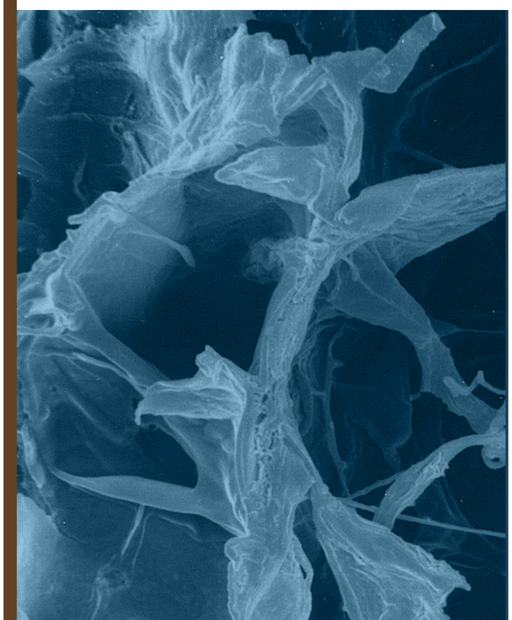
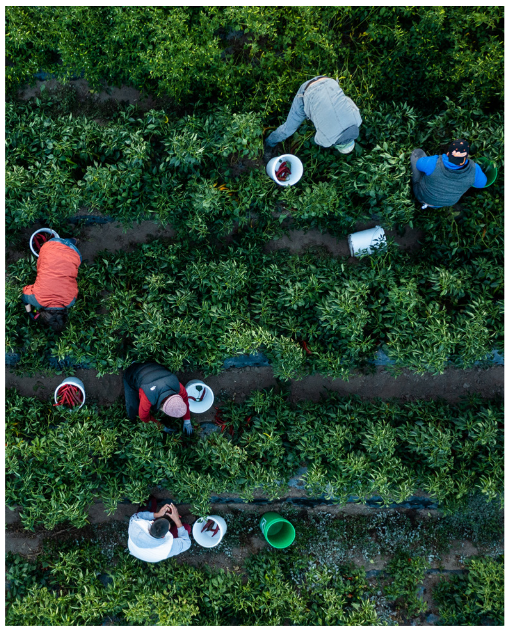
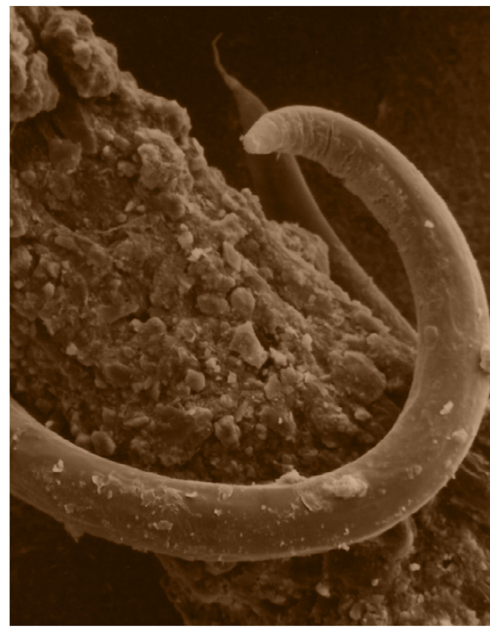
Soil Biodiversity in California Agriculture:

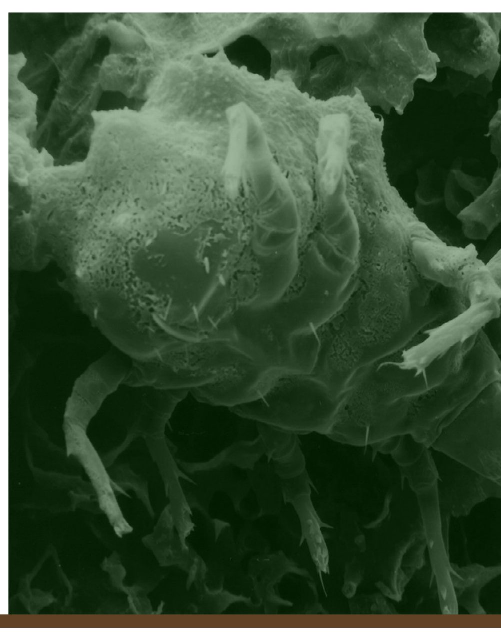
Framework and Indicators for Soil Health Assessment

Prepared by:

California Department of Food and Agriculture
Belowground Biodiversity Advisory Committee

July 2023

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Prepared by the Belowground Biodiversity Advisory Committee (BBAC)
For the California Department of Food and Agriculture (CDFA)

July 2023

CALIFORNIA DEPARTMENT OF FOOD & AGRICULTURE
Karen Ross, Secretary

Forward from Secretary Ross

Our soil is the foundation of continued healthy food production. Every time I walk through an orchard, field, or pasture, I am amazed to think about what's beneath my feet! The soil in the hands of our farmers and ranchers is also a solution to climate change!

