (25.1 to 92.0) compared to SHS2015 (11.8 to 27.9). For the individual HSHT parameters in the SS study, WEON and WEOC:WEON showed a

significantly lower value for MP while all other treatments were similar (Table 2.2). The WEOC showed no treatment differences. Solvita CO₂-C results displayed an interesting mix of differences, with NTW being significantly higher than MP, NT, and NTWD, but not different from CP or DP. The values under MP were significantly lower than NTW and DP, but were not significantly different from CP, NT, or NTWD.

In the CS study, soil health scores by both SHS2015 and SHS2018 methods differed for only one of the cover crop treatments (Fig. 2.2). In both cases, CR/HV yielded higher scores than SHM, W, and CTRL. For SHS2015, CR was not significantly different from any of the other treatments, while for SHS2018, CR/HV was significantly higher than CR as well. As for the SS study, SHS2018 scores were generally higher than the SHS2015 scores. As shown in Table 2.3, for the CS study, the WEOC, WEON, and WEOC:WEON did not vary across the cover crop treatments, but Solvita CO₂-C was significantly lower for the SHM (116 mg kg⁻¹) and higher for the (CR/HV) (212 mg kg⁻¹), but these treatments did not differ significantly from any of the other treatments. The soil health scores based on SHS2015 were higher for a combination of NT and 67 kg N ha⁻¹ treatment (47.2) compared to any other treatment (Fig. 2.3). Also, NT combined with no N application yielded a SHS2015 score significantly lower (25.2) than the combination of CT and 67 kg N ha⁻¹ (37.9). The SHS2018 scores showed no significant treatment differences. As with the other studies, the SHS2018 scores were higher (54 to 91 across the treatments) than the SHS2015 scores (25.2 to 47.2 across the treatments).

In examining the HSHT parameter values for the CC study (Table 2.4), there were no three-way interactions observed among the treatments, but the two-way interaction of N rates and tillage was significant ($p \leq 0.05$). Solvita CO₂-C and WEOC did not vary among the treatments. However, WEON concentration was the highest with NT plots supplied with 67 kg N

ha⁻¹ in comparison with all other tillage and N rate combinations. Among the cover crop treatments, WEON under W was 26.6 kg ha⁻¹, which was 26 % lower than no cover control (NC) (Table 2.4).

Separate regression analyses of the individual HSH parameters (Solvita CO₂-C, WEOC, WEON, WEOC:WEON) with SHS2015 and SHS2018 revealed that SHS2015 and SHS2018 were both related to WEON and WEOC:WEON ($0.43 < R^2 < 0.86$, $p < 0.001$) (Table 2.5). In addition, SHS2018 showed a weak relationship to Solvita CO₂-C ($R^2 = 0.16$, $p < 0.01$).

Potential N mineralization rates

In the SS study, Nmin for MP was 75.9 kg ha⁻¹, which was significantly lower than all the other treatments with means ranging from 152 kg ha⁻¹ to 205 kg ha⁻¹ (Table 2.6). The Nmin did not show differences among cover crop treatments in the CS study, with the mean ranging from 52.2 to 86.0 kg ha⁻¹. In the CC study, Nmin did not differ within tillage (254 to 281 kg ha⁻¹) or cover crop treatments (225 to 290 kg ha⁻¹) but varied between N rate treatments. The Nmin was 334 kg ha⁻¹ with 67 kg ha⁻¹ N application, which was 66.2 % higher compared to no N application. Interactions among the three treatments in this study were also not significant.

H3A and Mehlich-1 extractable soil nutrients

In general, the H3A solution extracted lower amounts of nutrients (P, K, Mg, Fe, Mn, Na, Ca, and Al) compared to the Mehlich-1 solution (Tables 2.7, 2.8, and 2.9). In the SS study, nutrients extracted by H3A and Mehlich-1 did not vary among treatments, except for Mehlich-1 extracted K and Fe (Table 2.7). Mehlich-1 extracted K was significantly higher under MP (127 mg kg⁻¹) and significantly lower under NTW (74.6 mg kg⁻¹). Mehlich-1 extractable Fe was significantly higher under MP (53.9 mg kg⁻¹) compared to all other treatments, which were similar to each other (17.4 to 24.5 mg kg⁻¹). However, in this study, H3A and Mehlich-1

extractable P, K, Fe, Mn, and Al were found to be strongly correlated ($0.59 < R > 0.73$; $p < 0.0001$) (Table 2.10).

In the CS study, H3A and Mehlich-1 extractable nutrients did not vary among cover crop treatments (Table 2.8) and there were no significant correlations between H3A and Mehlich-1 extractable nutrients (Table 2.10).

In the CC study, there was no three-way interaction for H3A nor Mehlich-1 extracted nutrients but there was a N x tillage interaction, which showed no differences except H3A for K and Fe (Table 2.9). Extractable K was significantly lower under NT with 67 kg ha⁻¹ N application (67-NT) (56.1 mg kg⁻¹) compared to other tillage and N rate combinations (0-CT, 0-NT, 67-CT) (86.4 mg kg⁻¹ to 106 mg kg⁻¹). Similarly, extractable Fe was also lower under 67-NT (28.3 mg kg⁻¹). Mehlich-1 extractable P and Ca responded significantly to cover crop treatments, and Fe responded to tillage treatments. Extractable P was 26.8 % higher under wheat cover crop than hairy vetch (85.2 mg kg⁻¹), whereas Ca was higher for hairy vetch (1780 mg kg⁻¹) compared to wheat (1440 mg kg⁻¹) and no cover (1460 mg kg⁻¹). All H3A extracted nutrients showed considerably high CV compared to Mehlich-1 extracted nutrients except Fe (Table 2.10). However, most H3A extractable nutrients showed significant positive correlations with Mehlich-1 extractable nutrients ($0.36 < R > 0.70$; $p < 0.05$) except Ca and Al, as Ca showed no correlation and Al showed a negative correlation ($R = -0.37$; $p < 0.05$).

Discussion

Haney soil health test scores (SHS2015 and SHS2018)

Both the original (SHS2015) and modified (SHS2018) equations of Haney soil health scoring responded differently to treatments. Based on SHS2015, a score of 7 or higher indicates “good” soil health (Presley, 2016), but SHS2018 does not rank soil’s health based on the overall

score (Bavougian et al., 2019) and emphasizes monitoring soil properties over a period to assess farm management effects on soil health (Haney et al., 2018). In our study, all the SHS2015 scores were >7 , indicating “good” soil health even after 39 years of intensive tillage (e.g., MP and DP) (Figs. 2.1, 2.2, and 2.3). The lack of significant differences in SHS among the long-term management treatments is inconsistent with well-established conservation management principles, which suggest that long-term adoption of NT and other conservation management practices (e.g., cover crops) improve soil health (Karlen et al., 2003; Kibblewhite et al., 2007; Mbuthia et al., 2015; Chalise et al., 2019; Maiga et al., 2019; Tobin et al., 2020). Soil health scores based on SHS2018 were higher than SHS2015 for all the treatments, however, SHS2018 also showed no obvious differences across diverse management practices similar to SHS2015. The numerical differences in SHS between SHS2015 and SHS2018 are problematic as this test, similar to other soil health tests, is intended for monitoring soil health changes from conservation management practices in cropping systems over the years. Since the duration of conservation management is a major factor in realizing soil health benefits (Daigh et al., 2018; Diaz-Zorita and Grove, 2002; Nouri et al., 2020), lack of responses in the CS study is attributed to its shorter duration (4 years) compared to CC and SS studies (37 and 39-year-old, respectively). Our results from the CS study are in agreement with previous findings from the same experiment that showed that SHS2015 scores did not differ among 4 years of cover crop treatments (Chu et al., 2019). Non-responsiveness of SHS2015 on cropland management practices were also reported by Roper et al. (2017) from North Carolina, Mitchell et al. (2017) from California, Bavougian et al. (2019) from Nebraska, and Chahal and Van Eerd (2018) from Canada.

Our regression analyses revealed strong positive relationships of SHS2015 and SHS2018 with WEON and WEOC:WEON (Table 2.5). However, WEOC was not significantly related to

soil health scores, and it did not vary among treatments in any of the field experiments. This exclusive linkage of soil health with soil N is problematic because it could lead to a false notion that soil N level primarily dictates soil health, while in reality soil nutrient status is just one component of soil health (Bhowmik et al., 2019; Cardoso et al., 2013; Delgado and Follett, 2002; Lal, 2016) and overapplication of N fertilizers has adverse environmental effects. Similar to our findings, Bavougian et al. (2019) also showed a strong influence of N rates on N-related HSHT parameters (WEON and WEOC:WEON).

Although Solvita CO₂-C was reported as the main driver of HSHT (Bavougian et al., 2019), our study it did not show a strong relationship to SHS (Table 5) or sensitivity to tillage, cover crop, or N rate treatments (Tables 2, 3, and 4). This lack of strong response implies that Solvita CO₂-C is probably not a reliable indicator to assess soil health across regions, or it could be simply due to high measurement variability of Solvita CO₂-C across labs (Kravchenko and Robertson, 2011; Morrow et al., 2016; Roper et al., 2017; Chu et al., 2019; Sullivan and Granatstein, 2015). Other studies also found inconsistent Solvita CO₂-C response to cropland management differences (e.g., Roper et al., 2017; Chahal and Van Eerd, 2018; Chu et al., 2019). Since Solvita CO₂-C is a measure of microbial respiration, it may lead to meaningful HSHT scores under those management practices that strongly influence soil respiration, such as different tillage treatments in SS study. On the other hand, WEON can strongly influence HSHT scores when N levels in the soil change profoundly, such as the N rate treatments in CC study. Overall, HSHT is conceptually and logistically an attractive approach but it lacked management-sensitivity when applied to cropping systems in Tennessee. This could also be due to the way the indicators were integrated into a soil health score. Through principal component analysis, Chu et al. (2017) showed that indicators used in the HSHT test better explained the variance in soil

properties compared to the overall HSHT score. We believe that the consideration of soil biological indicators alone and the unweighted approach to integrate indicators into soil health score are the two major issues limiting the sensitivity of HSHT test across the regions and cropping systems. Including representative indicators of soil physical and chemical conditions, and a weighted approach to integrate indicators into soil health score may improve the broader applicability of HSHT (Chahal and Van Eerd, 2019; Congreves et., 2015).

Potential N mineralization rates

The potential N mineralization (Nmin) rates intend to estimate plant-available N and predict N fertilizer recommendations (Haney et al., 2018) based on its correlation with C mineralization. For example, 100 mg kg⁻¹ WEOC and 25 mg kg⁻¹ Solvita CO₂-C indicate that 25 % of active C pool in the WEOC fraction is mineralized in one day. Since these are coupled microbial processes, it is assumed that 25 % of WEON will also be mineralized (Franzluebbers et al., 1996; Haney et al., 2001; Haney et al., 2008b; Haney et al., 2012). In the SS study, the response of Nmin to tillage treatments was identical to the response of WEON, while in the CC study, Nmin only responded to N rate treatment (Table 2.6). However, Nmin did not vary among treatments in the CS study, which may be due to its shorter duration (4 years). The lack of strong and consistent response of Nmin could also be attributed to its estimation from biological indicators in HSHT (Solvita CO₂-C, WEOC, and WEON), which also showed a generally poor response to management differences (Tables 2.2, 2.3, and 2.4). Other studies also reported a lack of strong response of Nmin estimated from HSHT parameters to agromanagement practices, including Castro Bustamante and Hartz (2016), Dick and Culman (2016), Tu (2016), and Moore et al. (2019). In addition, some studies discussed the limited applicability of using Nmin in predicting crop N fertilizer requirement due to the requirement of advanced yield estimation,

high measurement variability of HSHT indicators, and region-specific calibration and validation of such predictions (Stahl et al., 2016; Norris et al., 2018).

Extractable soil nutrients

The mass of nutrients extracted by H3A was considerably lower than that extracted by Mehlich-1 (Tables 2.7, 2.8, and 2.9). Since H3A is a mixture of three weak organic acids (oxalic, acetic, and citric acids), the extraction strength is lower than Mehlich-1 prepared from two strong acids (sulfuric and hydrochloric acids). Other studies also reported lower extraction efficiency of H3A than Mehlich-1 (Chu et al., 2019) and Mehlich-3 (Dari et al., 2019; Rogers et al., 2019a; Rogers et al., 2019b).

When treatment responses were analyzed, we found that only a few nutrients showed response to managements based on H3A and Mehlich-1 extractions. The H3A and Mehlich-1 extractable K and Fe were the most responsive to managements (Tables 2.7 and 2.9). For example, Mehlich-1 extractable K and Fe responded to tillage treatments in the SS system (Table 2.7) and H3A extractable K and Fe responses to the tillage and N rates interactions in the CC system (Table 2.9). However, other studies mainly reported H3A extractable P response to tillage and N rate treatments, with no other nutrient response to treatments (Karlen et al., 2013; Kaiser et al., 2016; Bavougian et al., 2019; Roper et al., 2017). Despite a few random responses of H3A and Mehlich-1 extractable nutrients to managements, in this study Mehlich-1 showed notably lower measurement variability than H3A (Table 2.10). Regardless, the content of nutrients extracted by both H3A and Mehlich-1 solutions were in accordance with other studies conducted in the region (Zhou et al., 2014; Chu et al., 2019; Singh et al., 2019; Singh et al., 2019; Bansal et al., 2020).

Mehlich-1 and Mehlich-3 solutions are commonly used for extracting nutrients from the soil, based on which fertilizer recommendations are calculated (Savoy, 2013). In Tennessee, where the current study was conducted, Mehlich-1 is the recommended extractant for nutrient analysis (Savoy and Joines, 2009; Singh et al., 2019; Bansal et al., 2020). Switching to H3A in place of Mehlich-1 and Mehlich-3 to comply with HSHT will require extensive calibration and validation research in the region. Additionally, high measurement variability could also contribute to inconsistent fertilizer recommendations within a region, further limiting the wider applicability of H3A at present.

Conclusions

To our knowledge, this is the first comprehensive evaluation of HSHT on multiple long-term cropping systems in the southeastern US. In this study, we evaluated the sensitivity of two versions of soil health score calculations in HSHT (SHS2015 and SHS2018) in response to agricultural management practices and compared the extraction efficiency of the recommended HSHT reagent (H3A) with that of the commonly used Mehlich-1 for nutrient analysis. The soil health scores calculated by SHS2015 and SHS2018 and the underlying parameters used to calculate the scores (Solvita CO₂-C, WEOC, WEON, WEOC:WEON) showed inconsistent responses to long-term N rates, tillage, and cover crops treatments under different row cropping systems. The results revealed that most, if not all, variation in soil health measured in HSHT was driven by N-related parameters (WEON and WEOC:WEON), which reflected an exclusive linkage of HSHT-estimated soil health with soil N status. When extractants were compared, H3A extracted considerably lower quantities of soil nutrients with notably higher variability than Mehlich-1, which suggests limited applicability of H3A in southeastern cropping systems.

Overall, the HSHT was not found to consistently capture long-term management-induced changes in soil health from these tested southeastern agroecosystems, despite several previous findings from these long-term experiments clearly indicating improved soil properties (e.g., soil organic C, bulk density, water holding capacity, active C fractions, enzymatic activity, and aggregation) under conservation management practices (e.g., Mbutia et al., 2015; Nouri et al., 2018; Nouri et al., 2019; Singh et al., 2020). Though the HSHT may be conceptually attractive because it strives to integrate soil biology and soil fertility into a soil health metric, it is imperative that researchers carry out extensive testing and/or modification of the soil health parameters or algorithms used to integrate those parameters into overall soil health score across soils, cropping systems, managements, and geographic locations in order to ensure these tools are useful and applicable.

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Appendix 2

Table 2.1. Description of study sites, cropping systems, and treatments.

Field Experiments	Location	Soil type and slope	Study design and replications	Treatments
Continuous soybean (SS) 1979 - current	University of Tennessee's West Tennessee Research and Education Center (WTREC) at Jackson, TN. (35°37' N, 88°50' W; elevation 125 m)	Lexington series (fine-silty, mixed, thermic, Ultic Hapludalf); 0 to 2 % slope	Completely Randomized Design; four replications	(i) Moldboard plowing to 25 cm depth + disking + roller harrowing (MP) (ii) Chisel plowing to 20 cm soil depth + roller harrowing (CP) (iii) Disc plowing to 10 cm depth + roller harrowing (DP) (iv) No tillage (NT) (v) NT with winter wheat (<i>Triticum aestivum</i> L.) cover crop (NTW) (vi) NT with wheat-soybean double crop (NTWD)
Corn-soybean (CS) 2013 - current	University of Tennessee's Research and Education Center at Milan (RECM), TN. (35°54' N, 88°44' W; elevation 129 m)	Loring series (fine-silty, mixed, thermic Oxyaquic Fragiudalf); 0 to 2 % slope	Randomized Complete Block Design (RCBD); four replications	No-till seeding of corn and soybeans in alternate years (i) Single species cover crop • Cereal rye (<i>Secale cereale</i> L.) (CR) • Winter wheat (W) (ii) Double species cover crop • CR + Hairy vetch (<i>Vicia villosa</i> L.) (HV) (iii) Soil health mixture (SHM) • CR + HV + Daikon radish (<i>Raphanus sativus</i> L. var. <i>Longipinnatus</i>) (DR) + Crimson clover (<i>Trifolium incarnatum</i> L.) (CRM) + Oats (<i>Avena sativa</i> L.) (O) (iv) No cover crop (CTRL)
Continuous cotton (CC) 1981 - current	WTREC at Jackson, TN. (35°37' N, 88°50' W; elevation 125 m)	Lexington series (fine-silty, mixed, thermic, Ultic Hapludalf); 0 to 2 % slope.	RCBD with split-split plot design; four replications	(i) N treatments (main-plots) • 0 kg N ha ⁻¹ (0N) • 67 kg N ha ⁻¹ (67N) (ii) Cover crop treatments (sub-plots) • No cover crop (NC) • HV • W (iii) Tillage treatments (sub-sub plots) • Conventional tillage to 10 cm depth by disking twice + harrowing (CT) • NT

Table 2.2. Tillage management effect on individual parameters of the Haney soil health test in the long-term continuous soybean (SS) study.

