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Laboratory Methods for Soil Health Assessment: An Overview

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The purpose for Volume II is to provide specific methods and guidelines available for individuals and laboratories to evaluate soil health indicators discussed in Volume I. This volume draws on and updates the 1996 Soil Science Society of America Special Publication Number 49 entitled *Methods for Assessing Soil Quality* that is commonly referred to as the “Green Book” for soil quality and soil health assessment. This volume, however, is not merely a revision of the 1996 book, but rather adds guidelines for several new soil health assessment tests and discusses advances in data interpretation made during the past two decades.

Soil health is defined widely as *the continued capacity of a soil to function as a vital living ecosystem that sustains plants, animals, and humans* (e.g., NRCS, 2020). In recent years, the concept of soil health has become better understood and more widely accepted in the United States and around the world. An important driver for increased interest and global acceptance of the concept is public recognition that to meet food, feed, fiber, and fuel demands associated with an increasing population, soil degradation through erosion and loss of soil organic carbon (SOC) must be stopped and reversed by enhancing desirable biological, chemical, and physical properties and processes within this living, dynamic resource. Thus, over the past 25 years, soil health has become a focal point for serious attention across a range of public- and private-sector agricultural, environmental, and conservation organizations. Collectively, these groups have identified numerous benefits to farmers; the agricultural industry as a whole; water, air and other natural resources; educators;

and the general public. This includes identifying and implementing soil health-promoting practices (e.g., cover crops, reduced intensity and frequency of tillage, improvements in and expanded use of perennials, site-specific soil and crop management) that can increase SOC (Ismail et al., 1994; Karlen et al., 1994; Ussiri and Lal, 2009; Varvel and Wilhelm, 2010; Wander et al., 1998), thereby increasing available water holding capacity, enhancing drought resistance and resilience (Emerson, 1995; Hudson, 1994; Olness and Archer, 2005), reducing wind and water erosion, and reducing nutrient loss to surface waters (Langdale et al., 1985; Tonitto et al., 2006; Yoo et al., 1988; Zhu et al., 1989). Additional benefits associated with improvements in soil health include increased suppression of pests and pathogens, increased crop yield and quality, improved return on investment, and many broad, nonpoint environmental benefits. Agricultural productivity, economic return, and environmental goals all benefit from enhancing soil health.

The literature on soil health, including the implementation of practices and technologies that promote it, has exploded in recent decades. In a search of literature covered by Google Scholar, Brevik (2018) identified more than 20,000 references using the term “soil health” from January 2000 through February 2018. This represented more than 93% of the total number of references recovered. However, our understanding of soil health and its benefits did not develop over just the past 20 years; indeed, the idea of promoting good soil health is more than 100 years old and has a surprising history. Brevik writes, “The earliest clear reference to soil health found in this review was made by Wallace (1910), who wrote about the importance of humus, particularly as obtained from manure, in maintaining soil health”. The author of this 1910 reference (a thesis submitted to Iowa State University) was a student who eventually would become President Franklin Roosevelt’s Secretary of Agriculture, then Roosevelt’s vice-president. The name of this earliest known user of the term “soil health” will be familiar to many, as it remains before us today on the largest research station operated by the U.S. Department of Agriculture: the Henry A. Wallace Agricultural Research Center in Beltsville, MD. Thus, the concept of soil health has a particularly illustrious pedigree in the history of agricultural science.

Research on soil properties— physical, chemical, and biological— has led to major advances in managing agricultural soils, contributing to significant crop yield increases throughout the 20th and 21st centuries. However, consensus on a holistic approach to understand, implement, and measure outcomes of soil management, with goals that include sustaining production and enhancing soil health, has escaped the scientific community. Reasons for this include: (i) ever-changing methods of measurement and how to interpret the data, especially for biological properties and processes; (ii) how to adapt analytical methods for soils having different properties, which may alter results and make data comparisons difficult;

(iii) the meaning of analytical results for different agricultural production systems and environments; (iv) unclear links among measurements, soil processes, and desired outcomes (ecosystem services such as agricultural yield, nutrient cycling, improved water quality, etc.); (v) complexity and costs for advanced measurement techniques; (vi) differences in sample handling and measurement protocols among analytical laboratories; (vii) producers' uncertainties about what the data mean and how to adjust management practices in response to the information, including potential risks and benefits; and (viii) inconsistent messaging about soil health and how to manage it to agricultural producers, natural resources managers, educators, policymakers, and other stakeholders.