Crops, trees, and grasses are supported by a vast and intricate network of soil biota that make life on earth possible. These tiny lifeforms enable plant productivity, nutrient cycling, and carbon sequestration; they mediate hydrological function, and determine biogeochemical cycles, including the water and carbon cycles. They decompose organic matter, define soil structure, and so much more. Science is only just beginning to understand the many functions of the life in our soil and its interplay with our agricultural systems.

California is unique in its agricultural productivity, diversity, and value. It is also unique in its leadership and investment in addressing climate change. To date, state investments in California Department of Food and Agriculture's (CDFA) Healthy Soils Program have amounted to over $156 million dollars; now we are asking how we can optimize the investment, the practices, and the impact by better understanding soil biodiversity and how it interacts with our cropping systems.

In his Executive Order, N-82-20, Governor Newsom called out the importance of soils in hosting over a quarter of the world's biodiversity, the wealth of California's 2,500 soil types, and the contribution of our working lands to the global food supply. He called for state agencies to pursue multiple pathways to inventory, preserve, and enhance biodiversity. As California turns toward a carbon-neutral future and a sustainable, resilient food system, it is essential that we develop a better understanding of this "belowground biodiversity," and how our soil management can play a role in helping us not only mitigate for and adapt to climate change, but also to restore degraded lands and enhance crop production – and, to use the term of the day - to truly regenerate our lands. We hope to support the effort of farmers and ranchers to ensure nutritional security for millions of people, while building resiliency and ameliorating climate change. To do this, they will need every tool in the toolbox.

I've had a longstanding interest in on-farm biodiversity, so I was excited to see a 2020 United Nations Food and Agriculture Organization report on the "State of Knowledge of Soil Biodiversity: Status, challenges, and potentialities." One of the lead authors was Dr. Kate Scow, a pre-eminent soil scientist and a Californian. The report stimulated my interest in doing a California-specific assessment, given the diversity of soils, crops, climatic conditions and the importance of California agriculture's contribution to the health and nutrition of citizens in our state, across the nation and around the world.

The work presented in this report is the result of world-class soil scientists working together for more than a year to identify key metrics for measuring soil biodiversity, as well as describing the importance of life beneath our feet for maintaining our agricultural systems and making them more resilient to climate change. This ad-hoc committee brought their expertise to bear, providing a deep exploration of soil biodiversity, all the while considering its relationship to California's agricultural systems. My hope is this document will help us enhance our understanding of the potential of soil to meet the dual challenges of meeting our nutritional needs while addressing climate change.

I am deeply grateful to the scientists and their collaboration to produce this report. I am excited the possibilities it highlights as we work with farmers and ranchers to be part of the solution, and look forward to sharing the findings with all our partners to make this a key focus of California's agricultural production.

Yours truly,

Karen Ross

Karen Ross
Secretary

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State of California
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ACRONYMS

BBAC	Belowground Biodiversity Advisory Committee
BBSK	Biological Soil Classification Scheme
BISQ	Biological Indicator System for Soil Quality
C	Carbon
CA	California
CalEPA	California Environmental Protection Agency
CASH	Comprehensive Assessment of Soil Health
CBD	Convention on Biological Diversity
CDFA	California Department of Food and Agriculture
CFU	Colony Forming Units
CH_4	Methane
CO_2	Carbon Dioxide
COP	Conference of the Parties
CSU	California State University
DNA	Deoxyribonucleic Acid
DOC	Dissolved Organic Carbon
EFA SAP	Environmental Farming Act Science Advisory Panel
ENVASSO	ENVironmental ASsessment of Soil for mOnitoring
EO	Executive Order
ESM	Equivalent Soil Mass
EU	European Union
FAIR	Findable, Accessible, Interoperable, and Reusable
FAME	Fatty Acid Methyl Ester Analysis
FAO	Food and Agriculture Organization of the United Nations
FFAR	Foundation for Food and Agriculture Research
FISH	Fluorescence In Situ Hybridization
GBSI	Global Soil Biodiversity Institute
GHG	Greenhouse Gas
GSBI	Global Soil Biodiversity Initiative
HSP	Healthy Soils Program
ISF	Indicator Selection Framework
ISO	International Organization for Standardization