Treatments	Solvita CO ₂ -C	WEOC	WEON	WEOC:WEON
	-----mg kg ⁻¹ -----			
MP	93.7 (5.12)c	173 (3.34)a	16.0 (3.08)b	12.1 (2.32)a
CP	122 (5.72)abc	183 (3.75)a	25.8 (1.56)a	7.18 (0.44)b
DP	127 (12.0)ab	184 (7.47)a	27.0 (2.48)a	6.96 (0.48)b
NT	116 (18.4)bc	176 (19.0)a	25.6 (2.84)a	6.88 (0.12)b
NTWD	116 (11.9)bc	194 (6.70)a	30.8 (1.67)a	6.34 (0.23)b
NTW	152 (4.52)a	200 (3.98)a	30.4 (2.06)a	6.67 (0.39)b

Means (standard error) followed by different lowercase letters within a column are significantly different at $p \leq 0.05$. MP: Moldboard plow; CP: Chisel plow; DP: Disk plow; NT: No-tillage; NTWD: No-tillage with wheat-soybean double crop; NTW: No-tillage with wheat cover crop; Solvita CO₂-C: cumulative flush of CO₂ released in 24 h; WEOC: water-extractable organic carbon; WEON: water-extractable organic nitrogen.

Table 2.3. Cover crop effect on individual parameters of the Haney soil health test in the corn-soybean (CS) study.

Treatments	Solvita CO ₂ -C	WEOC	WEON	WEOC:WEON
	-----mg kg ⁻¹ -----			
CTRL	164 (31.0)ab	168 (15.3)a	7.21 (0.21)a	23.2 (1.73)a
CR	177 (20.8)ab	181 (6.52)a	7.42 (0.42)a	24.7 (2.09)a
W	139 (8.19)ab	175 (3.47)a	7.32 (0.36)a	23.9 (0.96)a
CR/HV	212 (6.39)a	199 (13.0)a	9.14 (0.88)a	22.0 (1.61)a
SHM	116 (14.7)b	154 (5.57)a	7.68 (0.96)a	22.2 (2.83)a

Means (standard error) followed by different lowercase letters within a column are significantly different at $p \leq 0.05$. CTRL: No cover crop; CR: Cereal rye; W: Winter wheat; CR/HV: Cereal rye and hairy vetch mix; SHM: Soil health mix; Solvita CO₂-C: cumulative flush of CO₂ released in 24 h; WEOC: water-extractable organic carbon; WEON: water-extractable organic nitrogen.

Table 2.4. Nitrogen rates, tillage, and cover crop management effect on individual parameters of the Haney soil health test in the long-term continuous cotton (CC) study

Treatments	Solvita CO ₂ -C	WEOC	WEON	WEOC:WEON
	-----mg kg ⁻¹ -----			
N rate (kg ha ⁻¹)				
0	135 (8.62)a	154 (3.87)a	25.3 (1.86)b	6.95 (0.54)a
67	152 (5.30)a	153 (3.50)a	37.7 (2.62)a	4.63 (0.44)b
Cover crop				
NC	129 (7.49)a	147 (4.10)a	36.1 (3.57)a	4.80 (0.54)a
V	160 (9.96)a	162 (5.39)a	31.8 (2.94)ab	5.79 (0.57)a
W	141 (8.03)a	151 (3.01)a	26.6 (2.64)b	6.77 (0.80)a
Tillage				
NT	141 (8.43)a	153 (4.24)a	33.7 (2.83)a	5.45 (0.54)a
CT	146 (6.14)a	154 (3.04)a	29.3 (2.27)a	6.12 (0.55)a
N X Tillage				
0-CT	133 (10.2)a	152 (2.94)a	26.2 (2.99)b	6.75 (0.80)a
0-NT	136 (14.4)a	157 (7.26)a	24.4 (2.30)b	7.15 (0.76)a
67-CT	159 (5.0)a	157 (5.38)a	32.3 (3.32)b	5.50 (0.74)a
67-NT	146 (9.24)a	149 (4.43)a	43.1 (3.51)a	3.76 (0.36)a

Means (standard error) followed by different lowercase letters within a column treatment set are significantly different at $p \leq 0.05$. 0: 0 kg N ha⁻¹; 67: 67 kg N ha⁻¹; NC: No cover crop; V: Hairy vetch; W: Winter wheat; NT: No-tillage; CT: Conventional tillage; Solvita CO₂-C: cumulative flush of CO₂ released in 24 h; WEOC: water-extractable organic carbon; WEON: water-extractable organic nitrogen.

Table 2.5. Regression analysis of soil health scores based on SHS2015 and SHS2018 with individual parameters of the Haney soil health test, combining all studies.

	SHS2015	SHS2018
Solvita CO ₂ -C (mg kg ⁻¹)	0.06	0.16**
WEOC (mg kg ⁻¹)	0.08	0.03
WEON (mg kg ⁻¹)	0.86***	0.68***
WEOC:WEON	0.54***	0.43***

* $p \leq 0.05$ level, ** $p \leq 0.01$, *** $p \leq 0.001$. SHS2015: Haney soil health scores 2015; SHS2018: Haney soil health scores 2018; Solvita CO₂-C: cumulative flush of CO₂ released in 24 h; WEOC: water extractable organic carbon; WEON: water extractable organic nitrogen.

Table 2.6. Nitrogen mineralization (Nmin) rates across treatments in the continuous soybean (SS), corn-soybean no-till (CS), and continuous cotton (CC) studies.

Study	Treatments	Nmin rate (kg ha ⁻¹)
SS	MP	75.9 (11.8)b
	CP	155 (14.2)a
	DP	167 (22.1)a
	NT	152 (24.4)a
	NTWD	166 (21.4)a
	NTW	205 (8.25)a
CS	CTRL	62.5 (9.59)a
	CR	66.2 (13.5)a
	W	52.2 (3.60)a
	CR/HV	86.0 (10.4)a
	SHM	53.6 (5.38)a
CC	N rate (kg ha ⁻¹)	
	0	201 (19.5)b
	67	334 (22.0)a
	Cover crop	
	NC	290 (32.9)a
	V	286 (32.3)a
	W	225 (23.8)a
	Tillage	
	NT	281 (26.4)a
	CT	254 (23.2)a

Means (standard error) followed by different lowercase letters within a study (and within treatment set for CC study) are different among treatments at $p \leq 0.05$. MP: Moldboard plow; CP: Chisel plow; DP: Disk plow; NT: No tillage; NTWD: No tillage with wheat-soybean double crop; NTW: No tillage with wheat cover crop; CTRL: No cover crop; CR: Cereal rye; W: Winter wheat; CR/HV: Cereal rye and hairy vetch mix; SHM: Soil health mix; 0: 0 kg N ha⁻¹; 67: 67 kg N ha⁻¹; NC: No cover crop; V: Hairy vetch; W: Winter wheat; NT: No tillage; CT: Conventional tillage.

Table 2.7. H3A and Mehlich-1 extractable nutrients across treatments in the long-term continuous soybean (SS) study.

Treatments	H3A extractable nutrients								Mehlich-1 extractable nutrients							
	P	K	Mg	Fe	Mn	Na	Ca	Al	P	K	Mg	Fe	Mn	Na	Ca	Al
	----- mg kg ⁻¹ -----															
MP	19.7	65.9	36.7	82.1	110	11.8	373	152	33.8	127a	103	53.9a	166	6.52	1260	295
CP	17.4	53.3	31.6	48.0	97.5	10.8	311	112	50.0	106ab	98.1	24.5b	176	5.72	1170	308
DP	17.3	41.1	33.1	49.8	86.8	10.5	337	115	46.5	102abc	99.3	22.7b	161	6.25	1190	282
NT	14.5	37.9	35.1	42.0	75.0	9.67	327	106	42.1	83.8bc	101	18.9b	172	6.23	1290	278
NTWD	22.3	52.2	38.4	51.3	105	12.0	390	124	41.0	98.4abc	97.7	17.4b	213	6.01	1200	290
NTW	19.4	24.5	41.1	47.2	77.2	8.66	370	108	52.9	74.6c	111	17.8b	173	6.60	1400	265

Means followed by different lowercase letters within a column are different among treatments at $p \leq 0.05$. Columns with no lowercase letters showed no significant differences at $p \leq 0.05$. MP: Moldboard plow; CP: Chisel plow; DP: Disk plow; NT: No tillage; NTWD: No tillage with wheat-soybean double crop; NTW: No tillage with wheat cover crop.

Table 2.8. H3A and Mehlich-1 extractable nutrients across treatments in the corn-soybean no-till (CS) study.

Treatments	H3A extractable nutrients								Mehlich-1 extractable nutrients							
	P	K	Mg	Fe	Mn	Na	Ca	Al	P	K	Mg	Fe	Mn	Na	Ca	Al
	----- mg kg ⁻¹ -----															
CTRL	11.2	27.0	26.1	56.6	65.5	20.9	357	145	30.5	71.3	106	23.1	121	10.5	1730	291
CR	2.38	20.3	31.4	54.8	42.8	20.2	295	134	20.5	74.1	115	28.9	120	10.6	1600	285
W	10.3	25.5	28.4	47.3	49.2	20.1	316	130	23.2	69.1	96.9	28.6	133	9.88	1380	301
CR/HV	6.92	24.7	38.6	66.9	41.4	22.6	348	173	20.2	74.2	126	28.2	113	10.5	1710	295
SHM	4.59	21.3	27.3	52.6	36.2	19.7	234	131	19.4	71.2	115	28.1	117	11.1	1470	286

Means followed by different lowercase letters within a column are significantly different among treatments at $p \leq 0.05$. Columns with no lowercase letters showed no significant differences at $p \leq 0.05$. CTRL: No cover crop; CR: Cereal rye; W: Winter wheat; CR/HV: Cereal rye and hairy vetch mix; SHM: Soil health mix.

Table 2.9. H3A and Mehlich-1 extractable nutrients across treatments in the long-term continuous cotton (CC) study.

Treatments	H3A extractable nutrients								Mehlich-1 extractable nutrients							
	P	K	Mg	Fe	Mn	Na	Ca	Al	P	K	Mg	Fe	Mn	Na	Ca	Al
	-----mg kg ⁻¹ -----															
N rate (kg ha ⁻¹)																
0	38.2	103a	45.7	39.9	72.7	20.9a	334	124	106	208	113	20.5	138	6.73	1550	399
67	23.9	71.5b	41.6	35.2	63.0	16.3b	273	102	90.1	178	115	25.5	163	6.25	1570	405
Cover crop																
NC	31.1	90.0	42.2	34.5	64.5	17.6	262	105	101ab	199	114	22.9	155	6.62	1460b	409
V	25.4	75.7	46.8	35.0	69.8	20.8	343	117	85.2b	177	122	19.3	152	6.53	1780a	405
W	36.6	96.7	42.0	43.0	69.1	17.4	305	117	108a	203	105	26.9	143	6.33	1440b	392
Tillage																
NT	33.1	81.4	44.4	34.6	61.2b	18.4	293	110	103	184	115	190b	147	6.62	1470	392
CT	29.0	93.3	43.0	40.5	74.4a	18.8	314	116	93.3	201	112	270a	152	6.37	1650	407
N X Tillage																
0-CT	32.2	100a	43.8	38.3ab	77.1	20.5	322	121	105	209	111	22.9	145	6.73	1460	407
0-NT	44.2	106a	47.5	41.5a	68.2	21.4	346	127	107	207	114	18.2	128	6.73	1640	392
67-CT	25.7	86.4a	42.1	42.7a	71.8	17.2	307	111	82.2	193	114	31.2	159	6.00	1490	392
67-NT	19.8	56.1b	41.9	28.3b	54.8	15.6	239	95.3	98.0	162	116	19.9	167	6.50	1650	418

Means followed by different lowercase letters within a column treatment set are significantly different among treatments at $p \leq 0.05$. Column treatment sets with no lowercase letters showed no significant differences at $p \leq 0.05$. 0: 0 kg N ha⁻¹; 67: 67 kg N ha⁻¹; NC: No cover crop; V: Hairy vetch; W: Winter wheat; NT: No tillage; CT: Conventional tillage.

Table 2.10. Pearson correlation (*R*) and coefficient of variation (CV) for H3A and Mehlich-1 extractable nutrients under continuous soybean (SS), corn-soybean (CS), and continuous cotton (CC) studies.

Study	Extractable soil nutrient	CV (%)		Pearson correlation (<i>R</i>)
		H3A	Mehlich-1	
SS	P	64.5	38.0	0.69***
	K	50.0	25.1	0.73***
	Mg	28.6	13.8	0.23
	Fe	45.0	56.2	0.62***
	Mn	44.3	23.3	0.59**
	Na	57.3	11.6	0.05
	Ca	38.5	14.4	0.40
	Al	38.5	15.2	0.66***
CS	P	76.0	43.5	0.29
	K	27.0	12.5	0.09
	Mg	31.2	27.7	0.19
	Fe	25.8	16.6	0.01
	Mn	32.7	17.9	0.04
	Na	18.5	12.2	0.21
	Ca	33.0	18.8	0.28
	Al	23.3	6.46	0.23
CC	P	58.4	30.2	0.42**
	K	38.3	26.7	0.70***
	Mg	27.3	18.8	0.63***
	Fe	39.4	42.8	0.52***
	Mn	30.9	25.0	0.34*
	Na	29.1	17.7	0.36*
	Ca	41.2	26.0	0.12
	Al	35.5	18.5	-0.37*

* $p \leq 0.05$ level, ** $p \leq 0.01$, *** $p \leq 0.001$.

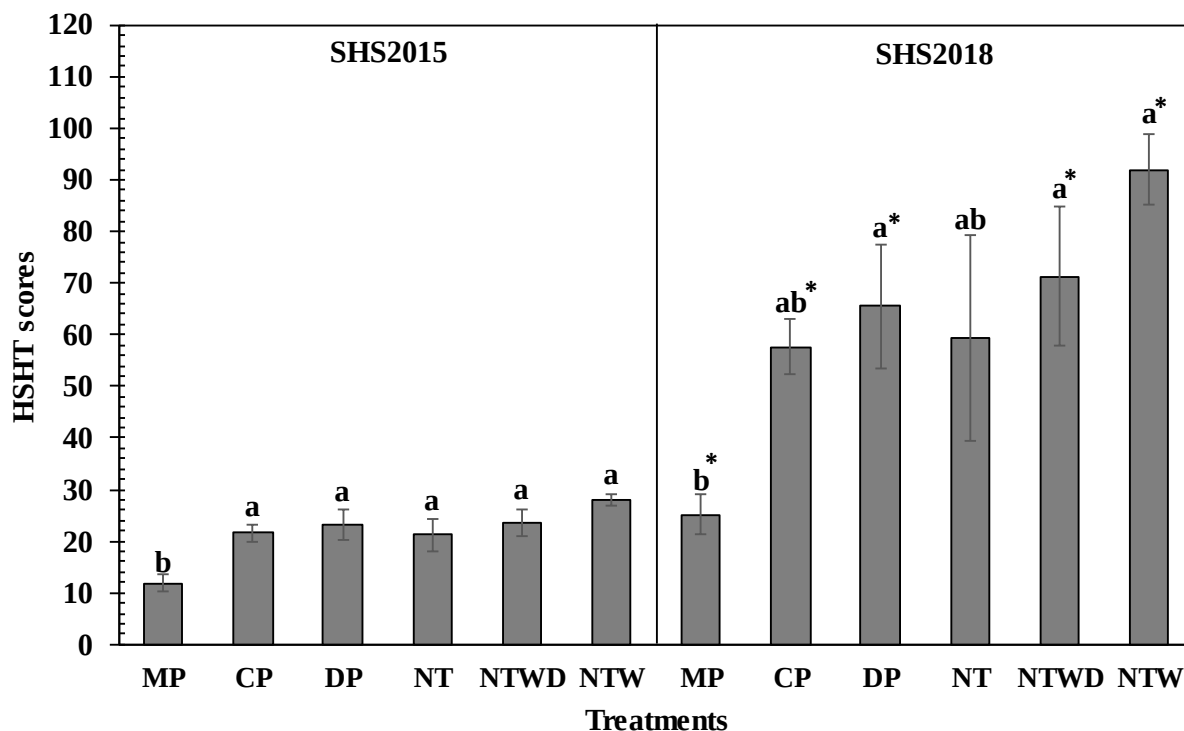


Figure 2.1. Haney soil health test scores under different tilled and no tilled treatments in the continuous soybean (SS) study.