Stakeholder diversity alone presents significant challenges to the community of scientists, practitioners, producers, and others who advocate making soil health the cornerstone of agricultural and environmental decision making. The needs of different segments of the community demand different kinds of data, information (interpretation of the data), and communication techniques. For example, the interests of a typical agricultural producer are unlikely to be met with a report on 20 to 30 laboratory measurements that quantify a range of physical, chemical, and biological properties of a soil. Such a report may be more than most producers would want to interpret. On the other hand, a small group of indicators, easily obtained and explained, might be helpful to a producer but insufficiently accurate, precise, and process-oriented for scientific research. The distinctly different needs of various stakeholders provide a critical starting point for any conversation about soil health.

How Can a Farmer Assess Soil Health in the Field?

Many producers are keen to learn about soil health on their farms and how they can alter their current soil and crop management practices to sustain or improve it. This interest has greatly increased opportunities for agricultural experts who can successfully bridge researcher and producer communities and is a key factor driving development of public and private programs that strive to strive for clear communication about soil health. For example, pasture and range scientists affiliated with the Noble Research Institute in Ardmore, OK, often advise farmers to consider five indicators (Jeff Goodwin, personal communication, 2018), which we summarize here as “the Five C’s of Soil Health”. They are:

- **Color**– A healthy soil’s dark brown color indicates the presence of a lot of carbon in the form of decomposed organic matter. In contrast, gray, yellow, or mottled colors indicate soil that has a low carbon content, is poorly drained and poorly aerated, and likely low in nutrients available to plants.

- **Crumbs**– A soil that is crumbly, like coffee grounds or cake crumbs, and holds that aggregate structure is likely in good physical condition supporting soil health. This is structure that allows water movement yet aeration, as well as root penetration. It holds up even when the soil is wet. If the dry soil can easily be ground to dust between the fingers, or it turns into a slick film when wet and rubbed between the forefinger and thumb, the aggregates are not stable and will not support a good crop.
- **Critters**– A healthy soil shows lots of evidence of life. Pulling the crop debris back from the surface should reveal earthworms, or their holes and castings. Turning over the soil with a shovel should uncover insects, pillbugs, and other arthropods essential in carbon and nutrient cycling. A low-power hand lens might allow observation of smaller arthropods such as mites that feed on debris and microbes, and perhaps even the filamentous hyphae of fungi or the near-microscopic worms that feed on them. A soil that lacks evidence of diverse life is not healthy.
- **Cooperation**, with roots, that is– A healthy soil does not constrain roots, its structure allows plant roots to grow vertically and laterally. When roots look stunted or turn at odd angles, it is likely that the soil is compacted or has a plow layer that obstructs root growth because it lacks good structure and aeration for a crop. Stubby, deformed, discolored, or rotten roots can also indicate the presence of parasitic nematodes, plant-feeding insect pests, or pathogenic microbes in the soil, none of which is desirable for a healthy soil.
- **Cologne**– A healthy soil has a fragrant, earthy aroma, indicative of the many aerated biological processes happening. A soil that has a sour or rotten-egg odor is poorly aerated, probably because of poor structure and poor drainage, and is not likely to be a hospitable environment conducive to plant root development or beneficial microbes.

What Do Researchers Need, and Can They Reach Consensus?

The Five C's of Soil Health may be useful to a farmer, and they can use them to consider modifications to production practices that could push the soil toward more desirable characteristics. For research purposes, however, these indicators are insufficiently quantitative, repeatable, and explanatory for statistical analyses and hypothesis testing about soils at different locations, under different production systems, or subjected to different management practices. For those needs, measurements that are highly repeatable and based on standardized protocols and techniques within research laboratories are needed.