ITPS	Intergovernmental Technical Panel on Soils
ITS	Internal Transcribed Spacer
N	Nitrogen
N_2	Nitrogen Gas
N_2O	Nitrous Oxide
NGO	Non-governmental Organization
NIFA	National Institute of Food and Agriculture
NINJA	Nematode INdicator Joint Analysis
NLFA	Neutral Lipid Fatty Acid
NO_2-	Nitrite
NO_3-	Nitrate
NRCS	Natural Resources Conservation Service
NSF NEON	National Science Foundation National Ecological Observatory Network
NSTC	National Science and Technology Council
O	Oxygen
P	Phosphorous
PAHs	Polycyclic Aromatic Hydrocarbons
PCR	Polymerase Chain Reaction
PLFA	Phospholipid Fatty Acids
PMC	Potentially Mineralizable Carbon
PMN	Potentially Mineralizable Nitrogen
rRNA	Ribosomal Ribonucleic Acid
SES	Soil Ecology Society
SGMA	Sustainable Groundwater Management Act
SIR	Substrate Induced Respiration
SOC	Soil Organic Carbon
SoilBON	Soil Biodiversity Observation Network
SOM	Soil Organic Matter
UC	University of California
UC ANR	UC Agriculture and Natural Resources
UN	United Nations
US	United States
USDA	United States Department of Food and Agriculture

GLOSSARY OF TERMS

Adaptive Management Framework	Provides managers with a way to proceed with management while learning about their system so that soil ecosystem service output can be increased, and critical thresholds avoided. (Birgé et al. 2016)
Alpha Diversity	The diversity of species in a specific location (e.g., the number of species of bacteria in a handful of soil).
Aridification	The gradual change of a region from a wetter to a drier climate.
Beta Diversity	The differences in species diversity between different locations.
Biodiversity	The variety of living organisms found in a given sample or habitat.
Biodiversity Indicator	A metric that shows the state or level of soil biodiversity or specific species/functions of interest. To be useful, an indicator must be sensitive to change, easily measured and interpretable at both scientific and policy levels and exist within a framework.
Biome	Biggest unit of ecosystem categorization. It is a complex biotic community characterized by distinct plant and animal species and maintained under the climatic conditions of the region. For example, all forests share certain properties regarding nutrient cycling, disturbance and biomass, which are different from the properties of grasslands. (Turbé et al. 2010)
Bioturbation	The disturbance of soil by living organisms.
Community	Any combination of populations from different organisms found living together in a particular environment, essentially the biotic component of an ecosystem. (Turbé et al. 2010)
Community Diversity	Diversity of biological communities and species, e.g., the number of nematode species in a field. (Geist 2011)
Community Fingerprinting	Techniques targeting all DNA or phospholipids in a sample that can be used to quickly profile the diversity of a microbial community.
Commensalism	A class of ecological relationships between two organisms where one benefits and the other is not significantly harmed or benefited. (Turbé et al. 2010)
Compound Disturbance	Disturbances of communities already stressed by abiotic or biotic forces.
Crust, Biological	An assemblage of organisms, including cyanobacteria, algae, lichens, liverworts, and mosses that forms an irregular living crust on soil surface, especially on otherwise barren arid-region soils. Also referred to ascryptogamic, cryptobiotic or microbiotic crusts. (Weil and Brady 2017)
Crust, Physical	A surface layer of soils, ranging in thickness from a few millimeters to as much as 3 centimeters, that physical-chemical processes have caused to be much more compact, hard, and brittle when dry than the material immediately beneath it. (Weil and Brady 2017)
Detritus	Debris from dead plants and animals. (Weil and Brady 2017)
Disturbance	Event that alters either the soil environment or soil biological communities.
Ecological Complexes	The interconnected suite of living organisms that work together in soil.