MP: Moldboard plow; CP: Chisel plow; DP: Disk plow, NT: No tillage; NTWD: No tillage with wheat-soybean double crop; NTW: No tillage with wheat cover crop; HSHT: Haney soil health test; SHS2015: Haney soil health scores 2015; SHS2018: Haney soil health scores 2018.

Different letters denote statistically different means based on Least Significant Difference (LSD) at $p \leq 0.05$. “*” denotes significantly higher SHS2018 score than SHS2015 score within each treatment at $p \leq 0.05$. The error bars represent standard error of the means (n=4).

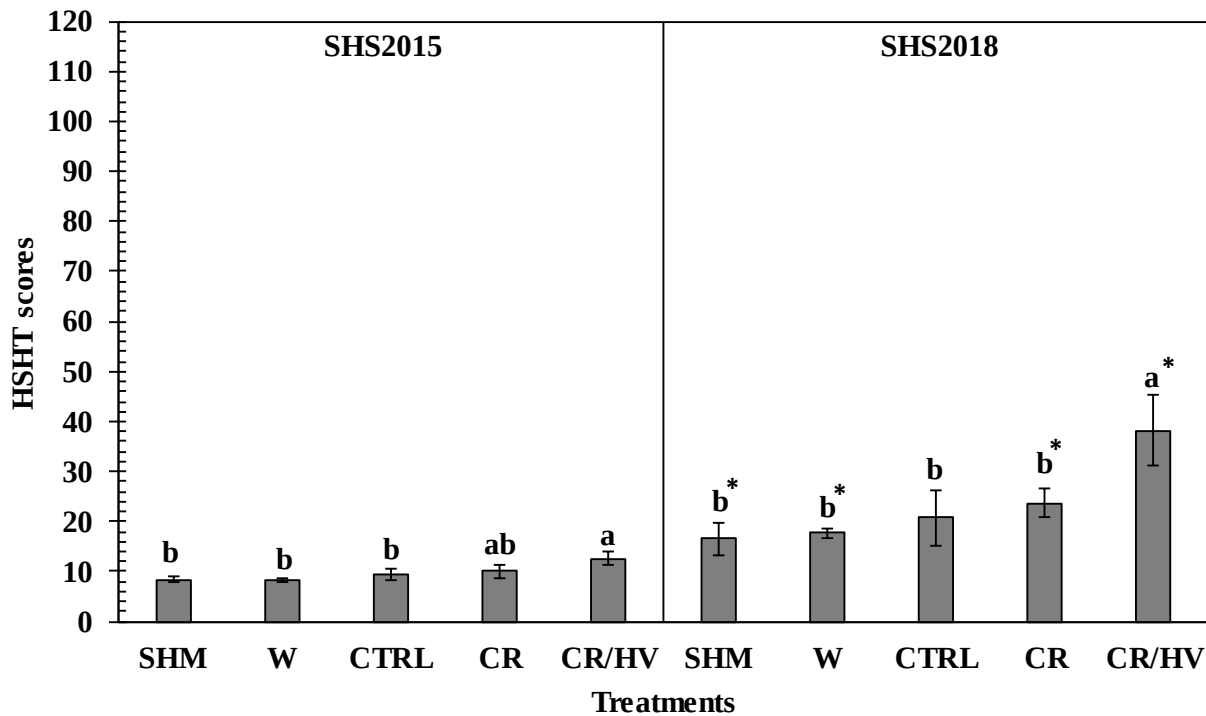


Figure 2.2. Fig. 2. Haney soil health test scores under different cover crop treatments in the corn-soybean no till (CS) study.

CTRL: No cover crop; SHM: Soil health mix; CR: Cereal rye; W: Winter wheat; CR/HV: Cereal rye and hairy vetch mix; HSHT: Haney soil health test; SHS2015: Haney soil health scores 2015; SHS2018: Haney soil health scores 2018. Different letters denote statistically different means based on Least Significant Difference (LSD) at $p \leq 0.05$. “*” denotes significantly higher SHS2018 score than SHS2015 score within each treatment at $p \leq 0.05$. The error bars represent standard error of the means (n=4).

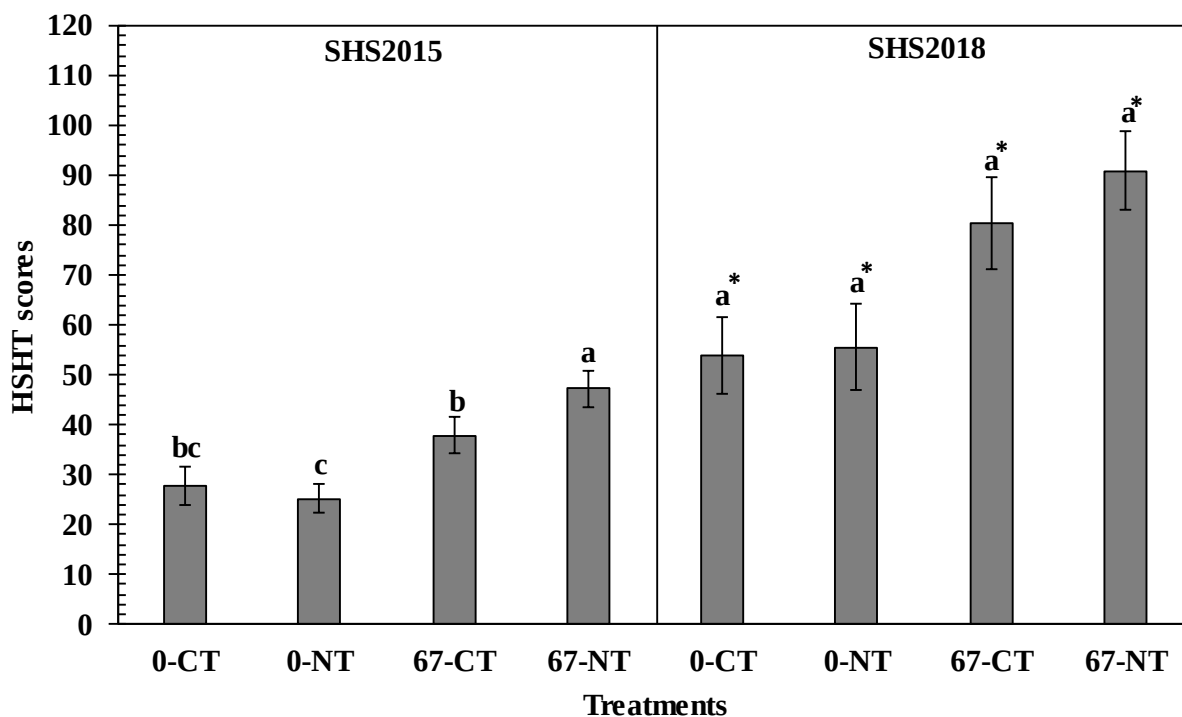


Figure 2.3. Haney soil health test scores under N rate and tillage interaction effects in the continuous cotton (CC) study.

0: 0 kg N ha⁻¹; 67: 67 kg N ha⁻¹; NT: No tillage; CT: Conventional tillage; HSHT: Haney soil health test; SHS2015: Haney soil health scores 2015; SHS2018: Haney soil health scores 2018. Different letters denote statistically different means based on Least Significant Difference (LSD) at $p \leq 0.05$. “*” denotes significantly higher SHS2018 score than SHS2015 score within each treatment at $p \leq 0.05$. The error bars represent standard error of the means (n=4).

**Chapter 3. Evaluating Cornell and Alabama Soil Health Assessment Approaches for the
Diverse Agroecosystems of West Tennessee**

Abstract

Assessing soil health is very important for ensuring agroecosystem sustainability. However, the unavailability of a standardized soil health assessment approach led to the development of several regional and commercial approaches. The applicability of such approaches across diverse agroecological regions remains debatable, mainly due to soil heterogeneity and its interactions with climate and managements. Therefore, this study was conducted to evaluate the feasibility of widely discussed Comprehensive Assessment of Soil Health (CASH) and regionally developed Alabama Soil Health Index (ASHI) to assess the soil health of Tennessee cropping systems. Since these indices were originally developed for specific regions, we hypothesized that they are not sensitive to detect the soil health changes from management differences for the croplands of Tennessee. The soil parameters (soil health indicators) included in CASH and ASHI approaches and the final soil health scores were determined leveraging three ongoing cropping system experiments in Tennessee: (i) continuous soybean (*Glycine max* L.) (SS); (ii) corn (*Zea mays* L.)-soybean rotation (CS), and (iii) continuous cotton (*Gossypium hirsutum* L.) (CC) systems. Our feasibility criteria depend on the sensitivity of soil health scores and underlying indicators to differentiate long-term management changes. In general, we found that CASH and ASHI scores did not differ strongly among tillage, cover crops, and N rate treatments. Soil health scores of both conventional and conservation management treatments were rated low to medium (scores <60) for CASH, and medium to high (scores >50) for ASHI. Overall, these soil health testing methods were not found to be sensitive to detect management-induced changes in soil health in various cropping systems of Tennessee. Our results highlight the need for extensive calibration and/or validation of CASH and ASHI approaches prior to wider adoption across agroecological regions.

Introduction

Given the concerns of sustainably feeding the rising global population, extensive research has been focused on ensuring agricultural sustainability and environmental quality by building healthy soils (Janzen, 2006; Karlen et al., 1997; Powlson et al., 2011). Soil health, defined as the “the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation” (Doran and Zeiss, 2000), has received increased attention in recent years among researchers, farmers, policymakers, consumers, and society as a whole. Healthy soils are regarded as optimally functional and naturally resilient to disturbances (Doran and Safley, 1997; Kibblewhite et al., 2007), so it is important to find ways to improve soil health in managed ecosystems. However, the selection of an effective method to measure soil health is an on-going challenge mainly because of the inherent complexity of soil systems and their interactions with a multitude of biotic and abiotic factors (Arias et al., 2005). Therefore, it is recognized that a standardized methodology or single criterion cannot assess or define universal soil health (Larkin, 2015; Mukherjee and Lal, 2014).

Broadening the focus in recent years from monitoring soil fertility to soil health entailed consideration of changes in soil physical, chemical, and biological properties in response to agricultural managements (Doran and Parkin, 1994; Doran et al., 1996; Larkin, 2015). However, different sets of soil properties, also known as soil health indicators, have been included in different soil health assessment methods (Andrews et al., 2004; Bünemann et al., 2018; Rinot et al., 2019) such as Haney soil health test (Haney et al., 2018), Comprehensive assessment of soil health (CASH) (Gugino et al., 2009), Alabama soil health index (Bosarge, 2015) (ASHI), and Soil biological quality index (QBS) (Menta et al., 2018). Ultimately, these multiple soil health

indicators are integrated into an overall soil health index or soil health score. Despite using different sets of soil health indicators in different tests, indicator scoring functions and integration approaches to derive the overall soil health index are also different. In addition, each of these tests were originally developed for specific agroecological zones. For example, the CASH (Fine et al., 2017; Gugino and 2007; Idowu et al., 2009a), developed by the Soil Health Testing Laboratory at the Cornell University, targeted soil health assessment for the Northeast and portions of Midwest US (Gugino et al., 2007; Moebius-Clune et al., 2016; Schindelbeck et al., 2008). On the other hand, the Alabama Soil Health Index (ASHI), developed by the Auburn University Extension, specifically targeted the cropland soils in Alabama (Bosarge, 2015; Mitchell et al., 2015). Although both CASH and ASHI tests were developed based on the Soil Management Assessment Framework (SMAF, Andrews et al., 2004), they differ considerably in terms of selection of soil health indicators, methods of soil analysis, scoring functions, and integration of indicators into an overall soil health score (Bosarge, 2015).

Since CASH is a comprehensive and popular soil health assessment approach and ASHI is originated from the southeast US region, we are particularly interested in evaluating these approaches on cropping systems and dominant soil types of Tennessee. The CASH has been evaluated on numerous field studies across different regions (Armenise et al., 2013; Chahal and Van Eerd, 2019; Congreves et al., 2015; Roper et al., 2017; Svoray et al., 2015) but ASHI has not been subjected to extensive evaluation outside Alabama. In addition, the CASH studies conducted so far revealed contrasting results (Birri, 2020; Chahal and Van Eerd, 2018; Chahal and Van Eerd, 2019; Roper et al., 2019a; Roper et al., 2017). For example, a study conducted in North Carolina reported inconsistent response of CASH scores among different managements (e.g., tillage treatments) and soil types (Roper et al., 2017). Therefore, this study was conducted

to evaluate if CASH and ASHI approaches can detect soil health differences from diverse agroecosystem management practices in Tennessee. Since ASHI was developed in Alabama, belongs to the southeast region, we hypothesized that this method is more suitable than CASH for measuring soil health in the agroecosystems of Tennessee.

Materials and methods

Study sites and treatments

This study was conducted on three ongoing agricultural experiments (hereafter mention as continuous soybean [*Glycine max* (L.) Merr.] study, corn (*Zea mays* L.)-soybean study, and continuous cotton (*Gossypium hirsutum*) study) located in West Tennessee on silt loam soils. The region experiences a 30-year mean annual temperature of 15.6 °C and a mean annual rainfall of 1375 mm.

Continuous soybean (SS) study

This study was started in 1979 (39-year old at the time of soil sampling) at the University of Tennessee's West Tennessee Research and Education Center (WTREC) in Jackson, TN (35°37' N, 88°50' W; elevation 125 m). The soil at the site is classified as a well-drained Lexington series (fine-silty, mixed, thermic, Ultic Hapludalf) on a 0 to 2 percent slope. Each experimental unit was 18 m by 6 m and contained four rows of soybean. This study includes six treatments with four replications in a completely randomized design (CRD): (i) moldboard plowing to 25 cm depth followed by disking and roller harrowing (MP); (ii) chisel plowing to 20 cm depth followed by roller harrowing (CP); (iii) disc plowing to 10 cm depth followed by roller harrowing (DP); (iv) no tillage (NT); (v) NT with winter wheat (*Triticum aestivum* L.) cover crop (NTW); and (vi) NT with wheat-soybean (*Glycine max* L.) double crop (NTWD). In tilled

treatments (MP, CP, and DP), tillage and harrowing operations were generally performed in late May before soybean planting. In the NTW system, a wheat cover crop was seeded after harvesting soybean grain in October-November, and the cover crop was chemically terminated using 0.71 kg ha⁻¹ paraquat (1-methyl-4-(1-methylpyridin-1-ium-4-yl) pyridin-1-ium) about two to three weeks prior to planting soybean. In NTWD system, wheat was grown for grain from mid-October to mid-June followed by soybean each year. All plots were treated with 3.36 kg ha⁻¹ alachlor (2-Chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide) and 0.42 kg ha⁻¹ metribuzin (4-Amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one) for pre-emergence weed suppression and 0.13 kg ha⁻¹ clethodim (2-[1-[[[(E)-3-chloroprop-2-enoxy] amino] propylidene]-5-(2-ethylsulfanylpropyl) cyclohexane-1,3-dione) for post-emergence weed control.

Corn-soybean (CS) study

This study was started in 2013 (4-year old at the time of soil sampling) at the University of Tennessee's Research and Education Center in Milan (RECM), TN (35°54' N, 88°44' W; elevation 129 m). The soil at the site is classified as a Loring B2 series (fine-silty, mixed, thermic, Oxyaquic Fragiudalf), which is moderately well-drained soils with a fragipan, and on a 0 to 2 percent slope. Each experimental unit was 9 m by 4 m with five crop rows. This study includes different cover crop treatments under corn-soybean rotation system with four replications in a randomized complete block design (RCBD): (i) cereal rye (*Secale cereale* L.) (CR); (ii) winter wheat (W); (iii) cereal rye-hairy vetch (*Vicia villosa* Roth.) mixture (CR/HV); (iv) soil health mix (SHM), a mixture of cereal rye, hairy vetch, daikon radish (*Raphanus sativus* L.) (DR), crimson clover (*Trifolium incarnatum* L.) (CRM) and oats (*Avena sativa* L.) (O); and (v) cover crop-free control (CTRL). Cover crop seeding rates were based on the recommendations of United States Department of Agriculture-Natural Resources Conservation

Service (USDA-NRCS) for Tennessee, which were 84 kg ha⁻¹ for W, 84 kg ha⁻¹ for CR, 26 kg ha⁻¹ CR and 24 kg ha⁻¹ HV for the CR/HV treatment, and a mixture of 17 kg ha⁻¹ CR, 7 kg ha⁻¹ HV, 2.3 kg ha⁻¹ DR, 4.5 kg ha⁻¹ CRM, and 23 kg ha⁻¹ O for the SHM treatment. A no-till planter was used to plant corn and soybean in April-May. Winter cover crops were seeded using a 1.83 m Tye drill in October-November after the harvest of corn or soybean and chemically terminated in April-May. Prior to row-crop planting, Gramoxone® SL 2.0 (N, N'-dimethyl-4, 4'-bipyridinium dichloride) was applied to terminate cover crops and weeds. Roundup PowerMax® (Glyphosate) was used as a post-emergence herbicide to control weeds during the row-crop growing season. All fertilizers were applied based on recommendations of the University of Tennessee (UT) for no till corn-soybean rotation systems.