At this other extreme, a new set of challenges arises—how to get a representative sample, how to handle and store it before it can be analyzed, which properties to measure, which measurement method to use, how to report the data, how to develop recommendations from those data. Just as a physician cannot adequately describe the health of a human patient with a small number of measurements or distillation of many measurements into a single number, scientists must rely on multiple different indicator measurements to provide a scientifically meaningful assessment of a soil's health. Preferences regarding specific measurements to make and methods to use are no doubt numerically equal to the number of scientists wanting to assess soil health. Reaching consensus in the community has been and continues to be a difficult task.

Currently, there are two integrated and coordinated efforts to identify suitable soil health indicator measurement protocols and to assess their utility throughout the country. One, led by the U.S. Department of Agriculture—Natural Resources Conservation Service (USDA-NRCS) Soil Health Division (SHD), is Soil Health Technical Note No. 450–03 (Stott, 2019) entitled “Standard Indicators and Laboratory Procedures to Assess Soil Health.” The other is a research project led by the Soil Health Institute (SHI), which is evaluating the utility of analytical methods to determine the usefulness of over 30 soil health indicators across much of North America. Methods addressed in this volume are applicable to both efforts and reinforce the concept that data on physical, chemical, and biological properties and processes all must be obtained for a full understanding of a soil's health.

Both the SHI and NRCS-SHD efforts obtained input from researchers, farmers, soil-testing laboratories, non-governmental organizations (NGOs), and representatives of state and federal agencies starting in 2013, when the two longest-serving agricultural foundations in the United States (Farm Foundation, established 1933; and The Samuel Roberts Noble Foundation, established 1945) partnered to design and initiate the Soil Renaissance effort. Several workshops were organized and facilitated to identify and strive for consensus regarding appropriate indicators of soil health. Each workshop was attended by a different mixture of university, government, and private industry scientists, field conservationists, and farmers. Technical discussion papers were written by teams of scientists from the U.S. Department of Agriculture and land-grant universities. Between 2014 and 2016, many measurement-related issues and challenges were assessed, including the status of existing soil health measurement frameworks; the benefits of a “tiered” approach for measurements at different stages of development and reliability; service lab adoption issues; data needs; communications plans; data interpretation, including issues related to different regions; sampling protocols; sample archives; quality assurance/quality control (QA/QC) protocols; and sampling frequency. To further the vision of the Soil Renaissance and implement its findings, the Soil Health Institute was created in 2015. In June 2017, the SHI used input

from the Soil Renaissance effort to conduct a survey of 179 individuals who were active in the measurement-related workshops organized by the SHI and/or the Soil Renaissance over the three years. A consensus emerged among the 48 respondents that many of the measurements used to characterize soil conditions for many years are also valuable for assessing soil health. These measurements—physical and chemical, supplemented with a few key biological—are well-accepted in the scientific community and thus were designated by the Soil Renaissance participants as “Tier 1” indicators. They can be used directly or as ancillary factors needed to improve the interpretation of yet other measurements. They include:

- Physical:
 - Soil texture
 - Water-stable aggregation
 - Bulk density
 - Water penetration resistance
 - Visual rating of erosion
 - Infiltration
 - Available water holding capacity
- Chemical:
 - Routine inorganic chemical analysis (N, P, K, micronutrients, pH, cation exchange capacity, base saturation, electrical conductivity)
 - Soil organic carbon
- Biological:
 - Short-term carbon mineralization (respiration)
 - Nitrogen mineralization
 - Crop yield