Ecological Process	An interaction among organisms; ecological processes frequently regulate the dynamics of ecosystems and the structure and dynamics of biological communities.
Ecosystem Diversity	Physical and chemical diversity of ecotones and habitats in fields and landscapes. (Geist 2011)
Ecosystem Function	The collective intraspecific and interspecific interactions of the biota, and between organisms and the physical environment, giving rise to functions such as organic matter decomposition and nutrient cycling.
Ecosystem Process	Changes in the stocks and/or flows of materials in an ecosystem, resulting from interactions among organisms and with their physical-chemical environment.
Ecosystem Service	The benefit that is derived from ecosystems. This comprises provisioning services such as food and water; regulating services such as flood and disease control; cultural services such as spiritual, recreational, and cultural benefits; and supporting services such as nutrient cycling that maintain the conditions for life on Earth. (Turbé et al. 2010)
Ecological Sieves	A series of filters an organism must pass through to become established in a community. First, dispersal sieve, to reach the location; second, environmental sieve to be able to survive there; and third, biotic sieve, ability to coexist with other organisms in that location.
Emergent Properties	Unique properties of a system arising from interactions and feedbacks between the components.
Function	The physical, chemical and biological processes that transform and translocate energy or materials in an ecosystem. (Naeem 1998)
Functional Diversity	Functional diversity refers to the different types of processes in a community that are important to its structure and dynamic stability.
Functional Potential	The genetic capacity or capability of a microbial community to perform specific biological processes and activities.
Genetic Diversity	Genetic variation within individuals or populations, to the genetic diversity of entire ecosystems. (Geist 2011)
Hyphae	Filaments of fungal cells. Many hyphae constitute a mycelium. (Turbé et al. 2010)
Infiltration	The downward entry of water into soil. (Weil and Brady 2017)
Intraspecific Diversity	Population diversity and phenotypic variation within species. (Geist 2011)
Metagenomics	The study of genetic material recovered directly from environmental or clinical samples by a method called sequencing.
Metric	Method used to measure an indicator.
Microbiome	A characteristic microbial community occupying a reasonable well-defined habitat which has distinct physio-chemical properties. The microbiome not only refers to themicroorganisms involved but also encompass their theatre of activity, which results in the formation of specific ecological niches.
Multifunctionality	Integration of processes, function and services to enable performance of many functions.
Mycorrhiza(e)	A symbiotic association between a fungus and plant roots. (Turbé et al. 2010)
Necromass	Dead biomass from organisms including soil microbes, soil fauna, and plants.

Parasitism	A relationship between two different organisms where one organism, the parasite, takes some advantages from one another, the host. (Turbé et al. 2010)
Pathogen	An organism that causes disease to its host. Common pathogens include bacteria, fungi, and viruses.
Pedon	A three-dimensional sample of a soil just large enough to show the characteristics of all its horizons.
Predation	The killing of one organism by another for food. Energy, carbon, and nutrients are transferred.
Process	A "series of events, reactions or operations, achieving a certain definite result." Ecosystem processes are seen therefore as the complex interactions among biotic and abiotic elements of ecosystems, encompassing in broad terms material cycles and flow of energy (Lyons et al. 2005).
Resilience	The capacity of an ecosystem to withstand negative impacts without falling into a qualitatively different state that is controlled by a different set of processes. (Turbé et al. 2010)
Resistance	The ability to withstand perturbation in the presence of a disturbance.
Rhizosphere	The zone around plant roots which is influenced by root secretion and by the root-associated soil microorganisms. (Turbé et al. 2010)
Root Exudation	The release from roots of exudates, comprising low-molecular weight organic compounds, including sugars, carbohydrates, and organic and amino acids, into soil. (Bardgett 2014)
Soil Aggregate	A group of primary soil particles that adhere to each other more strongly than to other surrounding particles, due to biological, chemical, and physical processes.
Soil Aggregate Stability	A measure of the proportion of aggregates in a soil that do not easily slake, crumble, or disintegrate. The ability of soil aggregates to resist stresses, such as wet/dry cycles, without breaking.
Soil Biodiversity	The variety of life belowground, from genes and species to the communities they form, as well as the ecological complexes to which they contribute and to which they belong, from soil micro-habitats to landscapes. (UN FAO 2020)
Soil Health	The continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans.
Soil Organic Matter	The organic fraction of the soil that includes plant, animal and microbial residues in various stages of decomposition, biomass of microorganisms, substances produced by plant roots and other soil organisms. It is commonly determined as the total organic (non-carbonate) carbon in a soil sample passed through a 2-mm sieve. (Weil and Brady 2017)
Soil Salinity	The amount of soluble salts in a soil, expressed in terms of percentage, milligrams per kilogram, parts per million (ppm), or other convenient rations. (Weil and Brady 2017)
Suppression	The ability of resident soil microbes to persistently reduce pathogen establishment or disease incidence and severity. (Schlatter et al. 2017)
Symbiosis	A close and long-term interaction between two species of organisms in which both species obtain a substantial benefit.

EXECUTIVE SUMMARY

Soil health depends on soil biodiversity.