Continuous cotton (CC) study

This study was started in 1981 (37-year old at the time of soil sampling) at WTREC in Jackson, TN. The soil at the site is classified as a well-drained Lexington series (fine-silty, mixed, thermic, Ultic Hapludalf) on a 0 to 2 percent slope under continuous cotton production. The size of each experimental unit was 12 m by 8 m with eight rows of cotton. Following UT recommendation, P fertilizer was applied as triple superphosphate at 101 kg P₂O₅ ha⁻¹ and K fertilizer was applied as muriate of potash at 134 kg K₂O ha⁻¹. The experimental design was RCBD with split-split plot treatment arrangement and the experiment includes four replications. The main plot treatment was N rates: 0 kg ha⁻¹ (0N) and 67 kg ha⁻¹ (67N) which was applied as ammonium nitrate (NH₄NO₃) a week after planting. The sub-plots were three cover crop treatments: winter wheat (W), hairy vetch (HV), and no cover crop (NCC). The cover crops were chemically terminated in late April to early May of each year using 3.51 L ha⁻¹ of Gramoxone® SL. The sub-sub plots were two levels of tillage: conventional tillage (CT) and no tillage (NT).

Tillage was done by disking twice to 10 cm depth using standard disc harrow followed by roller harrow leveling to smooth the land before planting in May.

Soil sampling

Soil samples were collected in July 2017 from each replicated experimental plot of all three field experiments. Six random cores were collected from the inter-row area from 0-15 cm depth using a bucket-type auger of 5 cm diameter. Samples from each plot were combined and homogenized to make a composite soil sample. The composite samples were air-dried and passed through an 8 mm sieve. Four penetration resistance (PR) measurements from 0 to 15 cm (surface hardness; PR15) and 15 to 45 cm (subsurface hardness; PR45) depths were recorded using a hand-held penetrometer (Soil compaction tester penetrometer, Dickey-john corporation, Illinois, USA) in each plot at approximately field capacity soil moisture.

Laboratory analyses

Quantification of CASH indicators

Soil samples were processed and analyzed for the physical, chemical, and biological indicators listed in the standard package of the CASH test. We strictly followed the standard operating protocols described in the CASH test (Schindelbeck et al., 2016). Accordingly, the physical soil health indicators included available water capacity (AWC) measured as volume (Reynolds and Topp, 2008), surface (PR15) and subsurface hardness (PR45) measured using penetrometer (Duiker, 2002), wet aggregate stability (WAS) measured using Cornell sprinkle infiltrometer (Ogden et al., 1997), and soil texture measured using the hydrometer method (Gee and Or, 2002). Biological soil health indicators included soil organic matter (SOM) measured using loss on ignition (LOI) at 500 °C for 2 hours (Broadbent, 1965), autoclaved-citrate extractable soil protein index (SPI) following Wright and Upadhyaya (1996) and Moebius-Clune

(2008) protocols, soil respiration from 4d incubation (4d CO₂) using potassium hydroxide (KOH) trap (Zibilske, 1994), and permanganate oxidizable C (POXC) as a surrogate for active C (Stiles et al., 2011; Weil et al., 2003). The chemical indicators included soil pH measured on a 1:1 soil:water (v/v) suspension, and macro- and micro-nutrients (P, K, Mg, Fe, Mn, and Zn) extracted using modified Morgan's solution (a mixture of ammonium acetate and acetic acid solution; pH 4.8) and analyzed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Schindelbeck et al., 2016). Measured values of individual CASH indicators were transformed into individual indicator indexed scores using scoring functions according to Fine et al. (2017). These indexed scores were then integrated into a final CASH soil health score using an unweighted average approach (Gugino and 2007; Idowu et al., 2009b). Detailed descriptions of the scoring functions and methods of analyses are described in the CASH framework guide (Moebius-Clune et al., 2016).

Quantifications of ASHI indicators

Soil samples were processed and analyzed for physical, biological, and chemical indicators specific to the ASHI test following procedures specified by the Auburn University (AU) Soil Testing lab (Hue and Evans, 1986; Alabama Soil Health Index, 2019). Accordingly, soil pH (measured on a 1:1 soil:water (v/v) suspension (Huluka, 2005)), Mehlich-1 extractable P and K (Mitchell and Huluka, 2012), estimated cation exchange capacity (ECEC), % base saturation (BS) (Huluka, 2005), soil organic matter (determined by LOI at 375 °C for 16 h (Ball, 1964), soil respiration measured by Solvita[®] 1d CO₂-C incubation (Haney and Haney, 2010), potentially mineralized N (PMN) estimated from 1d CO₂-C incubation (Franzluebbers et al., 1996; Linn and Doran, 1984), and wet aggregate stability (WAS) measured by Slaking method (Bosarge, 2015). Measured values of individual indicators were transformed into indexed scores

of indicators and the indexed scores were integrated into an ASHI soil health score using scoring functions as described in Bosarge (2015) and Alabama Soil Health Index (2019).

Statistical analyses

Analysis of variance (ANOVA) were conducted using the PROC GLIMMIX procedure in SAS v9.4 statistical package (SAS, 2012) for measured values of individual indicators, indexed scores of individual indicators, and final CASH and ASHI soil health scores. Separate ANOVA were conducted for each field experiment. Treatments were considered as fixed effects and replicates were considered as random effects in the model. Least square means were separated using the Least Significant Difference (LSD) at $p \leq 0.05$.

Results and discussion

Comprehensive Assessment of Soil Health (CASH)

The treatment response of CASH indicators for each field experiment is summarized with p -values in Table 3.1. In SS study, out of the 15 CASH indicators, four (WAS, 4d CO₂, SPI, and POXC) varied among the tillage treatments ($p \leq 0.05$). In the CS study, none of the indicators showed treatment differences. In the CC study, SPI, pH, P, K, and Mn varied with N rates (0 and 67 kg ha⁻¹), AWC, 4d CO₂, pH, and P differed with cover crop treatments, and only K varied with tillage treatments.

Index scores of each indicator, calculated based on the scoring functions on a scale of 0-100 (Fine et al., 2017), and the overall soil health score, calculated as the unweighted average of indexed scores of indicators (Moebius-Clune et al., 2016), were interpreted in five categories: (i) very low (0-20), (ii) low (20-40), (iii) medium (40-60), (iv) high (60-80), and (v) very high (80-100) (Moebius-Clune et al., 2016). In the SS study, the indexed scores of WAS, 4d CO₂, SPI,

and POXC varied among treatments (Table 3.2) with consistently low indexed scores under MP treatment compared to NTW and NTWD for WAS, 4d CO₂, and POXC. Indexed scores of SPI only differentiated NTWD from the rest of the treatments. However, none of the chemical indicators differed among treatments (Table 3.2). When mean scores of all physical, biological, and chemical indicators were calculated, we found that only mean biological scores varied among treatments (Table 3.2). In the CS study, none of the indexed indicator scores varied among treatments (Table 3.3), which was in accordance with the insignificant *p* values (> 0.05) for this study (Table 3.1). In CC study, indexed scores of SPI, K, and mean chemical scores differed with N rate treatments, AWC, 4d CO₂, and pH varied among cover crop treatments, and no indicators varied between the two tillage treatments (Table 3.4). In addition, the indexed scores of WAS were influenced by N rate x cover crop interaction (Table 3.4).

Regardless of management differences, a few indicators were scored >60 (high to very high). The PR15 and PR45 measurements were insignificant to managements and their indexed scores were mostly rated very low (<20), even for the treatments that strongly support sustainable agriculture (e.g., no-tilled and cover cropped soybean (NTW) in the SS study). Intensive tillage and/or no residue cover increases surface hardness (Pagliai et al., 2004) and cover cropping tends to ameliorate it (Blanco-Canqui et al., 2011; Nouri et al., 2019b; Villamil et al., 2006). However, increased compaction or crust formation under the no tillage system has also been frequently reported by other authors (Abdollahi and Munkholm, 2014; Nouri et al., 2018; Nouri et al., 2019b; Rosa et al., 2013). Although multiple past studies reported reduced soil compaction with conservation management practices, the measured values and indexed scores of PR15 and PR45 in our study did not vary among treatments. The indexed scores of WAS for the NT and NTWD treatments in the SS study were greater than MP and DP

treatments. In CC study, WAS indexed scores varied by N rates x cover crop interaction, where 0-NC treatment showed lower WAS indexed scores than all other N rate and cover crop combinations, except 60-W. Indexed scores of AWC followed the trend: W > V = NC. Although the indexed scores of WAS and AWC belonged to high category (>60) and showed some treatment differences in both SS and CC long-term studies, mean indexed scores derived by combining all the physical indicators did not show treatment differences and were mostly rated low to medium (<60).

Although indexed scores of individual biological indicators such as SPI, 4d CO₂, POXC, and the mean score for all the biological indicators combined differed among treatments in SS study, most scores were rated very low to medium (<60). In general, CASH biological indicators showed improved indexed scores under no-tilled systems (NT, NTW, and NTWD) in this study. In CC study, all biological indicators were rated very low to medium (<60), resulting in a low mean score (<40), when all the biological indexed scores were combined. The measured values and indexed scores of SOM, another biological indicator in the CASH test, did not differ among treatments in any of the studies. This could be due to the less precise LOI method used to determine SOM in CASH compared to more precise methods such as dry combustion. The LOI method is found to be less accurate in soils with relatively lower SOM content as the ones tested in this study (Roper et al., 2019b). Moreover, several studies reported that LOI method could result in overestimation of SOM because of volatilization of other soil compounds (e.g., structural water) in addition to SOC when exposed to high temperature for an extended period (Hoogsteen et al., 2015; Huang et al., 2012; Szava-Kovats, 2009). Previous studies that used the dry combustion method for SOM/SOC determination reported different results from the same long-term experiments. For example, Singh et al. (2020) reported greater SOC under no-tilled

systems (NT, NTW, and NTWD) than tilled systems (MP, CP, and DP) in SS study, and Mbuthia et al. (2015) reported greater SOC from NT-cover crop interactions compared to CT-no cover crop interaction in CC study. Indexed scores of 4d CO₂ and POXC showed treatment differences but the scores were below high category for all the treatments (Table 3.2). Indexed scores of most chemical indicators did not show treatment differences, except for soil pH under cover crop treatments in CC study (Table 3.4), which were greater under HV than NC and W treatments despite rating the lowest among chemical indicators (<45). Indexed scores of 4d CO₂ were also higher under HV than NC and W treatments in CC study. This could be due to relatively fast decomposing residues of leguminous cover crop of HV, which promotes higher microbial activity, thus, higher measured values and scores of 4d CO₂. Mbuthia et al. (2015) also reported higher microbial respiration under HV than W and NC treatments in the CC system.

Though indexed scores of modified Morgan extractable P and K did not differentiate among treatments in any of the studies, scores were rated high to very high (>80). Similar high scores for P and K were also observed in CC study when SMAF based scoring was conducted (Mbuthia et al., 2015). This could probably be due to P and K fertilizer applications, regardless of treatments, a few weeks prior to soil sampling (Mbuthia et al., 2015). Overall mean scores, combining all the chemical indicators, were high (60-80) but were similar among treatments. The lack of response of all indexed scores to cover crop treatments in CS study could be due to the short duration of the study (4-year at the time of sampling) to result in measurable changes in soil health indicators. Several past studies reported the need of continuing conservation management practices over a longer time to have positive effects on soil health indicators (Addiscott, 1995; Nouri et al., 2020; Ritchey et al., 2015).

Overall CASH soil health scores ranged from 45 to 56 in the SS study (Fig. 3.1a), 49 to 58 in the CS study (Fig. 3.1b), and 52 to 55 in the CC study (Fig. 3.1c), however, there were no treatment differences observed in any of the studies. This lack of response of overall scores could be due to that only a few indicators (in the case of SS study and CC study) or no indicators (in CS study) responded to treatments (Table 3.1). Despite short duration (4-year at the time of sampling) and lack of indicator responses in CS study, overall CASH scores were rated medium similar to long-term SS and CC studies. Based on the CASH test, soils receiving low soil health scores are recommended for the adoption of conservation practices (e.g., NT, cover crops) to improve soil health (Moebius-Clune et al., 2016). Such conservation practices are part of the treatments in the tested cropping systems (e.g., NT, NTW, and NTWD in SS study, cover crops in CS study, and cover crops and no tillage in CC study). However, overall soil health scores were similar across the treatments in all three studies regardless of the study duration and treatments (Fig. 3.1). Soil health indicators tend to respond differently with sampling time (De et al., 2019) and sampling depth (Jagadamma et al., 2019), therefore, monitoring CASH indicators overtime within a growing season and from different depths could yield better management sensitivity to treatments. This study was based on samples collected only once from a single depth (0-15 cm) from all the tested agricultural experiments.

We postulate multiple reasons for the lack of treatment differences in individual CASH indicators and overall CASH soil health scores:

- (i) Methodological differences: methodology/procedures used for analyzing indicators may not be sensitive enough to measure treatment differences. For example, as discussed earlier, a less precise LOI method, instead of dry combustion, was used for SOM determination. Also, a sprinkler infiltrometer was used for WAS instead of commonly used wet sieving.

Previous studies reported treatment differences in WAS from these tested cropping systems when wet sieving was used (Nouri et al., 2018; Nouri et al., 2019a; Nouri et al., 2019b; Singh et al., 2020).

- (ii) Scoring functions: sigmoidal scoring functions were used to transform measured indicator values into indexed scores, which were originally calibrated for regions with relatively higher values for several indicators (e.g., SOM in the northeastern US is normally higher than that in Tennessee). These functions mostly assigned relatively lower scores for indicators when used for croplands of Tennessee as those scores fell at the low end of the scoring curve (e.g., POXC received low to medium scores even for the long-term conservation practice of NTW). This may have minimized the response of indexed scores to treatments. These results indicate the need for regional calibration of scoring functions and using a regionally adaptable framework to assess soil health (Andrews et al., 2004; Fine et al., 2017; Roper et al., 2017; van Es and Karlen, 2019).
- (iii) Method of calculating soil health score: The indexed scores of individual indicators were integrated into final soil health score using an unweighted average approach (Fine et al., 2017; Moebius-Clune et al., 2016), which was shown to be less reliable in addressing relationships among indicators, soil health scores, and soil functions compared to weighted approach (Chahal and Van Eerd, 2019; Congreves et al., 2015; Vasu et al., 2016).

Alabama Soil Health Index (ASHI)

The p -values corresponding to the treatment response of each indicator under each field study are included in Table 3.1. Among all indicators, chemical indicators such as K and ECEC

showed treatment differences in SS study and no indicators showed treatment differences in CS study. In CC study, 1d CO₂, pH, K, and ECEC varied with N rates, pH and BS varied with cover crops, and only WAS varied with tillage treatments. Although several properties showed treatment responses in long-term SS and CC studies (Table 3.1), when they were converted to indexed scores, only soil pH under N rates and WAS under tillage treatments in the CC study showed treatment differences (Table 3.5). Final soil health scores were calculated as weighted addition of indexed scores of individual indicators and interpreted in three categories: (i) low (0-50), (ii) medium (50-80), and (iii) high (80-100). Overall ASHI scores ranged from 78 to 83 for SS study (Fig. 3.2a), 68 to 74 for CS study (Fig. 3.2b), and 75 to 83 for CC study (Fig. 3.2c), and the treatment differences were observed only for the cover crop treatments in the CC study with HV showed higher score than W and NCC.

The ASHI method also suffers from methodological issues in analytical protocols similar to the CASH method. For example, ASHI also utilizes the LOI method for SOM determination but at 375 °C for 16 h (Ball, 1964) compared to 500 °C for 2 h in the case of CASH (Broadbent, 1965; Moebius-Clune et al., 2016). A study conducted by Roper et al. (2019b) for comparing four methods of SOM determination showed that SOM determined by LOI at a relatively lower temperature and duration (360 °C for 2 hours) showed improved management-sensitivity and better correlation to SOC determined by dry combustion. In ASHI, the lower temperature was used, but the duration was longer. Also, ASHI used the slaking method for determining WAS (Bosarge, 2015; Seybold et al., 2001), which may not be sensitive enough to reflect long-term management differences. To reiterate this, we found evidence of improved sensitivity of WAS with long-term treatments in the tested field studies when wet sieving protocol of Kemper and Rosenau (1986) was used (Nouri et al., 2018; Nouri et al., 2019a; Nouri et al., 2019b; Singh et

al., 2020). In addition, the ASHI approach utilized Solvita[®] gel method to measure 1d CO₂ respiration and to estimate PMN, however many studies questioned the validity of this method to determine microbial activity and N mineralization (Kravchenko and Robertson, 2011; Sullivan and Granatstein, 2015). The lack of treatment response of PMN in our study underscores that the usefulness of Solvita-based 1d CO₂ to predict N mineralization is questionable (Chu et al., 2019; Sullivan and Granatstein, 2015; Wade et al., 2018).

