

SOIL HEALTH SERIES

Volume 2

# Laboratory Methods for Soil Health Analysis



Edited by Douglas L. Karlen

Diane E. Stott • Maysoon M. Mikha



**Soil Health Series: Volume 2 Laboratory Methods  
for Soil Health Analysis**

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# **Soil Health Series: Volume 2 Laboratory Methods for Soil Health Analysis**

*Edited by Douglas L. Karlen, Diane E. Stott,  
and Maysoon M. Mikha*

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## Dedication



These books are dedicated to Dr. John W. Doran, a retired USDA-ARS (Agricultural Research Service) Research Soil Scientist whose profound insight provided international inspiration to strive to understand the capacity of our fragile soil resources to function within ecosystem boundaries, sustain biological productivity, maintain environmental quality, and promote plant and animal health.

Understanding and quantifying soil health is a journey for everyone. Even for John, who early in his career believed soil quality was too abstract to be defined or measured. He initially thought soil quality was simply too dependent

on numerous, uncontrollable factors, including land use decisions, ecosystem or environmental interactions, soil and plant management practices, and political or socioeconomic priorities. In the 1990s, John pivoted, stating he now recognized and encouraged the global soil science community to move forward, even though perceptions of what constitutes a *good* soil vary widely depending on individual priorities with respect to soil function. Continuing, he stated that to manage and maintain our soils in an acceptable state for future generations, *soil quality (soil health)* must be defined, and the definition must be broad enough to encompass the many facets of soil function.

John had profound impact on our careers and many others around the World. Through his patient, personal guidance he challenged everyone to examine soil biological, chemical, and physical properties, processes, and interactions to understand and quantify soil health. For Diane, this included crop residue and soil enzyme investigations, and for Maysoon, interactions between soil physical and biological processes mediated by water-filled pore space. Recognizing my

knowledge of soil testing and plant analysis on Midwestern soils, as well as root-limiting, eluviated horizons and soil compaction in Southeastern U.S. soils, John encouraged me to develop a strategy to evaluate and combine the biological, chemical, and physical indicators that have become pillars for soil quality/health assessment. The Soil Management Assessment Framework (SMAF) was the first generation outcome of this challenge.

Throughout his life, John endeavored to involve all Earth's people, no matter their material wealth or status, in translating their lifestyles to practices that strengthen social equity and care for the earth we call home. Through development of the "soil quality test kit" John fostered transformation of soil quality into *soil health* by taking his science to farmers, ranchers, and other land managers. These two volumes have been prepared with that audience in mind to reflect the progress made during the past 25 years. Special thanks are also extended to John's life mate Janet, daughter Karin, son-in-law Michael, grandchildren Drew and Fayth, and all of his friends for their encouragement, patience and support as he continues his search for the "holy grail" of soil health. Without John's inspiration and dedication, who knows if science and concern for our fragile soil resources would have evolved as it has.

Thank you, John – you are an inspiration to all of us!

*Dough Z Kaul*     *Deane E. Stott*     *Maysoun M. Mikha*



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## Foreword

Soil science receives increasing attention by the international policy arena and publication of this comprehensive “Soil Health” book by the Soil Science Society of America (SSSA) and Wiley International is therefore most welcome at this point in time. Striving for consensus on methods to assess soil health is important in positioning soil science in a societal and political discourse that, currently, only a few other scientific disciplines are deeply engaged in. Specifically, increasing the focus on sustainable development provides a suitable “point on the horizon” that provides a much needed focus for a wide range of activities. Sustainable development has long been a likeable, but still rather abstract concept. The United Nations General Assembly acceptance of seventeen Sustainable Development Goals (SDGs) by 193 Governments in 2015 changed the status of sustainable development by not only specifying the goals but also defining targets, indicators, and seeking commitments to reach those goals by 2030 (<https://www.un.org/sustainabledevelopment-goals>). In Europe, the Green Deal, accepted in 2019, has targets and indicators corresponding to those of the SDGs (<https://ec.europa.eu/info/strategy/european-green-deal/soil>).