The Soil Renaissance, SHI, and NRCS-SHD communities also identified a group of measurements that have been designated “Tier 2”, mostly biological properties or processes in soil, for which there is scientific consensus that they are related to soil health but are less standardized with regard to measurement methods, interpretation, and known thresholds for management action. These indicators are identified in the SHI Action Plan (www.soilhealthinstitute.org, accessed February 20, 2020) as targets for research to develop sufficient response data to complete their development as reliable measurements. To achieve those goals, the Tier 2 indicators listed below need further development, testing, and evaluation on working farms so they can eventually be transferred and communicated to land-owners, operators, and retailers as tools for improving soil and crop management practices. They include:

- Beta-glucosidase activity (organic matter decomposition)
- Macro-aggregate stability (water partitioning)

- Permanganate oxidizable carbon (carbon food source for microbes)
- Soil protein (bioavailable nitrogen)
- Ester-linked fatty acid methyl ester; phospholipid fatty acid (microbial community structure, diversity)
- Nematode population densities (trophic levels)
- Pathogenic fungi populations or bioassays (pathogen activities and host ranges)

The SHI, SHD and Soil Renaissance communities also identified a category of measurements designated “Tier 3”, which are primarily measurements of soil biological properties or processes for which, again, there is scientific consensus that they are quite likely related to soil health, but they still require major research and development investments to determine whether they reveal information that can be used to improve soil and crop management decisions. Fundamental biological and agricultural principles suggest Tier 3 indicators may be very useful eventually for assessing soil health and making management decisions, provided significant research investments in their development are aggressively pursued. Therefore, Tier 3 measurements are worthy subjects of further research on long-term research sites and on-farm evaluations where there are detailed records of environmental conditions and management practices over enough years that Tier 3 measurements can be interpreted reliably. Prominent among such measurements are metagenomic analyses to reveal information about soil microbial populations, community structure, and diversity, as influenced by the status and trends of soil health and in relation to the history of environmental conditions and management practices on exceptionally well-characterized sites.

Consensus on *what* to measure is just part of the research associated with soil health measurements. There is also a need to reach consensus on *how* to measure each indicator, which can be very challenging and even contentious within the soil science and agronomic research communities. In the case of Tier 1 indicators, many analytical methods for measurements are widely accepted, for example, Soil Science Society of America Book Series 5, *Methods of Soil Analysis, second edition—Part 1, Physical and Mineralogical Methods* (1986); *Part 2, Chemical and Microbiological Properties* (1982); *Part 3, Chemical Methods* (1996). Variations in specific methods have been adapted in response to recommendations from research conducted in university, government, and private laboratories to obtain optimal, meaningful results for different soils collected from widely different locations and environments. These methods are in use for several different frameworks for soil health assessment (e.g., Karlen et al., 2014; Moebius-Clune et al., 2016).

Methods for Tier 2 indicators are under active development and evaluation, and the research community is not in full agreement on methods and interpretation. Tier 3 indicators, however, as might be expected, are still very much in

development, and their interpretation and value as soil health indicators that can be used to guide soil and crop management practice decisions remain uncertain.

To develop consensus that would support research on Tier 1 and Tier 2 indicator evaluation, in early 2018, the SHI assembled a panel of experts in soil health measurement from USDA agencies (Agricultural Research Service, Natural Resources Conservation Service), universities, and a private laboratory to meet and recommend a specific protocol for each indicator listed below. The goal for this gathering was to assemble a definitive list of widely-applicable, effective indicators for evaluating soil health *and* the specific methods to use for each indicator in many production environments across a wide geographical scale. To accomplish this, SHI is partnering with numerous investigators at long-term agricultural research sites (with appropriate experimental designs, controls, documented management histories, production records, etc.) that are being sampled and analyzed for over 30 soil health indicators (www.soilhealthinstitute.org/north-american-project-to-evaluate-soil-health-measurements/) (accessed February 20, 2020). Together, the indicator methods described in USDA-SHD Technical Note 450-03 (Stott, 2019), information provided in this volume, and methods under evaluation in the wide-scale SHI project (Tables 1.1 and 1.2) offer researchers and others who need scientifically justifiable procedures a good selection for current use, comparison, testing in different locations and agricultural production conditions, and further refinement.