Most of the indicators (WAS, SOM, 1d CO₂, PMN, pH, P, K, and ECEC) showed high to very high (60-100) indexed scores across all treatments. However, the overall soil health score fell in medium to high category (50-100) regardless of treatment types or length of management. The lack of response of ASHI's soil health score to management could be due to higher weightage assignment (relative importance) to soil chemical indicators (50 out of 100) as compared to soil physical (10 out of 100) and biological (40 out of 100) indicators (Bosarge, 2015). Soil chemical indicators (e.g., pH, P, K, BS, and ECEC) can be easily altered by fertilizer and/or lime applications. For example, the application of P and K fertilizers can improve P and K ratings as well as ECEC, ultimately generating higher overall soil health scores to indicate soil health improvement. Similarly, the application of lime to low pH soils can increase soil pH and improve BS. However, these improvements in soil health that strongly dependent on soil fertility changes, may not reflect long-term management-induced changes in soil health. Our study generated high soil health scores for both conservation and conventional managements, indicating that the ASHI method is not reliable to capture long-term management-induced changes in soil health in the tested cropping systems.

Conclusions

This study focused on a comprehensive evaluation of the Cornell Assessment of Soil Health (CASH) and Alabama Soil Health Index (ASHI) to assess soil health changes from different cropping system management practices in Tennessee. We assessed in detail the management-sensitivity of CASH and ASHI final soil health scores, underlying soil health indicators, and their indexed scores to differentiate changes in soil health across different tillage, cover crops, and N rate treatments in corn and soybean production systems. Despite a few individual indicator responses, CASH and ASHI scores showed no or inconsistent differentiation of treatments in tested cropping systems. The sensitivity of these methods is plausibly limited by the issues related to indicator selection, analytical methods, scoring functions, and final score calculation. Although CASH and ASHI showed promise for their respective regions of origin, applicability across different agroecological regions is questionable. Our results highlight the need for developing regionally suitable soil health assessment approaches to guide soil health improvement by adopting conservation management practices.

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Appendix 3

Table 3.1 The *p*-values for the F-test statistics of soil health indicators of comprehensive assessment of soil health (CASH) and Alabama soil health index (ASHI) for each field experiment.

Soil health test	Soil Health Indicators	Study				
		SS study (tillage levels)	CS study (cover crop types)	CC study		
				N rate	Cover crop	Tillage
CASH	Available water capacity (AWC)	0.703	0.340	0.942	0.027	0.072
	Surface hardness (PR15)	0.952	0.742	0.850	0.716	0.289
	Subsurface hardness (PR45)	0.200	0.552	0.273	0.464	0.220
	Wet aggregate stability (WAS)	0.014	0.278	0.997	0.078	0.147
	Soil respiration (4d CO ₂)	0.015	0.115	0.119	0.030	0.794
	Soil protein index (SPI)	0.028	0.203	0.001	0.339	0.602
	Soil organic matter (SOM)	0.167	0.395	0.432	0.603	0.990
	Active carbon (POXC)	0.017	0.459	0.302	0.199	0.959
	Soil pH	0.055	0.430	0.035	0.005	0.350
	Modified Morgan P	0.655	0.530	0.011	0.023	0.870
	Modified Morgan K	0.178	0.396	0.025	0.205	0.039
	Minor elements					
	Modified Morgan Mg	0.662	0.859	0.690	0.358	0.793
	Modified Morgan Fe	0.501	0.900	0.963	0.121	0.656
	Modified Morgan Mn	0.236	0.800	0.002	0.628	0.948
	Modified Morgan Zn	0.109	0.839	0.120	0.198	0.923
ASHI	Wet aggregate stability (WAS)	0.364	0.063	0.977	0.089	0.001
	1d soil respiration (1dCO ₂)	0.276	0.380	0.013	0.361	0.915
	Potential mineralized N (PMN)	0.052	0.099	0.992	0.999	0.997
	Soil organic matter (SOM)	0.336	0.896	0.070	0.320	0.908
	Soil pH	0.055	0.430	0.035	0.010	0.350
	Mehlich-1 extractable P	0.693	0.536	0.057	0.070	0.255
	Mehlich-1 extractable K	0.028	0.941	0.050	0.330	0.956
	Soil ECEC	0.007	0.531	0.021	0.520	0.663
	Base saturation (BS)	0.412	0.428	0.517	0.020	0.186

SS, continuous soybean; CS, corn-soybean; CC, continuous cotton; ECEC, Estimated cation exchange capacity.

Table 3.2: Indexed CASH indicator scores in response to treatments in continuous soybean study

CASH indicators	Treatments					
	CP	DP	MP	NT	NTW	NTWD
Physical indicators						
Available water capacity (AWC)	31.3a	71.8a	38.3a	30.5a	49.0a	54.0a
Surface hardness (PR15)	5.00a	19.8a	15.8a	11.3a	9.25a	11.5a
Sub surface hardness (PR45)	22.5a	26.3a	18.5a	39.8a	44.0a	45.8a
Wet Aggregate Stability (WAS)	91.5ab	82.3bc	77.5c	94.5a	93.5ab	96.3a
Mean physical scores	37.6a	50.0a	37.5a	44.0a	48.9a	51.9a
Biological indicators						
Soil Respiration (4d CO ₂)	29.8bc	30.8bc	12.0c	37.8ab	44.8ab	54.3a
Soil Protein Index (SPI)	36.5b	39.5b	29.0b	43.8b	45.3b	71.0a
Soil Organic Matter (SOM)	18.3a	17.8a	17.0a	19.3a	26.5a	22.3a
Active Carbon (POXC)	16.5c	15.8c	11.3c	18.7bc	33.3ab	42.8a
Mean biological score	25.3bc	25.9bc	17.3c	32.3abc	37.4ab	47.5a
Chemical indicators						
Soil pH	44.0a	56.8a	74.5a	57.5a	51.0a	34.0a
Modified Morgan P	93.3a	87.0a	82.8a	87.3a	96.3a	85.0a
Modified Morgan K	100a	100a	100a	100a	100a	100a
Minor Elements	56.0a	56.0a	56.0a	41.0a	56.0a	56.0a
Mean chemical scores	73.3a	74.9a	83.9a	73.5a	75.8a	68.8a

Means followed by different lowercase letters within a row are significantly different across tillage treatments at $p \leq 0.05$. CP, Chisel plow; DP, Disk plow; MP, Moldboard plow; NT, No tillage; NTW, No tillage with wheat cover crop; NTWD, No tillage with wheat-soybean double crop.

Table 3.3: Indexed CASH indicator scores in response to treatments in corn-soybean study.

CASH indicators	Treatments				
	CTRL	CR	CR/HV	SHM	W
Physical indicators					
Available Water Capacity (AWC)	72.8a	84.3a	84.3a	86.5a	81.0a
Surface Hardness (PR15)	6.75a	17.0a	15.8a	9.33a	9.75a
Subsurface Hardness (PR45)	7.25a	7.75a	23.8a	13.7a	4.0a
Wet Aggregate Stability (WAS)	100a	100a	100a	100a	99.8a
Mean physical scores	46.7a	52.3a	55.9a	52.1a	48.7a
Biological indicators					
Soil Respiration (4d CO ₂)	22.5a	25.8a	36.5a	23.0a	31.5a
Soil Protein Index (SPI)	26.5a	40.0a	47.3a	44.5a	38.0a
Soil Organic Matter (SOM)	47.8a	67.0a	79.0a	43.0a	55.0a
Active Carbon (POXC)	56.8a	58.5a	62.3a	49.8a	40.0a
Mean biological score	38.0a	47.8a	56.3a	40.1a	41.1a
Chemical indicators					
Soil pH	21.0a	14.3a	11.3a	14.8a	11.5a
Modified Morgan P	100a	100a	100a	100a	100a
Modified Morgan K	100a	100a	100a	100a	100a
Minor Elements	33.5a	11.0a	33.5a	22.3a	44.8a
Mean chemical scores	61.4a	59.8a	61.2a	59.3a	64.1a

Means followed by different lowercase letters within a row are significantly different across cover crop treatments at $p \leq 0.05$. CTRL: no cover crop control; CR: Cereal rye; CR/HV: Cereal rye and hairy vetch; SHM, soil health mix; W: Winter wheat.

Table 3.4: Indexed CASH indicator scores in response to treatments in continuous cotton study

CASH indicators	Treatments						
	N rate (kg ha ⁻¹)		Cover crop			Tillage	
	0	67	NCC	HV	W	NT	CT
Physical indicators							
Available Water Capacity (AWC)	83.2a	85.1a	83.3b	81.3b	87.8a	82.5a	85.7a
Surface Hardness (PR15)	5.58a	3.83a	4.38a	4.69a	5.06a	3.79a	5.63a
Subsurface Hardness (PR45)	13.1a	20.0a	15.1a	15.2a	19.4a	16.2a	16.9a
Wet Aggregate Stability (WAS)	98.3a	98.5a	97.9a	99.0a	98.3a	98.7a	97.1a
Mean physical scores	48.3a	50.2a	49.5a	48.2a	50.2a	48.7a	49.9a
Biological indicators							
Soil Respiration (4d CO ₂)	24.7a	28.2a	23.5b	31.2a	24.6b	26.1a	26.8a
Soil Protein Index (SPI)	48.5b	62.4a	53.8a	59.3a	53.4a	56.3a	54.7a
Soil Organic Matter (SOM)	46.8a	42.3a	47.3a	46.9a	39.3a	43.6a	45.4a
Active Carbon (POXC)	19.0a	20.4a	18.9a	23.4a	16.8a	20.4a	19.0a
Mean biological score	35.2a	38.3a	36.5a	40.2a	33.5a	36.7a	36.8a
Chemical indicators							
Soil pH	38.2a	26.1a	25.1b	45.4a	25.9b	34.4a	29.8a
Modified Morgan P	100a	100a	100a	100a	100a	100a	100a
Modified Morgan K	100a	100a	100a	100a	100a	100a	100a
Minor Elements	69.9a	59.7a	58.7a	64.3a	67.0a	61.5a	65.1a
Mean chemical scores	76.3a	72.1a	71.2a	77.4a	73.2a	74.6a	73.7a

Treatment interactions

CASH indicator	N rate X cover crop					
	0-NCC	60-NCC	0-V	60-V	0-W	60-W
Wet Aggregate Stability (WAS)	97.1b	98.8a	99.3a	98.8a	98.6a	98.0ab

Means followed by different lowercase letters within a row (within treatment levels) are significantly different across treatments at $p \leq 0.05$. 0: 0 kg ha⁻¹ N; 67: 67 kg ha⁻¹ N; NC: No cover crop control; HV: Hairy vetch; W: Winter wheat; NT: No tillage; CT: Conventional tillage.

Table 3.5: Indexed ASHI indicator scores in response to treatments in continuous soybean (SS), corn-soybean (CS), and continuous cotton (CC) studies.

Study	Treat	ASHI indicators								
		WAS	1dCO ₂	PMN	SOM	pH	P	K	ECEC	BS
SS	CP	8.50a	10.0a	10.0a	14.7a	10.0a	10.0a	9.00a	5.00a	6.00a
	DP	8.00a	10.0a	10.0a	13.0a	11.3a	7.00a	8.00a	5.00a	5.25a
	MP	8.50a	10.0a	10.0a	13.0a	13.8a	7.50a	8.00a	5.00a	6.00a
	NT	9.50a	10.0a	10.0a	14.0a	11.3a	8.75a	7.00a	5.00a	6.00a
	NTW	9.00a	10.0a	10.0a	16.0a	11.3a	10.0a	6.00a	5.00a	6.00a
	NTWD	9.00a	10.0a	10.0a	15.0a	10.0a	7.50a	8.00a	5.00a	4.50a
CS	CTRL	7.50a	10.0a	10.0a	20.0a	10.0 a	6.25a	4.50a	5.00a	5.25a
	SHM	7.50a	10.0a	10.0a	20.0a	11.3a	2.50a	6.00a	5.00a	5.25a
	CR	6.50a	10.0a	10.0a	20.0a	10.0a	2.50a	6.00a	5.00a	6.00a
	W	6.00a	10.0a	10.0a	18.0a	10.0a	2.50a	6.00a	5.00a	4.50a
	CR/HV	7.50a	10.0a	10.0a	20.0a	7.50a	5.00a	4.50a	5.00a	4.50a
CC	N rate									
	0	8.75a	4.83a	7.92a	15.7a	11.0a	5.83a	6.83a	5.00a	5.63a
	67	8.50a	5.33a	8.00a	17.3a	9.24b	6.04a	7.33a	5.00a	5.50a
	Cover crop									
	NCC	8.63a	4.63a	7.88a	16.2a	8.85a	5.31a	7.50a	5.00a	5.44a
	V	8.75a	5.38a	8.00a	17.3a	11.3a	6.88a	7.00a	5.00a	6.00a
	W	8.50a	5.25a	8.00a	16.0a	10.3a	5.63a	6.75a	5.00a	5.25a
	Tillage									
	NT	9.17a	4.92a	8.00a	16.6a	10.3a	5.83a	7.33a	5.00a	5.75a
	CT	8.33b	5.25a	7.92a	16.3a	10.0a	6.04a	6.83a	5.00a	5.38a

Means followed by different lowercase letters within a column (within the study) are significantly different across treatments at $p \leq 0.05$. SS, continuous soybean; CS, corn-soybean; CC, continuous cotton; CP, Chisel plow; DP, Disk plow; MP, Moldboard plow; NT, No tillage; NTW, No tillage with wheat cover crop; NTWD, No tillage with wheat-soybean double crop..0: 0 kg ha⁻¹ N; 67: 67 kg ha⁻¹N; NC: No cover crop control; HV: Hairy vetch; W: Winter wheat; NT: No tillage; CT: Conventional tillage. WAS, Wet aggregate stability; 1d CO₂, 1d soil respiration; PMN, Potential mineralized N; SOM, Soil organic matter; Soil ECEC, estimated cation exchange capacity; BS, Base saturation.

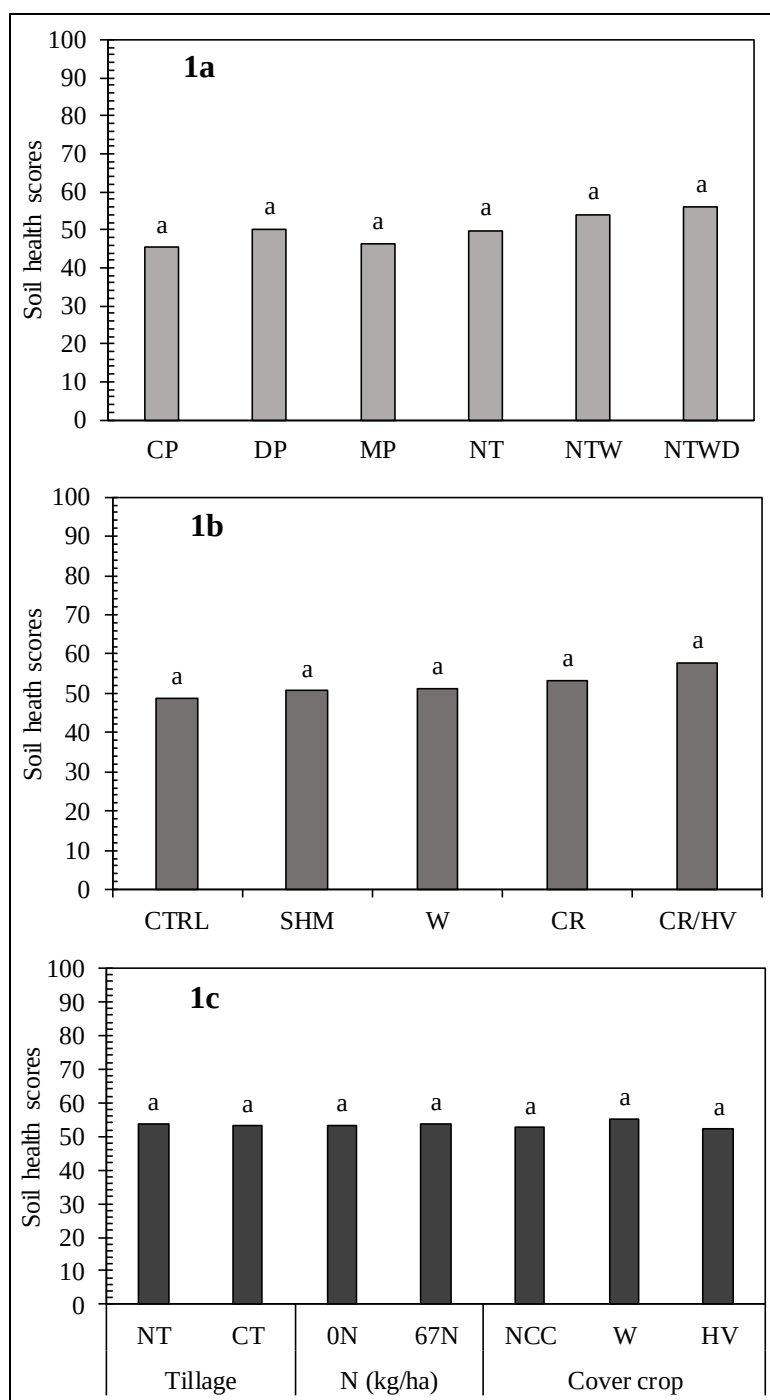


Figure 3.1 Soil health scores calculated by the Comprehensive Assessment of Soil Health (CASH) method under (1a) continuous soybean (SS), (1b) corn-soybean (CS), and (1c) continuous cotton (CC) field studies.