However, external pressures from land-use change, climate change and certain agricultural practices threaten the biotic networks that underpin the delivery of soil's many ecosystem services. Yet measuring soil biodiversity is a complex task, with a wide variety of possible indicators, and methodologies that are evolving with recent technological advances. This report, prepared by the Belowground Biodiversity Advisory Committee (BBAC) convened by the California Department of Food and Agriculture (CDFA), focuses on how best to assess soil biodiversity in the context of working lands and considers current and future challenges faced by California agricultural producers, policy makers, governing agencies, and related stakeholders. The report presents information on the taxonomic and functional diversity of soil organisms, ecosystem services they provide, threats to soil biodiversity, assessment frameworks, and biodiversity indicators. Examples of how biodiversity indicators can be applied to specific use cases provide insights for soil health, sustainable and climate-smart agriculture, and biodiversity conservation in California.

Soil biodiversity is the interconnected 'social' network of numerous species of living organisms that contribute to soil functioning. As these organisms grow, die, and interact with soil's abiotic components, they perform essential functions in carbon, water and nutrient cycling and plant growth, collectively described as multifunctionality, benefiting ecosystems and humans alike. Comprehensive assessment of soil biodiversity involves measurements of organism abundance, identity, and functional diversity or traits, ideally in tandem with measurements of soil processes, as well as interactions among organisms. Soil biodiversity and soil processes vary in space and time due to factors like location, climate, vegetation, and land management practices across California's diverse landscapes.

Soils are incredibly biodiverse habitats, containing a vast array of organisms ranging from macroscopic organisms like gophers to microscopic worms, fungi, and billions of bacterial cells. The physical and chemical properties of soils – soil texture, pH, water and oxygen content, salinity, organic matter inputs, and nutrients – determine the types of organisms found in a particular habitat. The array of organisms inhabiting soil spans over six orders of magnitude in size, and includes microorganisms (viruses, bacteria, archaea, and fungi); microfauna (protists, nematodes, and tardigrades); mesofauna (mites and springtails); and macrofauna (earthworms). Life in soil exists in ecological communities that are complex and interconnected. These interconnections provide stability to soil functions. Soil organisms are critical to regulation of greenhouse gases, both by consuming and producing gasses such as nitrous oxide, carbon dioxide, and methane. Mycorrhizal fungi in symbiosis with most plant species promotes root growth and availability of water and nutrients. A broad range of soil organisms mediate the decomposition of organic inputs and enhance nutrient cycling. Other functions of biodiverse soils include soil structure formation, organic matter formation, carbon storage, water regulation, and pathogen suppression. But despite these critically important functions, the diversity and complexity of soil biodiversity makes it challenging to decipher these intricate relationships and understand the impact of human activities.

Soil biodiversity faces many of the major threats from human activities and global change that also impact soil health and sustainability of California's agroecosystems. Land use changes, intensive agriculture, climate change, pollution, invasive species, overexploitation, and loss of habitat connectivity all pose risks. These threats disrupt soil biological networks, reduce biodiversity, impair ecosystem functions, and degrade soil structure and fertility. Soil biodiversity loss reduces multifunctionality and the provision of ecosystem services, highlighting the need to recognize the value of belowground communities to overcome challenges such as climate change, land degradation, and overall biodiversity loss. Addressing these challenges through sustainable land management, agroecological approaches, and awareness campaigns is crucial for preserving belowground biodiversity to maintain provision of essential ecosystem services.

Measuring and characterizing soil biodiversity requires identifying specific indicators which can be classified into five major categories: abundance, identity, functional traits, interactions, and processes. **Abundance** indicators quantify the biomass or numbers of organisms, while **identity** indicators use morphological traits or DNA sequencing to determine taxonomic composition (types, or species present). **Functional trait** indicators assess the capacity of soil organisms to perform specific functions. For example, the anatomy and morphology (shape, structure) of nematodes provide information on their functions, while the analysis of DNA extracted from microscopic soil organisms provides the blueprint for their functional diversity. **Interactions** among soil organisms, such as predation and symbiosis, are essential indicators of biodiverse and balanced soil networks. **Process** indicators measure the rates and transformation products of soil processes, which are influenced by the abundance, identity, and interactions of soil organisms. Integrating data and establishing relationships among these different categories of biodiversity is needed to connect this information to the goals of agricultural sustainability and healthy soils. In turn, database systems and computing infrastructure are needed to bring biodiversity information into practice.

This report includes an overview of institutional and project-based efforts to identify soil biodiversity indicators and monitor soil health – these highlight the growing recognition in various international initiatives, of soil biodiversity's importance, as well as the threats faced by soil biodiversity and potential solutions.

In choosing indicators for biodiversity assessment, the needs, priorities, and constraints of involved stakeholders must be part of the selection process. The BBAC developed a set of specific **Indicator Selection Criteria** to facilitate the incorporation of specific goals and needs, as well as identification of constraints, in the process of selection of indicators for soil biodiversity assessment. Selection criteria were identified based on scientific and policy relevance, as well as review of other programs identifying criteria for biodiversity indicators. Core criteria identified by the BBAC for biodiversity indicator selection include (i) meaningful alignment with goals, (ii) relevance to organism scale and biology, (iii) feasibility and interpretability, and (iv) adherence to standardized sampling methods.