Measurements and methods in Tables 1.1 and 1.2 are the subjects of ongoing research being conducted by the SHI with university, government, and private-sector partners with funding (2017–2020) from the Foundation for Food and Agriculture Research, General Mills, The Samuel Roberts Noble Foundation, and matching-fund sources. The indicators under investigation by NRCS are a subset of those being evaluated by SHI, and both organizations coordinated to use the same methods for those specific indicators.

What Do Commercial Analytical Laboratories Need?

The primary interest of researchers usually is a level of accuracy, precision, and explanatory linkage to processes occurring in soil, so that results can be used to explain and predict soil health in a way that leads to new ways of managing the soil resource. In most cases, the limits on accuracy and precision, and the QA/QC procedures to ensure desired data quality and curation, are specified by the individual researcher as needed for the goals of the research and as constrained by the research budget.

Analytical laboratories that measure soil properties for a fee are also concerned with accuracy and precision that reflect the reliability and reputation of their

Table 1.1 Tier 1 Soil Health Indicators and Methods to be Assessed.

Indicator	Method	Reference
Soil pH	1:2 soil:water, standard pH electrode system	Thomas, 1996
Soil Electrical Conductivity (EC)	1:2 soil:water, standard electrical conductivity meter system	Rhoades, 1996
Cation Exchange Capacity (CEC)	Sum of cations: Soil pH $\geq$ 7.2: use ammonium acetate extractant; Soil pH $<$ 7.2: use Mehlich 3 extractant	Knudsen et al., 1982 Sikora and Moore, 2014
% Base Saturation (BS)	Calculation: For soil pH $\geq$ 7.2: use ammonium acetate extractant; for soil pH $<$ 7.2: use Mehlich 3 extractant	Knudsen et al., 1982 Sikora and Moore, 2014
Extractable Phosphorus	Soil pH $\geq$ 7.2: use sodium bicarbonate extractant; Soil pH $<$ 7.2: use Mehlich 3 extractant	Olsen and Sommers, 1982 Sikora and Moore, 2014
Extractable Potassium, Calcium, Magnesium, Sodium	pH $\geq$ 7.2: use ammonium acetate extractant; Soil pH $<$ 7.2: use Mehlich 3 extractant	Knudsen et al., 1982 Sikora and Moore, 2014
Extractable Iron, Zinc, Manganese, Copper	DTPA extractant derivatives	Lindsay and Norvell, 1978
Total Nitrogen	Dry combustion	Nelson and Sommers, 1996
Soil Organic Carbon (SOC)	Dry combustion; corrected for inorganic C, if present, using pressure calcimeter	Nelson and Sommers, 1996 Sherrod et al., 2002
Soil Texture	Pipette method with a minimum of 3 size classes. Weight/volume measurements	Gee and Bauder, 1986
Aggregate Stability	Wet sieve procedure. Weight measurement Water slaking image recognition	Kemper and Roseneau, 1986 Mikha and Rice, 2004 Fajardo et al., 2016

(Continued)

Table 1.1 (Continued)

Indicator	Method	Reference
Available Water Holding Capacity	Ceramic plate method measured at –33 kPa (–10 kPa for sandy soils) and –1500 kPa	Klute, 1986
Bulk Density (BD)	Core method: diameter to be determined, (most likely 2-inch or 5.08 cm)	Blake and Hartge, 1986
Saturated Hydraulic Conductivity	Two-ponding head method in field with Saturo	Reynolds and Elrick, 1990
Crop Yield	Obtained from historical and current plot yield data provided by site manager	
Short-Term Carbon Mineralization	4-d incubation followed by CO ₂ –C evolution and capture at 50% water-filled pore space.	Zibilske, 1994
Potentially Mineralizable Nitrogen	Short-term anaerobic incubation with ammonium and nitrate measured colorimetrically pre- and post-incubation	Bundy and Meisinger, 1994

service. Relationships between measurements and soil processes elucidated in research laboratories underlie a service lab’s analytical offerings, but in most cases, such relationships have been worked out by the research community. Although cost is certainly a consideration in a research budget, a service lab must offer analyses in a consistent, cost-effective, and competitive way to remain in business. Selection of specific methods often relies on recommendations from researchers at universities located within the general region from which a service lab draws customers; such methods are most likely to yield reliable results for the region in which they were developed.