CP, Chisel plow; DP, Disk plow; MP, Moldboard plow; NT, No tillage; NTWD, No tillage with wheat-soybean double crop; NTW, No tillage with wheat cover crop; CTRL, No cover crop; SHM, Soil health mix; CR, Cereal rye; W, Winter wheat; CR/HV, Cereal rye and hairy vetch mix; NCC, No Cover Crop; W, Wheat; HV, Hairy Vetch; 0, 0 kg N ha⁻¹; 67, 67 kg N ha⁻¹; NT: No tillage; CT, Conventional tillage.

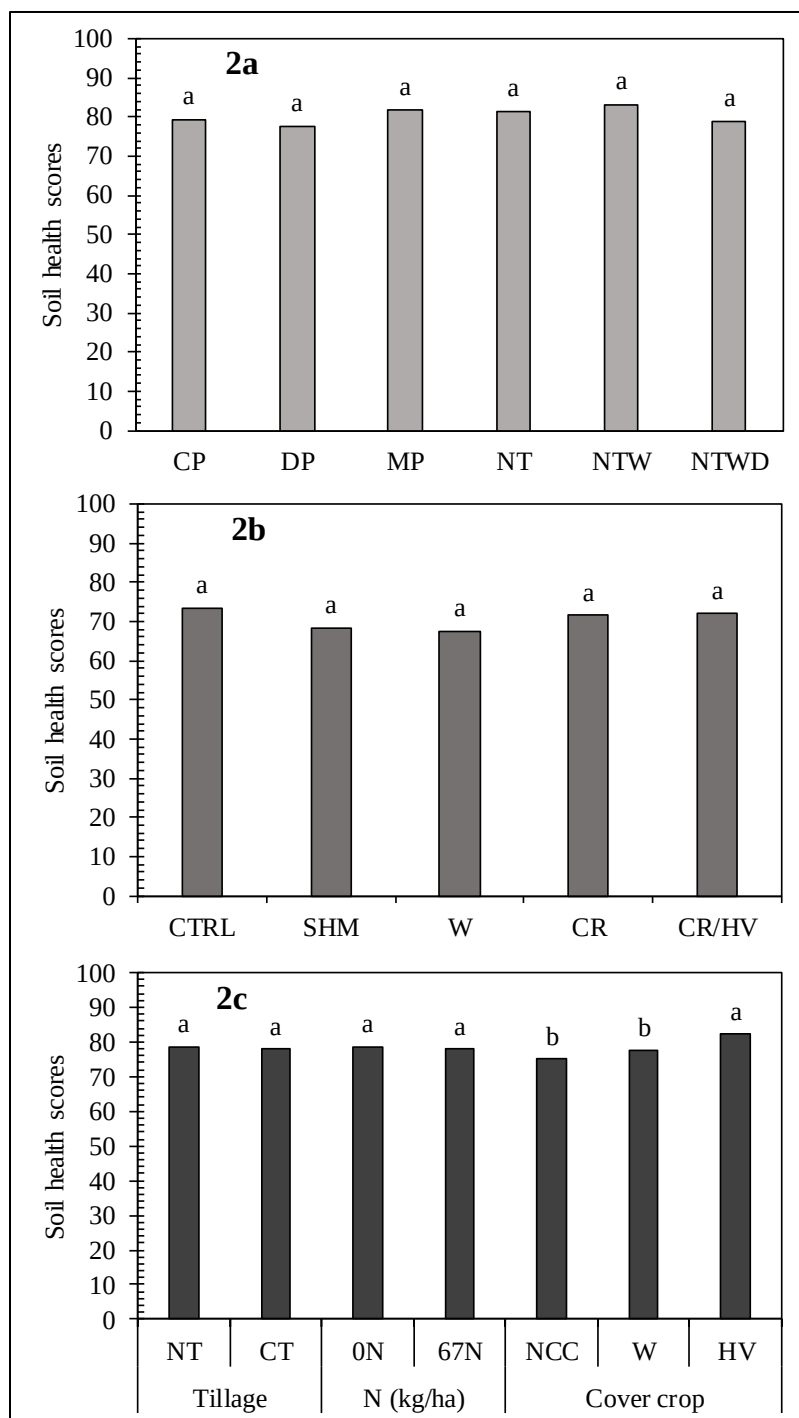


Figure 3.2 Soil health scores calculated by the Alabama soil health index (ASHI) method under (2a) continuous soybean (SS), (2b) corn-soybean (CS), and (2c) continuous cotton (CC) field studies.

CP, Chisel plow; DP, Disk plow; MP, Moldboard plow; NT, No tillage; NTWD, No tillage with wheat-soybean double crop; NTW, No tillage with wheat cover crop; CTRL, No cover crop; SHM, Soil health mix; CR, Cereal rye; W, Winter wheat; CR/HV, Cereal rye and hairy vetch mix; NCC, No Cover Crop; W, Wheat; HV, Hairy Vetch; 0, 0 kg N ha⁻¹; 67, 67 kg N ha⁻¹; NT: No tillage; CT, Conventional tillage.

Chapter 4. A weighted soil health index approach for refined assessment of soil health in cropping systems

Abstract

An effective method to assess soil health to determine regionally attainable soil health goals by adopting conservation management practices is needed for promoting sustainable crop production. However, measuring soil health is challenging due to multiple interactions among dynamic soil properties (i.e., soil health indicators) across management practices and agroecological regions. We tested the currently most popular soil health assessment methods for the cropping systems of the southeastern US and found that these methods failed to differentiate soil health under long-term conservation management from that under conventional management. Therefore, this study was conducted to develop a weighted soil health index (WSHI) based on a set of management-sensitive soil health indicators, assigning meaningful weights to indicators, and normalizing the scores based on regionally-relevant undisturbed natural reference sites. The undisturbed natural systems include Jackson woodlot (JW), Milan grassland (MG), and Milan woodlot (MW) in West Tennessee. The cropping systems include moldboard plow (MP) in continuous soybean (SS) system, no tillage (NT) in SS system, NT with wheat cover (NTW) in SS system, no cover and chisel plow (NCCT) in continuous cotton (CC) system, no cover and no tillage (NCNT) in CC system, and hairy vetch cover and no tillage (VCNT) in CC system. Out of 22 management-sensitive indicators, six were selected as a minimum dataset (MDS), which are: particulate organic C (POM-C), soil respiration from 4-day incubation (4d CO₂), small macroaggregate (0.250-2mm)-associated C (SMA-C), surface hardness (PR15), microbial biomass N (MBN), and bulk density (BD). Measured values of MDS indicators were transformed into unitless normalized scores (based on the regional range of the indicator), and finally integrated into WSHI scores using a weighted-addition approach. Additionally, the soil health gap (SHG) between the soil health of the regional reference system

and different cropping systems was calculated. Results revealed that WSHI strongly differentiated soil health between long-term conservation and conventional managements practices. The WSHI scores for cropland soils varied as follows: $VCNT = NTW > NT > NCNT \geq NCCT \geq MP$. The SHGs under MP, NCCT, NCNT, NT, NTW, and VCNT were 85.5, 79.9, 68, 45.1, 25.2, and 24.3, respectively. Results showed that the WSHI approach is effective in the realistic regional assessment of soil health and SHG can be a potential metric for comparing soil health across agroecological regions.

Introduction

Healthy soil, an important natural resource, is closely associated with an array of soil functions and ecosystems services, and considered as optimally functional and naturally resilient to disturbances (Karlen et al., 2001; Karlen et al., 2003; Kibblewhite et al., 2007). Therefore, improving soil health is key for maintaining and improving environmental quality and agricultural sustainability (Lal, 2011; Nakajima et al., 2015). Adoption of conservation agricultural management practices (e.g., reduced or no tillage, cover crops, crop rotations, etc.) tend to improve soil functions such as nutrient cycling, aggregation, and overall soil health as compared to conventional practices (e.g., intensive tillage, monoculture, etc.) (Al-Kaisi et al., 2014; Blanco-Canqui and Francis, 2016; Chalise et al., 2019; Lal, 2016; Wegner et al., 2018). However, the extent of soil health improvement/degradation in response to management also depends on agroecological regions with specific climatic and edaphic characteristics (Karlen et al., 2001; Wienhold et al., 2004). This region-specificity can act as a greater control on soil health compared to differences in agromanagement practices. Therefore, despite the pivotal role of healthy soils in building sustainable production systems, standardize/universal protocol for soil health measurement remain challenging and is currently under active debate (Bünemann et al., 2018; Larkin, 2015; Rinot et al., 2019; Roper et al., 2019; Roper et al., 2017; van Es and Karlen, 2019).

Several soil health assessment approaches currently exist including Haney soil health test (HSHT) (Haney et al., 2008; Haney et al., 2018), comprehensive assessment of soil health (CASH) (Gugino and 2007; Idowu et al., 2009; Schindelbeck et al., 2008), and Alabama soil health index (ASHI) (Bosarge, 2015). Although these methods were developed to target specific agroecological regions (i.e., HSHT for Texas, CASH for New York, and ASHI for Alabama),

their use have been reported from other regions in recent years. However, most of those studies revealed that the region-specificity of these tests limits the applicability across regions due to differences in the major soil types and climatic factors. For example, CASH showed no difference in soil health to long-term management when applied to hot-humid southeastern US (Birri, 2020; Roper et al., 2019; Roper et al., 2017), and HSHT showed the same in humid-temperate (Canada) and hot-humid (southeastern US) regions (Chahal and Van Eerd, 2018; Chahal and Van Eerd, 2019; Chu et al., 2019; Roper et al., 2017). We also found that soil health scores from HSHT (Chapter 2), CASH (Chapter 3), and ASHI (Chapter 3) did not differentiate soil health under long-term conservation management from conventional management in cropping systems of Tennessee. Therefore, this study aims to develop a new soil health indexing specific for croplands and dominant soil types of Tennessee to discern soil health of systems with conservation management from those with conventional management.

A weighted approach for developing soil health index is considered more useful in explaining relationships among soil functions, soil properties (soil health indicators), and soil health scores as the weight assignment to individual soil health indicator is based on the variability and relative importance of that particular indicator within a suite of indicators (Congreves et al., 2015; Mukherjee and Lal, 2014; Vasu et al., 2016). This process involves the selection of soil health indicators, determination of minimum dataset (MDS) using principal component analysis (PCA), scoring of indicators using scoring functions that are calibrated to the regional range of soil health indicators, assignment of weight to indicators by a variance-based method, and integration of indicators into a soil health score by a weighted-addition method (Andrews et al., 2004; Askari and Holden, 2014; Obade and Lal, 2016).

Because of the regional-specificity in management response to soil health, a set of reference or benchmark values to set upper and lower boundaries for regional soil health is important to evaluate if management practices indeed improved soil health. An undisturbed or uncultivated native soil in the vicinity of the croplands can be used as a reference for the upper bound of soil health as this reference soils most likely show the highest possible scores for soil health (Bünemann et al., 2018; Maharjan et al., 2020; Nortcliff, 2002). Similarly, a very disturbed or highly managed site can be used for setting the lower regional bound of soil health. This way of calibrating scoring functions for soil health assessment on the basis of reference sites has been suggested by several past studies (Andrews et al., 2004; Maharjan et al., 2020; Rinot et al., 2019). Such normalization of soil health scores is particularly relevant for the southeast region, including Tennessee, to avoid over and/or underestimation of soil health by using already available methods such as HSHT, CASH, and ASHI. For example, soil organic carbon (SOC) content in southeast US soils is generally lower than the soils in the midwest and northeast regions (Buol et al., 2011). So, the use of SOC scoring functions of CASH, developed using soils predominantly from the northeast region, can underestimate the potential of conservation management in building SOC in the croplands and soils of Tennessee. Similarly, using scoring functions developed predominantly from relatively degraded soils, as in the case of the ASHI test, can overestimate the soil health of degraded soils in Tennessee. Both over and underestimation of soil health scenarios were evident when we applied CASH and ASHI (chapter 3) for croplands of Tennessee. We found that all conventional and conservation management treatments showed low to medium soil health based on CASH and medium to high soil health based on ASHI. Therefore, a realistic soil health assessment of specific cropping systems and agroecological regions requires a method that takes into account region-specific

upper and lower bounds of soil health. This study aims to develop a weighted soil health index framework for the row cropping systems of Tennessee using regionally relevant upper and lower bounds of soil health and regionally sensitive set of soil health indicators.

Materials and methods

Study sites description and treatments

Soil samples for this study were collected from two ongoing long-term agricultural field experiments (continuous soybean, SS and continuous cotton, CC) and three undisturbed sites (two woodlots and one grassland) located in west Tennessee on silt loam soils. The region experiences a 30-year mean annual temperature of 15.6 °C and a mean annual rainfall of 1375 mm. Details of the SS and CC studies are presented in Chapter 2 and Chapter 3. Three undisturbed and uncultivated sites - (i) Jackson woodlot (JW), Milan woodlot (MW), and Milan grassland (MG) - were selected within the region to represent a relatively “natural undisturbed” soil conditions with no known recent agricultural history. These undisturbed natural sites were considered as benchmark or reference to determine the upper bound of regional soil health (Bünemann et al., 2018; Maharjan et al., 2020). In the SS study, treatments studied were: (i) moldboard plowing to 25 cm depth followed by disking and roller harrowing (MP); (ii) no tillage (NT); and (iii) NT with winter wheat (*Triticum aestivum* L.) cover crop (NTW). The MP treatment, representing the most disturbing conventional agricultural management, is considered to set the lower bound of regional soil health. In the CC study, treatments were: (i) no cover crop and chisel plow (NCCT), (ii) no cover crop and no tillage (NCNT), and (iii) hairy vetch cover crop and no tillage (VCNT).

Soil sampling

In January 2020, replicated (n=4) soil samples were collected from all the treatments. Approximately, 10-15 random cores were collected from each replicated treatment at a depth of 0-15 cm using a sampling probe of 2.5 cm diameter. These cores were combined and homogenized to make a composite soil sample. Additionally, four cores of 5 cm diameter and 15 cm length were collected from each treatment for bulk density (BD) measurements.

Quantification of soil health indicators

Bulk soil analysis

Soil samples were processed and analyzed for an array of physical, chemical, and biological indicators. The soil physical indicators include surface and subsurface hardness (PR15 and PR45, respectively) measured using penetrometer (Soil compaction tester penetrometer, Dickey-john corporation, Illinois, USA) (Duiker, 2002), wet aggregate stability (WAS) measured using a wet sieving apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands) (Kemper and Rosenau, 1986), soil texture measured using the hydrometer method (Gee and Or, 2002), gravimetric soil moisture, and BD measured using the core method (Grossman and Reinsch, 2002). Soil biological indicators include the soil protein index (SPI) Wright and Upadhyaya (1996) and Moebius-Clune et al. (2016), soil respiration from 4-day incubation (4d CO₂) using potassium hydroxide (KOH) trap method (Zibilske, 1994), permanganate oxidizable C (POXC) (Weil et al., 2003), soil respiration measured by Solvita[®] gel method (1d CO₂-C) (Haney and Haney, 2010), Solvita[®] labile amino N (SLAN) (Brinton (2020), soil organic C (SOC) measured by dry combustion at 950 °C using a CN analyzer (Elementar vario TOC cube in solid mode, Hanau, Germany), water extractable C (WEC) and N (WEN) (Jones and Willett, 2006), potential mineralizable N (PMN) using 7-day incubation (Drinkwater

et al., 1997), microbial biomass C (MBC) and N (MBN) using chloroform fumigation method (Brookes et al., 1985; Vance et al., 1987), extracellular enzyme activities of four C-acquiring enzymes (α -glucosidase (AG), β -glucosidase (BG), cellobiohydrolase (CBH), and β -xylosidase (XYL)), one P-acquiring enzyme (phosphatase; PHOS), and one N-acquiring enzyme (N-acetyl glucosamine; NAG) (German et al., 2011), and density fractionation based particulate organic C (POM-C) and mineral-associated organic C (MOM-C) using sodium polytungstate solution at a density of 1.6 Mg m^{-3} (Diekow et al., 2005). The soil chemical indicators include soil pH measured on a 1:1 soil:water (v/v) suspension, Mehlich-1 extractable macro- and micro-nutrients (P, K, Mg, Fe, Mn, and Zn) analyzed by Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Mehlich, 1953), and ammonium N and nitrate N using 2 M potassium chloride (KCl) extraction followed by measurement on a Skalar Continuous Flow Analyzer (Mulvaney, 1996).

Aggregate analysis

Aggregate size distribution was determined using dry sieving method by placing 100 g of air-dried and 8 mm sieved soil sample on top of a stack of sieves of sizes 2, 0.25 and 0.053 mm, and shaken using a vertical sieve shaker apparatus (CSC sieve shaker, Fairfax, VA) for 5 min at an amplitude of 0.1 mm. Aggregates were fractionated into four size classes: >2 mm (large macroaggregates, LMA), 0.25-2 mm (small macroaggregates, SMA), 0.053-0.25 mm (microaggregates, MiA), and <0.053 mm (clay- and silt-size particles, CSP). Aggregate size distribution was calculated by weighing aggregates retained on top of each sieve and expressing as a fraction of the initial amount of sample used. The mean weight diameter (MWD) was calculated using equation (1) (Youker and McGuinness, 1957):

$$\text{MWD} = \sum_{i=1}^n x_i w_i \dots\dots\dots(1)$$

where x_i is the mean diameter of aggregates size fraction on each sieve (mm), w_i is the mass of aggregates retained on each sieve, and n is the number of aggregate size fractions. After aggregate size separation, aggregate-associated SOC concentration (g kg^{-1} aggregates) in each aggregate size class was determined by dry combustion using the Elementar CN analyzer (Elementar Vario, Hanau, Germany). Aggregate-associated C fractions were classified into four categories: large macroaggregate-associated C (LMA-C), small macroaggregate-associated C (SMA-C), microaggregate-associated C (MiA-C), and clay- and silt-size particles-associated C (CSP-C).