So far, soil scientists have not been actively engaged in defining SDG targets, which is unfortunate considering soil functions contribute significantly to ecosystem services that, in turn, contribute to the SDGs. The connections are all too obvious for soil scientists, but not necessarily so for scientists in other disciplines, politicians, or the public at large. For example, adequate production of food (SDG2) is impossible without healthy soil. Ground- and surface-water quality (SDG6) are strongly influenced by the purifying and infiltrative capacities of soils. Carbon capture through increases in soil organic carbon (SOC) is a major mechanism contributing to the mitigation of an increasingly variable climate (SDG13) and living soils as an integral part of living landscapes are a dominant source of biodiversity (SDG15) (Bouma, 2014; Bouma et al., 2019). With complete certainty, we can show that healthy soils make better and more effective contributions to ecosystem services than unhealthy ones! This also applies when considering the

recently introduced Soil Security concept, which articulates the 5 C's: soil capability, condition, capital, connectivity, and codification (Field et al., 2017). A given soil condition can be expressed in terms of soil health, whereas soil capability defines potential conditions, to be achieved by innovative soil management, thus increasing soil health to a characteristically attainable level for that particular soil. Healthy soils are a capital asset for land users; connectivity emphasizes interactions among land users, citizens, and politicians that are obviously important, especially when advocating measures to increase soil health that may initially lack societal support. Finally, codification is important because future land use rules and regulations could benefit by being based on quantitative soil health criteria, thus allowing a reproducible comparison between different soils.

These volumes provide an inspiring source of information to further evaluate the soil health concept, derive quantitative procedures that will allow more effective interaction among land users, and information needed to introduce soil science into laws and regulations. The introductory chapters of Volume 1 present a lucid and highly informative overview of the evolution of the soil health movement. Other chapters discuss data needs and show that modern monitoring and sensing techniques can result in a paradigm shift by removing the traditional data barriers. Specifically, these new methods can provide large amounts of data at relatively low cost. The valuable observation is made that systems focusing only on topsoils cannot adequately represent soil behavior in space and time. Subsoil properties, expressed in soil classification, have significant and very important effects on many soil functions. Numerous physical, chemical and biological methods are reviewed in Volume 2. Six chapters deal with soil biological methods, correctly reflecting the need to move beyond the traditional emphasis on physical and chemical assessment methods. After all, soils are very much alive!

The book *Soil Health* nicely illustrates the “roots” of the soil health concept within the soil science profession. It also indicates the way soil health can provide “wings” to the profession as a creative and innovative partner in future environmental research and innovation.

**Johan Bouma**  
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## References

- Bouma, J. (2014). Soil science contributions towards Sustainable Development Goals and their implementation: Linking soil functions with ecosystem services. *J. Plant Nutr. Soil Sci.* 177(2), 111–120. doi:10.1002/jpln.201300646
- Bouma, J., Montanarella, L., and Vanylo, G.E. (2019). The challenge for the soil science community to contribute to the implementation of the UN Sustainable Development Goals. *Soil Use Manage.* 35(4), 538–546. doi:10.1111/sum.12518
- Field, D.J., Morgan, C.L.S., and Mc Bratney, A.C., editors. (2017). *Global soil security*. Progress in Soil Science. Springer Int. Publ., Switzerland. doi:10.1007/978-3-319-43394-3



## Preface

This two-volume series on Soil Health was written and edited during a very unique time in global history. Initiated in 2017, it was intended to simply be an update for the “Blue” and “Green” soil quality books entitled *Defining Soil Quality for a Sustainable Environment* and *Methods for Assessing Soil Quality* that were published by the Soil Science Society of America (SSSA) in the 1990s. In reality, the project was completed in 2020 as the United States and world were reeling from the Covid-19 coronavirus pandemic, wide-spread protest against discriminatory racial violence, and partisan differences between people concerned about economic recovery versus protecting public health.

Many factors have contributed to the global evolution of soil health as a focal point for protecting, improving, and sustaining the fragile soil resources that are so important for all of humanity. Building for decades on soil conservation principles and the guidance given by Hugh Hammond Bennett and many other leaders associated with those efforts, soil health gradually is becoming recognized by many different segments of global society. Aligned closely with soil security, improving soil health as a whole will greatly help the United Nations (UN) achieve their Sustainable Development Goals (SDGs). Consistent with soil health goals, the SDGs emphasize the significance of soil resources for food production, water availability, climate mitigation, and biodiversity (Bouma, 2019).