Service labs must maintain consistent quality of data if they are to remain in business. A farmer must have confidence that analyses conducted in different years or on different parts of the farm reflect real properties of the soil, and if changes in a measurement are occurring, that these really do reflect changes in soil on the farm. Service labs may strive to achieve this reliability through associations with organizations that provide independent testing and verification of laboratory results.

One example of laboratory validation is offered through the North American Proficiency Testing (NAPT) Program delivered by the Soil Science Society of America. The NAPT program supports soil, plant, and water testing laboratories

Table 1.2 Tier 2 Soil Health Indicators and Methods to be Assessed

Indicator	Method	Reference
Sodium Adsorption Ratio (SAR)	Saturated paste extract followed by atomic absorption or inductively coupled plasma spectroscopy	Miller et al., 2013
Soil Stability Index	Combination of wet and dry sieving at multiple sieve sizes	Franzluebbers et al., 2000
Active Carbon	Permanganate oxidizable carbon (POXC). Digestion followed by colorimetric measurement	Weil et al., 2003
Soil Protein Index	Autoclaved citrate extractable	Schindelbeck et al., 2016
B-Glucosidase	Assay incubation followed by colorimetric measurement	Tabatabai, 1994
N-acetyl-B-D-glucosaminidase	Assay incubation followed by colorimetric measurement	Deng and Popova, 2011
Phosphomonoesterase	Assay incubation followed by colorimetric measurement	Acosta-Martínez and Tabatabai, 2011
Arylsulfatase	Assay incubation followed by colorimetric measurement	Klose et al., 2011
Phospholipid Fatty Acid (PLFA)	Bligh-Dyer extractant, solid phase extraction, transesterification; gas chromatography	Buyer and Sasser, 2012
Genomics	18S, 16S or ITS analysis or a combination of 16S and 18S/ITS and/or shotgun metagenomics	Earth Microbiome Project 500; Marotz et al., 2017 Thompson et al., 2017
Reflectance	Diffuse reflectance spectroscopy	Veum et al., 2015

by providing interlaboratory sample exchanges and statistical analyses of data. American and Canadian experts from scientific organizations, state (U.S.) and provincial (Canada) departments of agriculture, regional working groups, and public and private analytical labs provide organization and oversight (SSSA, 2020).

Several layers of certifications or validations may be in place to ensure data quality for commercial analytical labs. For example, the Agricultural Laboratory Proficiency (ALP) Program provides soil samples to laboratories for analysis and then assesses that laboratory's results relative to the test soil's known properties. In turn, the ALP program is accredited by the American National Standards Institute/American Society of Quality Control National Accrediting Board (ANAB) through testing of ISO/IEC 17043, itself an international standard of

the International Organization for Standardization and the International Electrotechnical Commission for laboratory proficiency testing that determines the performance of individual laboratories for specific tests or measurements and is used to monitor laboratories' continuing performance through interlaboratory comparisons. Thus, commercial laboratories conducting analyses for soil health indicators select measurements and indicators that are understood in the research community to relate to processes in soil important to soil health; and methods that are affordable to the farming community, as well as verifiable for accuracy and precision (= reliability to the producer) by independent tests.

Summary and Conclusions

This introduction to Volume II provides those interested in soil health assessment methods a brief background regarding identification and selection of the indicators within this volume and to provide context regarding current and future soil health assessment efforts. It also demonstrates that multiple groups (*i.e.*, SHI and the NRCS-SHD) that engaged in workshops and conferences during 2013 to 2015 continue to coordinate soil health assessments to protect and enhance our fragile, yet life-sustaining soil resource for current and future generations.

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