In addition to the above, the BBAC has designed an **Indicator Selection Framework (ISF)** to provide an approach to standardize how indicator selection criteria may be applied to meet the goals and constraints of specific use cases. This framework guides users through the process of identifying the problem to solve, formulating clear goals and identifying the intended audience for the particular soil biodiversity assessment. Then the Indicator Selection Criteria are used to compare and select appropriate biodiversity indicators for the specific needs of the assessment. To facilitate these steps, the BBAC has prepared a template and step-by-step instructions to guide reader consideration. Similarly, the BBAC has prepared four example case studies illustrating the application of the ISF, including: (i) conduct a general assessment of California soil biodiversity; (ii) assess impacts of the CDFA Healthy Soils Program on soil biodiversity; (iii) assist growers to manage the functions of healthy soils using information on soil biodiversity and processes; and (iv) enliven soil biodiversity for growers, gardeners, ranchers, and consumers. An example table is provided to present the information involved in indicator selection for a particular use case.

To close this report, the BBAC presents **targeted recommendations** as a roadmap for policymakers and stakeholders to conserve biodiversity and enhance soil health. Specific recommendations include (i) use soil biodiversity as a key metric to assess, preserve, and prioritize soil health and help meet climate and sustainability goals in California agroecosystems; (ii) integrate soil biodiversity assessment into CDFA's Healthy Soils Program; (iii) use and refine the preliminary ISF to assess soil biodiversity under a range of applications and conditions; (iv) develop an Adaptive Management Framework for soil biodiversity assessment, expand data management infrastructure, and increase capacity to support soil biodiversity assessments.

Broader recommendations and opportunities identified by BBAC include (i) optimize regional, statewide, and global partnerships to promote California soil biodiversity through education, outreach, and cooperation, including with the California Biodiversity Network 30x30 Partnership, and ii) build State capacity within the public and private sector to provide services and training for soil biodiversity analysis and assessment.

Suggestions for future research and initiatives include: (i) create a monitoring program to determine the status and trajectory of soil biodiversity in California working lands; (ii) investigate relationships between soil biodiversity and soil health in California working lands; (iii) establish causal relationships between soil biodiversity and human health, (iv) investigate impacts of climate and land use change on soil biodiversity and identify roles of soil biodiversity in mitigation and adaptation to climate change, and v) conduct further research on soil biodiversity indicators.

Overall, the recommendations in this report aim to promote the importance of soil biodiversity in soil health assessments and climate-smart and sustainable food systems, and provide guidance for its assessment, management, and conservation in California's working lands.

INTRODUCTION

Soil organisms (macrofauna, microarthropods, microbes, and other soil biota) are central to ecosystem functions and provide numerous ecosystem services. Taken together, *soil biodiversity* refers to the different types and interconnected network of these living soil organisms that play a vital role in carbon, water, and nutrient cycling, and supporting plant growth. In agricultural systems, soil biodiversity is a critical component of system health and function, highlighting a need to monitor the effects of agricultural and soil management practices - both positive and negative - on belowground organisms. This understanding is important not only for conservation and stewardship of the important and diverse group of organisms that live in soil, but also to help producers and agencies sustainably foster the multiple soil-based ecosystem services that support humanity.

Under Executive Order (EO) N-82-20[1] issued by Governor Newsom (September 2020), the California Natural Resources Agency, in consultation with the California Department of Food and Agriculture (CDFA), the California Environmental Protection Agency (CalEPA) and other state agencies, were directed to establish the California Biodiversity Collaborative to bring together government partners, California Native American tribes, experts, business and community leaders and other California stakeholders to protect and restore the State's biodiversity. The EO emphasizes the importance of equitable climate-mitigation and climate-readiness practices: *"Whereas as we work to mitigate greenhouse gas emissions, we must also work to accelerate actions to enable the State to adapt and become more resilient to the impacts of climate change, including expanding nature-based solutions - the use of sustainable land management practices to tackle environmental, social and economic challenges."*

In this context the EO also highlights the need to prioritize investments in biodiversity measurement and protection. It calls on the State to *"expand the communication and use of information, indicators and tools to monitor, track and protect California's biodiversity and natural resources"* and directly calls on the CDFA to *"enhance soil health and biodiversity through the Healthy Soils Initiative."*

The priority given to soil biodiversity in the EO is particularly noteworthy given that the topic was not explicitly called out in the "30 X 30" Global Biodiversity Framework at the Conference of the Parties (COP) 15 meeting[2] in Montreal, nor is it currently a measured outcome of any California State (State) government program. Indeed, soil biodiversity is rarely measured during routine soil tests used on working farms and ranches in the State. California is no different from many other states or nations in this regard. Despite the central role of soil organisms to provide ecosystem services, nature conservation initiatives across the world frequently overlook the vast diversity of organisms that live in soil (Guerra et al., 2021), and historically, soil monitoring in agricultural systems has focused on chemical (e.g., fertility) and physical (e.g., tilth) properties.