Statistical analysis

Analysis of variance (ANOVA) were conducted using the PROC GLIMMIX procedure in SAS v9.4 statistical package (SAS, 2012) for individual soil health indicators based on a completely randomized design. Treatments were considered as fixed effects and replicates were considered as random effects in the model. Least square means were separated using the Least Significant Difference (LSD) at $p < 0.05$ to determine treatment response to each indicator. To identify a minimum data set (MDS) of indicators, only those indicators that responded statistically to at least one of the treatments ($p < 0.05$) were selected PCA, which resulted in 22 indicators. These 22 indicators were subjected to PCA using OriginPro[®] software to calculate the individual indicator weight and relationship among indicators. Based on the PCA scree plot (point of inflection), four principal components (PC) were selected. Within each PC, the indicator with the highest eigenvector value was selected for the MDS. Also, if there were other indicators in each PC with eigenvector values within 10% of the highest eigenvector values, those were selected for MDS (Andrews et al., 2002; Vasu et al., 2016). After this step, in case there were more than one indicators retained within each PC, correlation analysis was conducted

using PROC CORR procedure in SAS ($r > 0.75$, $p < 0.05$) to eliminate redundant variables showing stronger autocorrelations and low eigenvector values (Andrews et al., 2002; Flury, 1988; Li et al., 2013; Rezaei et al., 2006). Finally, the MDS comprised of six indicators (POM-C, 4d CO₂, SMA-C, PR15, MBN, and BD). The next step was to transform the measured indicator values to indicator scores on a scale of 0 to 100 using cumulative normal distribution functions (Andrews et al., 2002; Andrews et al., 2004; Moebius-Clune et al., 2016). The POM-C, 4d CO₂, SMA-C, and MBN were scored using “more is better” approach, and PR15 and BD were scored using “less is better” approach (Andrews et al., 2004; Rezaei et al., 2006). In these scoring functions, for an individual indicator, lower and upper limits of the curve were fixed based on the lower and upper limits of that particular indicator for the region. Thus, these scoring functions represent the region-specific ranges of soil health indicators (Andrews et al., 2002; Li et al., 2013). After transforming the selected indicators into unitless indexed scores, the variance-based weighting factors generated from PCA were assigned to each indicator. The weighting factor was calculated as a fraction of the proportion of variance accounted by each PC and total cumulative variance of all the selected PCs (Chahal and Van Eerd, 2019; Ray et al., 2014). In each PC, all selected indicators were assigned the same weight that was determined by dividing the weighting factor of that PC by the total number of indicators selected in that PC (Vasu et al., 2016). A weighted score of individual indicators was determined as the product of the indexed indicator score (0-100) and the assigned weight. Finally, a weighted soil health index (WSHI) score was calculated by adding weighted scores of all selected indicators (based on equation 2) (Chahal and Van Eerd, 2019; Doran and Parkin, 1994; Li et al., 2013).

$$WSHI = \sum_{i=1}^n (W_i \times Q_i) \dots\dots\dots (2)$$

Where, n = number of indicators in MDS, W_i = assigned weight of individual indicators, and Q_i = indexed score of individual indicators.

Results and discussion

Minimum dataset of soil health indicators (MDS)

From the PCA with a total of 22 indicators (15 indicators related to bulk soil and 7 indicators to soil aggregates), four PCs were selected that accounted for 82.5% cumulative variance in the dataset with the first two PCs (PC1 and PC2) accounted for 73% of the variance (Table 4.1). From each PC, indicators associated with the greatest absolute eigenvector values and within 10% of the greatest eigenvector values were selected for multiple correlation analysis to eliminate indicators which were autocorrelated (Sharma et al., 2008; Sharma et al., 2005). Accordingly, POM-C, WAS, POXC, SOC, 4d CO₂, SLAN, BG, and 1d CO₂ were selected from PC1 (Table 4.1). Due to significant positive correlation ($r > 0.75$, $p < 0.05$), WAS, POXC, SOC, SLAN, BG, and 1d CO₂ were removed, and POM-C and 4d CO₂ were retained for the MDS. In PC2, SMA-C, LMA-C, and CSP-C were selected first and then SMA-C (eigenvector of 0.42) was retained as LMA-C (eigenvector of 0.38) and CSP-C (eigenvector of 0.39) showed strong positive correlations with SMA-C ($r = 0.89$, $p < 0.05$) and lower eigenvectors than SMA-C. Following the same method, only PR15 was selected from PC3, and both BD and MBN were selected from PC4. Both BD and MBN from PC4 were retained for MDS as no strong positive correlation was found between BD and MBN ($r < 0.50$, $p < 0.05$). So, the MDS included POM-C, 4d CO₂, SMA-C, PR15, BD, and MBN.

The POM-C, reservoir of labile C pool and primary energy source for microorganisms, mainly consists of partially decomposed plant materials. This C pool is widely recognized as more responsive to management than total SOC (Christensen, 2001; Gregorich et al., 2006;

Jagadamma and Lal, 2010; Mirsky et al., 2008; Tan et al., 2007). Soil respiration, measured based on 4-day incubation of rewetted dry soil and capturing CO₂ in KOH solution, has been used as a very sensitive indicator of soil health differences among management practices (Fine et al., 2017; Franzluebbers and Veum, 2020; Moebius-Clune et al., 2016; Schindelbeck et al., 2016). Since agricultural management practices affect soil aggregation and C protection in aggregates (Al-Kaisi et al., 2005; Six et al., 1999; Six et al., 2000), aggregate-associated C fractions can serve as an early indicator for SOC changes. Bulk density has also been emphasized as an indicator for soil health assessment because of its role in deciding soil physical conditions for supporting plants (Andrews et al., 2004). Consistent with our finding, many other studies in the southeastern US region have also reported management-sensitivity of indicators such as POM-C (Motta et al., 2007), 4d CO₂ (Roper et al., 2017; van Es and Karlen, 2019), SMA-C (Singh et al., 2020), PR15 (Nouri et al., 2018; Nouri et al., 2019), BD (Mbuthia et al., 2015; Nouri et al., 2018; Nouri et al., 2019), and MBN (Mbuthia et al., 2015).

Response of indicators, indicator scores, and soil health scores to management practices

Measured values of all the indicators in the MDS varied significantly among treatments. Undisturbed natural systems showed consistently improved soil health indicator values compared to cropping systems (Table 4.2). A better response among cropping system treatments was observed when undisturbed natural systems were excluded from the model. This is attributed to the greater magnitude of weighted scores under undisturbed natural systems and associated standard deviation of means, as reported by Williams et al. (2020). Among cropping system treatments, POM-C were: MP<NCCT<NCNT=NT<NTW=VCNT. Undisturbed natural systems showed the highest 4d CO₂ compared to cropping systems. However, 4d CO₂ among the three undisturbed natural systems were statistically similar, while it was higher under NT, NTW, and

VCNT compared to MP, NCCT, and NCNT in cropping systems. Similar to POM-C and 4d CO₂, SMA-C was also higher in undisturbed natural systems as compared to cropping systems but did not differ among undisturbed natural systems.

However, when undisturbed natural systems were excluded from the model to compare cropping systems, NTW and VCNT showed higher SMA-C than MP. Among cropping systems, almost all indicators showed numerically the lowest measured values under MP treatment. Statistically, however, SMA-C was the lowest for both MP and NCNT treatments, and MBN was the lowest for both MP and NCCT. Undisturbed natural systems MG and MW showed significantly lower penetration resistance (PR15) than JW, and all undisturbed natural systems showed lower PR15 than cropping systems. Among cropping systems, higher BD were observed under no-tilled treatments (VCNT, NTW, NT, and NCNT) than tilled treatments (MP and NCCT) but MBN followed the opposite trend. However, MBN in no-tilled treatments were not different from that in the undisturbed natural systems.

Increase in POM-C, 4d CO₂, SMA-C, and MBN under undisturbed natural systems compared to cropping systems, and under no-tilled systems compared to tilled systems can be attributed to less soil disturbance in undisturbed natural and no-tilled systems compared to tilled systems, and high organic matter inputs in undisturbed natural systems and cover cropped cropping systems. In cropping systems, cover crops (e.g., hairy vetch and wheat) add more above-and below-ground biomass to soil (Blanco-Canqui et al., 2015; Kuo et al., 1997; Sainju et al., 2003), which increases C accumulation as well as stimulates microbial growth and activity. Several recent studies conducted in croplands of Tennessee reported the response of one or more indicators included in our MDS to management practices. For example, Mbuthia et al. (2015) reported enhanced MBN under hairy vetch cover crop treatment and BD as an overall key soil

health indicator in CC system. Also, BD under CC and PR15 under SS systems showed differences among treatments in Nouri et al. (2019) and Nouri et al. (2018). In addition, an increase in SMA-C under NTW and NT as compared to MP treatment in the SS system was reported by Singh et al. (2020). Similar soil properties also showed improved responses in no-tilled and cover cropped systems compared to tilled and fallow systems in other agroecological regions (Babujia et al., 2010; Blanco-Canqui et al., 2011; Poeplau and Don, 2015; Sapkota et al., 2012; Steenwerth and Belina, 2008).

The measured values of MDS indicators were transformed into unitless scores (indexed scores) using “more is better” or “less is better” scoring functions. Out of all the indicators in the MDS, the highest weighting factor (0.36) was assigned to indicators in PC1 (POM-C and 4d CO₂) as PC1 accounted for the greatest proportion of variance among selected PCs (Table 4.1). Weighting factors of 0.165, 0.063, and 0.054 were assigned to indicators in PC2 (SMA-C), PC3 (PR15), and PC4 (BD and MBN), respectively. Thus, the relative contribution of individual indicators in overall weighted soil health index (WSHI) were 36, 36, 16.5, 6.3, 2.6, and 2.6 for POM-C, 4d CO₂, SMA-C, PR15, BD, and MBN, respectively. Weighted scores for individual indicators (product of indexed scores and weight) varied significantly among management practices when compared with and without undisturbed natural systems (Table 4.3). In general, among all the treatments, undisturbed natural systems showed significantly higher weighted scores for most indicators than cropping system treatments.

Among cropping systems treatments, weighted scores of POM-C, 4d CO₂, SMA-C, and MBN were greater under no-tilled treatments, NTW and VCNT than under tilled treatments, MP and NCCT (Table 4.3). However, weighted scores of PR15 and BD were relatively higher under tilled treatments despite no statistical difference between tilled treatments except for PR15 which

was higher under MP than NCCT. Overall, weighted scores of MDS indicators followed an almost identical trend to measured indicator values under different managements (Tables 4.2 and 4.3). Among SS system treatments (MP, NT, and NTW), weighted indicator scores of all indicators were lower for the intensive MP tillage. Among CC system treatments (NCCT, NCNT, and VCNT), weighted scores of all indicators were the lowest for NCCT. Consequently, relative WSHI scores were statistically different among these treatments (Fig. 4.1).

The relative WSHI scores were calculated by assigning the maximum WSHI score of 100 to the MW system because MW had the highest absolute WSHI score of 83 (sum of weighted scores of indicators in MDS, Table 4.3). The WSHI scores of MW did not vary from other undisturbed natural systems (JW, and MG), however, WSHI scores of all undisturbed natural systems were higher than that of cropping system treatments. The intensively tilled MP treatment received the lowest WSHI score, however, it was not different from NCCT and NCNT scores when undisturbed natural systems were included in the model. When cropping systems were compared without undisturbed natural systems in the model, VCNT and NTW showed greater WSHI scores than other treatments. Overall, WSHI scores varied as follows: VCNT (70.3) = NTW (69.5) > NT (51) > NCNT (29.7) ≥ NCCT (18.6) ≥ MP (13.5). The treatment response of WSHI scores were consistent with the treatment response of individual indicators (Tables 4.2 and 4.3).

The multivariate analysis combined with the weighted scoring approach we used for quantitative soil health evaluation has been assessed in many past studies for a wide range of managements (Bastida et al., 2008; Sinha et al., 2009; Zhang et al., 2011). Many such studies revealed that the weighted approach could discern efficient interrelationships among indicators and generate meaningful overall soil health index (Congreves et al., 2015; Sinha et al., 2009;

Vasu et al., 2016; Zornoza et al., 2015). Although the region-specific determination of MDS and the consequent indicator weighting process used in our study is mostly applicable to the cropland soils in the west Tennessee, this process of generating MDS and WSHI could easily be adapted to other regions and cropping systems.

Soil health gap (SHG)

Due to the regional specificity of soil health assessments, the need for accounting for regional reference or benchmark soil health is obvious. Since the historical mismanagement of soil is attributed to the deterioration of soil health (Karlen and Rice, 2015), assessing the soil health gap (SHG) between cropping system management and a nearby undisturbed natural system could quantify the magnitude of soil health degradation/improvement under specific management (Maharjan et al., 2020). Soil health gap, a concept similar to extensively used “yield gap”, could be utilized as a universal standard to assess the performance of conservation management practices to build soil health. Additionally, SHG can provide a realistically achievable regional soil health goal when conventional systems are transitioned to more sustainable systems by adopting conservation practices (Maharjan et al., 2020). The SHG (the difference between the WSHI score of undisturbed natural system and cropping system treatments) from our study is presented in Fig. 4.2. Since WSHI scores among the three undisturbed natural systems were not significantly different, we used a single reference system, that was generated by averaging the WSHI scores across undisturbed natural systems and assigning a maximum score of 100 to the undisturbed natural system.

The SHGs under MP, NCCT, NCNT, NT, NTW, and VCNT were 85.5, 79.9, 68, 45.1, 25.2, and 24.3, respectively (Fig. 4.2). Higher SHG under MP is expected and it reiterates the degraded soil health due to 41 years of intensive MP tillage in a soybean monoculture system.

This also demonstrates a great opportunity for soil health enhancement in heavily tilled monoculture cropping systems. Since undisturbed or native soils are most probably reached equilibrium under a given climatic condition, it is logical to consider them as reference for most attainable soil health (Bünemann et al., 2018; Nortcliff, 2002). Use of SHG for regional soil health assessment and as a potential universal soil health index deserves more attention as the SHG concept can address multiple ongoing challenges in soil health assessment due to differences in indicator selection, analytical protocols, soil types, climates, and conservation management practices.

Conclusions

To our knowledge, this is the first study that assessed the soil health of Tennessee croplands using a weighted soil health index (WSHI) approach utilizing regional undisturbed natural systems as benchmark soils. This approach under a multivariate framework showed promise in efficiently discerning the differences in soil health among various conservation and conventional managements in the tested cropping systems. Though we proposed a region-specific approach, this process of deriving WSHI can easily be adapted to other regions and cropping systems. Further, the soil health gap (SHG) concept applied in this study revealed the extent of degradation of soil health under intensive cropping systems management practices and showed promise to be used as a standardized tool to assess soil health across agroecological regions.

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Appendix 4

Table 4.1 Eigenvectors of soil health indicators used in the principal component analysis for developing minimum dataset.

Indicators	Eigenvectors			
	PC1	PC2	PC3	PC4
Bulk soil analysis				
WAS (%)	0.24	-0.04	0.25	-0.11
BD (g cm ⁻³)	0.17	-0.17	-0.07	0.58*
PR15 (MPa)	0.13	-0.30	0.57*	0.16
MWD (mm)	0.22	-0.27	0.20	-0.24
POM-C (mg kg ⁻¹ soil)	0.26*	-0.06	-0.15	0.02
SOC (mg kg ⁻¹ soil)	0.25	0.09	-0.24	-0.03
POXC (mg kg ⁻¹ soil)	0.25	0.07	-0.20	-0.07
MBC (mg kg ⁻¹ soil)	0.21	0.01	-0.16	-0.24
MBN (mg kg ⁻¹ soil)	0.17	-0.05	0.14	0.52
BG (nmol g ⁻¹ soil)	0.24	0.00	-0.34	0.07
MOM-C (mg kg ⁻¹ soil)	0.23	-0.11	-0.07	0.21
4d CO ₂ (mg g ⁻¹ soil)	0.24	0.11	-0.04	0.00
1d CO ₂ (mg CO ₂ -C kg ⁻¹ soil)	0.25	0.14	-0.17	-0.09
SLAN (mg NH ₃ -N kg ⁻¹)	0.24	-0.08	-0.01	0.06
Nitrate-N (mg kg ⁻¹ soil)	0.19	0.02	-0.19	0.09
Aggregate fraction analysis				
LMA-C (mg kg ⁻¹)	0.15	0.39	0.16	0.06
SMA-C (mg kg ⁻¹)	0.14	0.42*	0.33	-0.02
MiA-C (mg kg ⁻¹)	0.21	0.29	0.22	-0.16
CSP-C (mg kg ⁻¹)	0.19	0.38	0.15	0.08
LMA (%)	0.22	-0.27	0.07	-0.25
MiA (%)	-0.21	0.25	-0.10	0.25
CSP (%)	-0.21	0.20	0.00	0.10
PCA outputs				
Eigenvalue	13.04	2.98	1.13	0.98
Proportion of variance (%)	59.3	13.6	5.15	4.45
Cumulative variance (%)	59.3	72.9	78.0	82.5
Weighting factor	0.719	0.164	0.062	0.054

WAS, wet aggregate stability; BD, bulk density; PR15, surface hardness; MWD, mean weight diameter; POM-C, particulate organic matter C; SOC, soil organic C; POXC, permanganate oxidizable C; MBC, microbial biomass C; MBN, microbial biomass N; BG, β -glucosidase activity; MOM-C, mineral-associate organic matter C; 4d CO₂, KOH trap based CO₂ from 4-day incubation; 1d CO₂, Solvita based CO₂ from 1-day incubation; SLAN, Solvita labile amino N; LMA-C, large macroaggregate-associate C; SMA-C, small macroaggregate-associate C; MiA, microaggregate-associate C; CSP-C, clay- and silt-sized particle-associated C; LMA, macroaggregate; MiA, microaggregate; CSP, clay- and silt-sized particles. *Indicator with the highest eigenvector value within each PC.