The paradox of completing this project during a period of social, economic, and anti-science conflicts associated with global differences in response to Covid-19, is that the pandemic’s impact on economic security and life as many have known it throughout the 20th and early 21st centuries is not unique. Many of the same contentious arguments could easily be focused on humankind’s decisions regarding how to use and care for our finite and fragile soil resources. Soil conservation leaders such as Hugh Hammond Bennett (1881–1960), “Founder of Soil Conservation,” W. E. (Bill) Larson (1921–2013) who often stated that soil is “the thin layer covering the planet that stands between us and starvation,” and many current conservationists can attest that conflict regarding how to best use soil

resources is ancient. Several soil science textbooks, casual reading books, and other sustainability writings refer to the Biblical link between soil and humankind, specifically that the very name “Adam” is derived from a Hebrew noun of feminine gender (*adama*) meaning earth or soil (Hillel, 1991). Furthermore, Xenophon, a Greek historian (430–355 BCE) has been credited with recording the value of green-manure crops, while Cato (234–149 BCE) has been recognized for recommending the use of legumes, manure, and crop rotations, albeit with intensive cultivation to enhance productivity. At around 45 CE, Columella recommended using turnips (perhaps tillage radishes?) to improve soils (Donahue et al., 1971). He also suggested land drainage, application of ash (potash), marl (limestone), and planting of clover and alfalfa (N fixation) as ways to make soils more productive. But then, after Rome was conquered, scientific agriculture, the arts, and other forms of culture were stymied.

Advancing around 1500 yr, science was again introduced into agriculture through Joannes Baptista Van Helmont’s (1577–1644 CE) experiment with a willow tree. Although the initial data were misinterpreted, Justice von Liebig (1803–1873 CE) eventually clarified that carbon (C) in the form of carbon dioxide (CO<sub>2</sub>) came from the atmosphere, hydrogen and oxygen from air and water, and other essential minerals to support plant growth and development from the soil. Knowledge of soil development, mineralogy, chemistry, physics, biology, and biochemistry as well as the impact of soil management (tillage, fertilization, amendments, etc.) and cropping practices (rotations, genetics, varietal development, etc.) evolved steadily throughout the past 150 yr. **SO**, what does this history have to do with these 21st Century Soil Health books?

First, in contrast to the millennia throughout which humankind has been forewarned regarding the fragility of our soil resources, the concept of soil health (used interchangeably with soil quality) per se, was introduced only 50 yr ago (Alexander, 1971). This does not discount outstanding research and technological developments in soil science such as the physics of infiltration, drainage, and water retention; chemistry of nutrient cycling and availability of essential plant nutrients, or the biology of N fixation, weed and pest control. The current emphasis on soil health in no way implies a lack of respect or underestimation of the impact that historical soil science research and technology had and have for solving problems such as soil erosion, runoff, productivity, nutrient leaching, eutrophication, or sedimentation. Nor, does it discount contributions toward understanding and quantifying soil tilth, soil condition, soil security, or even sustainable development. All of those science-based accomplishments have been and are equally important strategies designed and pursued to protect and preserve our fragile and finite soil resources. Rather, soil health, defined as an integrative term reflecting the “capacity of a soil to function, within land use and ecosystem boundaries, to sustain biological productivity, maintain environmental quality, and promote



plant animal, and human health” (Doran and Parkin, 1994), is another attempt to forewarn humanity that our soil resources must be protected and cared for to ensure our very survival. Still in its infancy, soil health research and our understanding of the intricacies of how soils function to perform numerous, and at times conflicting goals, will undoubtedly undergo further refinement and clarification for many decades.

Second, just like the Blue and Green books published just twenty years after the soil health concept was introduced, these volumes, written after two more decades of research, continue to reflect a “work in progress.” Change within the soil science profession has never been simple as indicated by Hartemink and Anderson (2020) in their summary reflecting 100 yr of soil science in the United States. They stated that in 1908, the American Society of Agronomy (ASA) established a committee on soil classification and mapping, but it took 6 yr before the first report was issued, and on doing so, the committee disbanded because there was no consensus among members. From that perspective, progress toward understanding and using soil health principles to protect and preserve our fragile soil resources is indeed progressing. With utmost gratitude and respect we thank the authors, reviewers, and especially, the often-forgotten technical support personnel who are striving to continue the advancement of soil science. By developing practices to implement sometimes theoretical ideas or what may appear to be impossible actions, we thank and fully acknowledge all ongoing efforts. As the next generation of soil scientists, it will be through your rigorous, science-based work that even greater advances in soil health will be accomplished.