In support of the EO, and to further the goals of the State's Healthy Soils Program[3], the CDFA **Environmental Farming Act Science Advisory Panel (EFA SAP)** voted to establish a committee of scientists to evaluate whether soil biodiversity metrics could be identified and defined and used to indicate the soil health of working lands. The committee was not tasked with developing new regulations but rather, to envision activities that might complement the existing CDFA Healthy Soils Program. The EFA SAP acknowledged the possibility that practical and reliable biodiversity measurement schemes may not yet be available, and that barriers should also be identified by the committee as part of a roadmap towards the development and use of biodiversity indicators in support of CDFA's goals.

The Belowground Biodiversity Advisory Committee (BBAC) was formed in May 2022 for this purpose, and actively engaged in analysis, discussion, and synthesis of their findings from May 2022 – June 2023. Members of the BBAC include 14 scientists with expertise in soil health, plant science and soil biodiversity. CDFA supported the BBAC with facilitation services provided by California State University Sacramento's Collaboration and Consensus Program and staff resource support from CDFA's Healthy Soils Program.

This report is the result of the BBAC's efforts to determine soil health biodiversity indicators used to classify a soil as healthy under a variety of conditions.

1 EO N-82-20 Addressing the Biodiversity Crisis: https://www.gov.ca.gov/wp-content/uploads/2020/10/10.07.2020-EO-N-82-20-.pdf
2 https://www.unep.org/un-biodiversity-conference-cop-15
3 https://www.cdfa.ca.gov/oefi/healthysoils/

Soils are complex systems composed of many biotic and abiotic components that interact. The components can be physical, such as soil particles that influence texture or density of soil; chemical, such as its mineral composition or nutrient content; or biological, the living organisms that have co-evolved together with these other components to make soil their habitat.

The interactions among all components of soil result in unique properties that the components on their own do not display (termed *emergence*), often resulting from feedback between the components. For example, fungi that form partnerships with plant roots (mycorrhizae), extend the distribution of new carbon from roots further into soil, supplying fuel to otherwise carbon starved bacteria. These bacteria then mobilize nutrients from soil organic matter, and become a source of carbon, energy, and nutrients for grazing microorganisms such as protozoa that help liberate those nutrients and support plant growth.

These types of interactions have co-evolved over many millions of years to result in soil communities that efficiently transform and transfer resources among their members. Such ecological interactions are essential to healthy and productive soils (described further in this report) and require that soil functioning be considered from a whole-system perspective.

Complex systems often operate as a network–with many interdependent points of connection (nodes), that function through diverse interactions. Soils (like energy distribution and social communication networks) have certain configurations and network level properties, such as the number and connectivity of nodes (Figure 1.1). Together, these properties determine how effectively the whole system functions. Thus, it is not merely the number of organisms or species present in soil that matters to assess a soil's health but also, their interactions with each other and with their environment.

Globally, soils contain about one quarter of all species on Earth (Guerra et al. 2021), and an estimated 40% of living organisms in terrestrial ecosystems are directly associated with soil during their life-cycle (Decaëns et al. 2006). These organisms have evolved and adapted to interact with one another in soil, together performing numerous processes that regulate the stocks and flow of materials (e.g., water, carbon, nutrient, contaminants) in ecosystems. In carrying out these ecosystem processes, soil organisms shape the soil environment in ways that promote not just their own needs, but also those of their neighbors, and their neighbor's neighbors – building a network of interdependence leading to healthy functioning soils that other non-soil dwelling organisms, including humans, derive benefit from. From a human perspective, these benefits are called *ecosystem services*. Life in soil enables a broad range of processes, functions, and services, collectively described as *multifunctionality*. Soil multifunctionality is directly related to the diversity of organisms present (Wagg et al. 2014), highlighting the need to monitor, conserve, restore, and enhance soil biodiversity (Creamer et al. 2022).