Table 4.2 Response of measured values of indicators included in the minimum dataset under different treatments

Treatments	POM-C	4d CO ₂	SMA-C	PR15	BD	MBN
	g kg ⁻¹ soil	mg g ⁻¹ soil	g kg ⁻¹	MPa	g cm ⁻³	g kg ⁻¹ soil
JW	2.4ab	0.60a	16.2a	1.13e	1.27d	22.0bcd
MG	2.1b	0.60a	14.1a	0.302f	1.20e	36.2a
MW	2.8a	0.57ab	17.5a	0.200f	1.15e	33.2ab
MP	0.6dD	0.17dB	7.5bBC	1.61dD	1.42cB	19.0cdB
NCCT	0.9dC	0.23dB	8.8bAB	1.76cdCD	1.43bcB	15.6dB
NCNT	1.6cB	0.21dB	7.0bC	2.18aA	1.51aA	31.1abcA
NT	1.5cB	0.48cA	9.2bAB	2.13abAB	1.48abcAB	31.4abA
NTW	2.2bA	0.50bcA	9.4bA	1.93abcBC	1.50abA	29.8abcA
VCNT	2.4abA	0.47cA	9.6bA	1.91bcBC	1.52aA	29.6abcA

Numbers followed by different lowercase letters within a column are significantly different across all treatments at $p < 0.05$. Numbers followed by different uppercase letters within a column are significantly different across only cropping system treatments at $p < 0.05$. JW, Jackson woodlot; MG, Milan grassland; MW, Milan woodlot; MP, moldboard plow under continuous soybean (SS) system; NCCT, no cover with chisel plow under continuous cotton (CC) system; NCNT, no cover with no tillage under CC system; NT, no tillage under SS system; NTW, NT with wheat cover under SS system; VCNT, hairy vetch cover with no tillage under CC system; POM-C, particulate organic matter C; 4d CO₂, KOH trap-based CO₂ from 4-day incubation; SMA-C, small macroaggregate-associate C; PR15, surface hardness; BD, bulk density; MBN, microbial biomass N.

Table 4.3 Response of index score values of indicators included in the minimum dataset under different treatments

Treatments	POM-C	4dCO ₂	SMA-C	PR15	BD	MBN
JW	26.8ab	29.9a	10.4a	4.29b	2.12b	0.639c
MG	23.8b	30.5a	8.46a	6.1a	2.47ab	2.34a
MW	31.7a	28.5ab	11.7a	6.21a	2.55a	2.02ab
MP	2.02dC	2.73dB	2.19bBC	2.74cA	1.12cA	0.338cB
NCCT	3.46dC	5.13dB	3.3bABC	2.25cdAB	1.07cdAB	0.178cB
NCNT	15.1cB	4.16dB	1.97bC	1.08eD	0.546eC	1.77bA
NT	12.7cB	22.1cA	3.63bAB	1.24eCD	0.706deABC	1.8abA
NTW	25.7abA	24bcA	3.78bA	1.71deBCD	0.628eBC	1.61bA
VCNT	28.2abA	22.2cA	3.96bA	1.78deBC	0.502eC	1.59bA

Numbers followed by different lowercase letters within a column are significantly different across all treatments at $p < 0.05$. Numbers followed by different uppercase letters within a column are significantly different across only cropping system treatments at $p < 0.05$. JW, Jackson woodlot; MG, Milan grassland; MW, Milan woodlot; MP, moldboard plow under continuous soybean (SS) system; NCCT, no cover with chisel plow under continuous cotton (CC) system; NCNT, no cover with no tillage under CC system; NT, no tillage under SS system; NTW, NT with wheat cover under SS system; VCNT, hairy vetch cover with no tillage under CC system; POM-C, particulate organic matter C; 4d CO₂, KOH trap-based CO₂ from 4-day incubation; SMA-C, small macroaggregate-associate C; PR15, surface hardness; BD, bulk density; MBN, microbial biomass N.

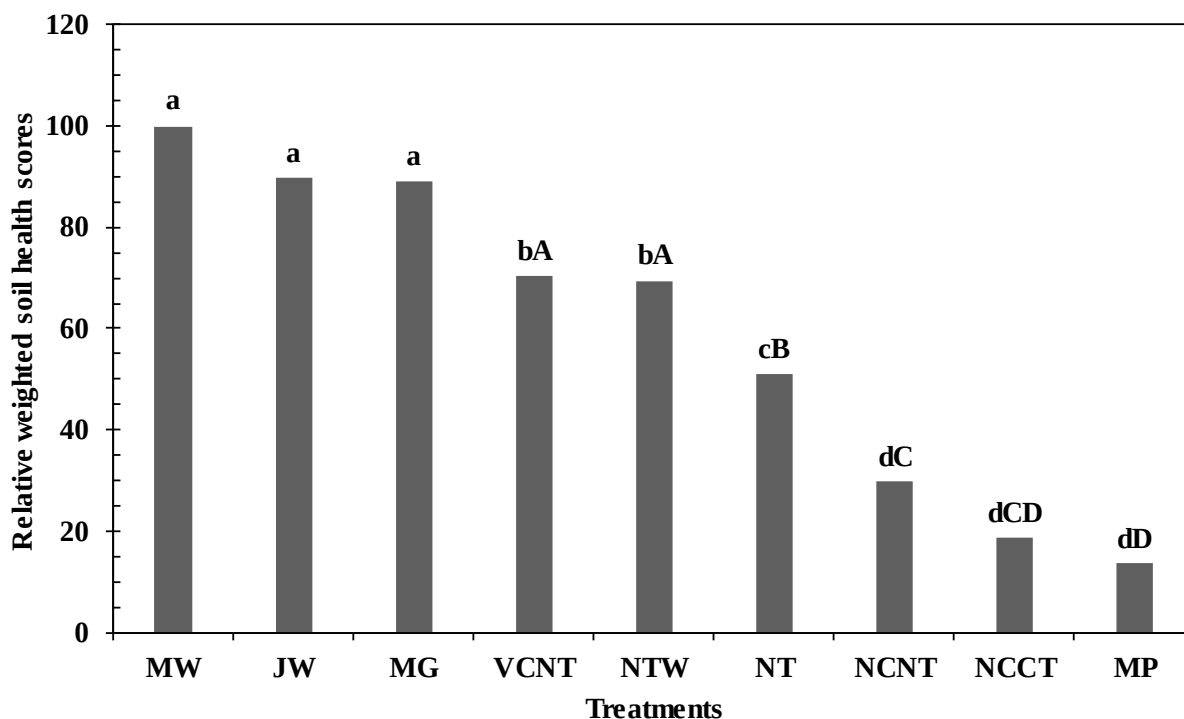


Figure 4.1 Relative weighted soil health index (WSHI) scores under different cropping and undisturbed natural systems.

Different lowercase letters denote significantly different across all treatments at $p < 0.05$. Different uppercase letters denote significantly different across only cropping system treatments at $p < 0.05$. MW, Milan woodlot; JW, Jackson woodlot; MG, Milan grassland; VCNT, hairy vetch cover with no tillage under continuous cotton (CC) system; NTW, no tillage with wheat cover under continuous soybean (SS) system; NT, no tillage under SS system; NCNT, no cover with no tillage under CC system; NCCT, no cover with chisel plow under CC system; MP, moldboard plow under continuous SS system.

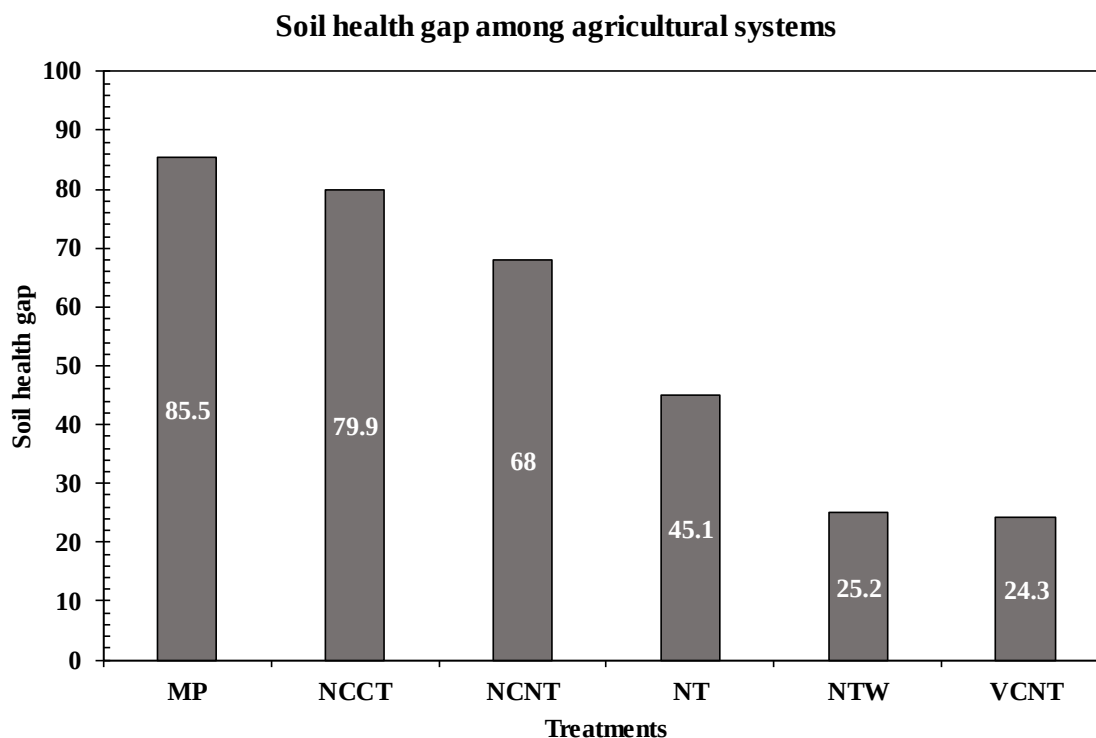


Figure 4.2 Soil health gap (SHG) of agricultural systems under different managements in relation to the natural undisturbed systems.

MP, moldboard plow under continuous soybean (SS) system; NCCT, no cover with chisel plow under continuous cotton (CC) system; NCNT, no cover with no tillage under CC system; NT, no tillage under SS system; NTW, no tillage with wheat cover under SS system; VCNT, hairy vetch cover with no tillage under CC system.

Chapter 5. General conclusions

This dissertation research evaluated the feasibility of Haney's Soil Health Test (HSHT), Comprehensive Assessment of Soil Health (CASH), and Alabama Soil Health Index (ASHI) to assess soil health in diverse cropping systems of Tennessee. Specifically, the overall soil health index and the underlying soil health indicators derived from these three tests were evaluated for their sensitivity to management changes under three existing cropping system trials in Tennessee such as continuous soybean (SS), corn-soybean (CS), and continuous cotton (CC) trials. Despite some individual indicator responses, HSHT, CASH, and ASHI failed to consistently differentiate soil health across different tillage, cover crops, and N rate treatments in the tested cropping systems. These results revealed the limited applicability of the existing soil health tests for Tennessee cropping systems. Therefore, a set of soil health indicators sensitive to management treatments in the tested cropping systems were selected and integrated using regionally-meaningful scoring functions (taking into account the relatively undisturbed benchmark soils) to develop a weighted soil health index (WSHI) to effectively differentiate soil health of conservation systems from conventional systems. The principal component analysis was used to develop a minimum data set of soil health indicators. For better explaining the interrelationships of selected indicators, weights were assigned to indicators followed by using a weighted-addition approach to calculate WSHI scores. The WSHI was found to be more sensitive to conservation and conventional management practices compared to existing tests such as CASH, HSHT, and ASHI. Based on WSHI, 40 years of intensive tillage (moldboard plow; MP) in the SS cropping system received the lowest, and undisturbed natural systems received the highest scores. Among cropping system treatments, hairy vetch cover crop combined with no tillage in the CC system (VCNT) and wheat cover crop combined with no tillage in the SS system (NTW) showed the highest soil health scores. Further, the soil health gap (SHG), calculated as the difference in the

WSHI of cropping systems from that of undisturbed natural sites, showed the utility of the SHG approach for assessing the extent of agricultural management-induced soil health degradation.

The highest SHG was observed under intensively tilled MP treatment, while the lowest was found under VCNT and NTW treatments. The SHG approach shows promise as a potential tool to compare soil health across agroecological regions. Our research highlights the need for further testing the usefulness of existing soil health tests across regions and cropping systems, and/or developing regionally-weighted soil health tools like our WSHI and SHG for more meaningful soil health assessments.

Chapter 6. Future research directions

1. Temporal variability in soil health

Even though soil health encompasses the continued capacity of soil to optimally function in the long-term, it is measured largely from a single snapshot of soil sampling. If measured over time within a single growing season and over multiple years, it is highly likely that the management sensitivity of many soil health indicators increases with resultant variations in final soil health score. For example, Nouri et al. (2019) reported that the effect of 34 years of disc tillage on bulk density (BD) was variable when measured at different time points. The BD measured before annual tillage application did not vary significantly between tilled and no-tilled treatments but that measured after the tillage application was significantly different between tillage treatments. Similarly, the significant influence of two sampling times (June vs. October) on several soil health indicators (e.g., total organic matter, microbial biomass C and N, microbial community structure, and extractable soil N, P, and K) were found (Zhao et al., 2014). Albuquerque et al. (2012) also reported that treatment influence on soil physical (e.g., wet aggregate stability), chemical (e.g., extractable nutrients, soil pH, electrical conductivity, total N, and ammonium-N, and nitrate-N), and biological (e.g., total organic C, microbial biomass C and N, enzymatic activities, and soil respiration rate) indicators varied significantly at five pre- and post-crop sampling times. This indicates that time and frequency of soil sampling for indicator measurement is nontrivial. Various studies had also reported temporal variability in soil health dynamics, especially when comparing agricultural systems to native or undisturbed reference sites (Reeder et al., 1998; Matamala et al., 2008; Rosenzweig et al., 2016; De et al., 2019). Therefore, future research should focus on monitoring the temporal variability in soil health from the tested agroecosystems.

2. Need for defining the sustainable level of inputs needed in managed systems

Assessing soil health of agroecosystems in relation to a native or unmanaged site offers a realistic and regionally relevant measurement of soil health (Maharjan et al., 2020). However, this approach assumes that native sites offer higher soil health status than the managed sites, which is not the case always. For example, at a particular time point, measured values of many soil health indicators, and therefore the overall soil health score, can be improved in managed sites by applying a substantial level of inputs (e.g., synthetic fertilizers, organic amendments, irrigation, etc.). Once these inputs are discontinued, the health of these soils will most probably return to their pre-input application state. Therefore, there is a need to focus future research to define the sustainable levels of inputs needed in managed systems to maintain soil health over a longer period.

3. Variability in sampling depth, sampling methods, and analytical methods for measuring soil health indicators

Variability in the measurement of soil health indicators is an ongoing challenge in soil health assessment. For example, many studies reported variability of soil health indicators due to sampling depth (Černohlávková et al., 2009; Jagadamma et al., 2019), sampling methods (Sharma et al., 2020; Crozier et al., 2017; Cornell University, 2016), and analytical methods (Sainju et al., 2006; Roper et al., 2019; Stott, 2019). Jagadamma et al. (2019) reported that management practices influenced total and active C greatly in the top few centimeters of the soil profile. Also, BD measurement from the same site varied with the type of sampling probes, leading to different total C pool values (Sharma et al., 2020). Similarly, different results were observed when wet aggregate stability was determined using the Cornell sprinkler infiltrometer (Schindelbeck et al., 2016) and the wet sieving method (Kemper and Rosenau, 1986). Regardless

of these differences, the existing soil health assessment methods follow different sampling and analytical methods. These variations play a huge role in hindering the cross-regional comparison and applicability of soil health assessment methods (Stott, 2019). Therefore, future research should be focused on developing either standardized sampling and analytical protocols for measuring soil health indicators for wider application or regionally-suited methods that are used only for the region in which they were developed.

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VITA

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