Third, my co-authors and I recognize and acknowledge soil health assessment is not an exact science, but there are a few principles that are non-negotiable. First, to qualify as a meaningful, comprehensive assessment, soil biological, chemical, and physical properties and processes must all be included. Failure to do so, does not invalidate the assessment, but rather limits it to an assessment of “soil biological health”, “soil physical health”, “soil chemical health”, or some combination thereof. Furthermore, although some redundancy may occur, at least two different indicator measurements should be used for each indicator group (*i.e.*, biological, chemical, or physical). To aid indicator selection, many statistical tools are being developed and evaluated to help identify the best combination of potential measurements for assessing each critical soil function associated with the land use for which an evaluation is being made.

There is also no question that any soil health indicator must be fundamentally sound from all biological, chemical, physical and/or biochemical analytical perspectives. Indicators must have the potential to be calibrated and provide meaningful information across many different types of soil. This requires sensitivity to not only dynamic, management-induced forces, but also inherent soil properties and processes reflecting subtle differences in sand, silt, and clay size particles

derived from rocks, sediments, volcanic ash, or any other source of parent material. Soil health assessments must accurately reflect interactions among the solid mineral particles, water, air, and organic matter contained within every soil. This includes detecting subtle changes affecting runoff, infiltration, and the soil's ability to hold water through *capillarity*– to act like a sponge; to facilitate gas exchange so that with the help of CO<sub>2</sub>, soil water can slowly dissolve mineral particles and release essential plant nutrients– through *chemical weathering*; to provide water and dissolved nutrients through the soil *solution* to plants, and to support exchange between oxygen from air above the surface and excess CO<sub>2</sub> from respiring roots.

Some, perhaps many, will disagree with the choice of indicators that are included in these books. Right or wrong, our collective passion is to start somewhere and strive for improvement, readily accepting and admitting our errors, and always being willing to update and change. We firmly believe that starting with something good is much better than getting bogged down seeking the perfect. This does not mean we are discounting any fundamental chemical, physical, thermodynamic, or biological property or process that may be a critical driver influencing soil health. Rather through iterative and ongoing efforts, our sole desire is to keep learning until soil health and its implications are fully understood and our assessment methods are correct. Meanwhile, never hesitate to hold our feet to the refining fire, as long as collectively we are striving to protect and enhance the unique material we call soil that truly protects humanity from starvation and other, perhaps unknown calamities, sometimes self-induced through ignorance or failing to listen to what our predecessors have told us.

Douglas L. Karlen (Co-Editor)

## References

- Alexander, M. (1971). Agriculture's responsibility in establishing soil quality criteria  
In: *Environmental improvement– Agriculture's challenge in the Seventies*.  
Washington, DC: National Academy of Sciences. p. 66–71.
- Bouma, J. (2019). Soil security in sustainable development. *Soil Systems*. 3:5.  
doi:10.3390/soilsystems3010005
- Donahue, R. L., J. C. Shickluna, and L. S. Robertson. 1971). *Soils: An introduction to soils and plant growth*. Englewood Cliffs, N.J.: Prentice Hall, Inc.
- Doran, J.W., Coleman, D.C., Bezdick, D.F., and Stewart, B.A., editors. (1994).  
*Defining soil quality for a sustainable environment*. Soil Science Society of America (SSSA) Special Publication No. 35. Madison, WI: SSSA Inc.
- Doran, J.W., and Parkin, T.B. (1994). Defining and assessing soil quality. In:  
J.W. Doran, D.C. Coleman, D.F. Bezdick, and B.A. Stewart, editors, *Defining soil*

- quality for a sustainable environment. SSSA Special Publication No. 35. Madison, WI: SSSA. p. 3–21. doi:10.2136/sssaspecpub35*
- Doran, J.W., and Jones, A.J. (eds.). (1996). *Methods for assessing soil quality. Soil Science Society of America (SSSA) Special Publication No. 49. Madison, WI: SSSA Inc.*
- Hartemink, A. E. and Anderson, S.H. (2020). 100 years of soil science society in the U.S. *CSA News* 65(6), 26–27. doi:10.1002/csann.20144
- Hillel, D. (1991). *Out of the earth: Civilization and the life of the soil.* Oakland, CA: University of California Press.



## 1

## Laboratory Methods for Soil Health Assessment: An Overview

*Steven R. Shafer, Douglas L. Karlen, Paul W. Tracy, Cristine L.S. Morgan, and C. Wayne Honeycutt*

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;

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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](http://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/](http://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.

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

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