(A) Western US Power Grid Network **(B) Social Network** **(C) Microbial Network**

Figure 1.1. Interactions between components of complex systems contribute to the overall function of the systems and ultimately their ability to withstand stress and disturbance. Like energy networks (A), and social networks (B), soil organisms, including microbes (C) display network properties that connect the behavior of individual components. *Image credits: (A) Western US Power Grid Network. Modified from: Requião da Cunha et al (2015). https://doi.org/10.1371/journal.pone.0142824 CC BY-SA 4.0. (B) Social network. From Martin (2015). Geschichte und Informatik. https://commons.wikimedia.org/wiki/File:SocialNetworkAnalysis.png. CC BY-SA 4.0. (C) Soil bacterial network. Credit: Shi Wang, Lawrence Berkeley National Lab.*

Soil biodiversity can be defined in many ways, but in essence, all living organisms that spend at least part of their life cycle in soil are part of this interconnected network. Collectively this includes the **abundance** and **identity** of organisms, their **functional traits** (from genes to genomes to traits), as well as the network of **interactions** between these organisms that regulate soil **processes** and contribute to overall soil function, as well as the capacity of soils to resist or recover from disturbance (L. Brussaard, De Ruiter, and Brown 2007).

Soil biological networks vary over spatial and temporal scales. Spatial variation is linked to location, soil type, climatic variation, moisture gradients and depth. Temporal variations in soil networks range from hours (the lifespan of some soil microorganisms), to daily and seasonally (e.g., with temperature and moisture fluctuations), through short to long term climate and vegetation changes, and up to geological time scales. Biological networks emerge as energy flows from sunlight through plants. The sunlight is captured and converted during photosynthesis, stored as carbon and transferred to soil either directly through the roots, by microbes, or by animals who consume the plant carbon, transforming it further and leaving their non-living residues (termed necromass) within the soil. This energy transfer–with carbon as stored energy–is mediated by plants and the numerous organisms they host, both above and belowground.

The Food and Agriculture Organization (FAO) of the United Nations (UN) published a recent synthesis on the State of Knowledge of Soil Biodiversity (FAO 2020), where soil biodiversity is defined as:

> *"...the variety of life belowground, from genes and species to the communities they form, as well as the ecological complexes to which they contribute and to which they belong, from soil micro-habitats to landscapes."*

This definition invokes an interconnected suite of living organisms that work together in soil (ecological complexes) to influence soil functioning across scales. As a definition, it is broadly encompassing, but it does not explicitly connect the functioning of soil to its biological components. **This connection between types of living organisms in soil and their functional roles is central to establishing a framework for biodiversity assessment and relating biodiversity goals to agronomic and other environmental goals.** The FAO report highlights the hierarchical nature of soil biodiversity. Soil organisms come in all shapes and sizes, ranging from 20 nanometers (10-9 m) to 30 centimeters (from viruses to plant roots that can extend for meters), all assembling into a network of interdependencies, often described as a food web, but more accurately represented as a network of interactions (e.g., de Castro et al. 2021).

The term *soil health* describes the state of a living system, and "the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans" (Lehmann et al. 2020). Soil biodiversity underpins soil health through the living organisms that possess capabilities to support soil processes (e.g., organic matter mineralization, nitrification) that interact to sustain ecosystem services (e.g., nutrient cycling) (Box 1). Healthy soils sustain numerous such processes

Box 1. Relationship between soil processes, functions and ecosystem services

Soil **processes** include physical, chemical, and biological processes that occur within the soil, such as organic matter decomposition, nutrient cycling, and water retention. These processes support soil functions, which are the benefits that the soil provides to ecosystems and human societies.

Soil functions are typically classified into three main categories: provisioning, regulating, and supporting. **Provisioning functions** include food, fiber, and fuel production. **Regulating functions** include water quality, climate, and disease regulation. **Supporting functions** include nutrient cycling, providing habitats for biodiversity, and providing cultural services.

Ecosystem services are the benefits that humans derive from ecosystem processes and includes soil processes and soil functions. Soil processes support the provision of ecosystem services. For example, soil processes such as organic matter decomposition and nutrient cycling support the production of crops, while soil functions like water retention and nutrient regulation support the provision of clean water.

Soil processes, functions, and ecosystem services are connected, and these connections are complex and interdependent. Changes in one can affect the others, with implications for the longer-term sustainability of ecosystems and human societies that rely on their services. For this reason, it is important to both understand these connections, and to manage soil resources, including its biodiversity, in a sustainable manner that supports the provision of ecosystem services.

that enhance the multifunctionality of soil. External pressures like land use change or climate change, impact all components of soil, and can have profound impacts on soil biodiversity, thus impacting soil multifunctionality, ecosystem services, and the benefits and values that soil provides (Bloor et al. 2021) (Figure 1.2).

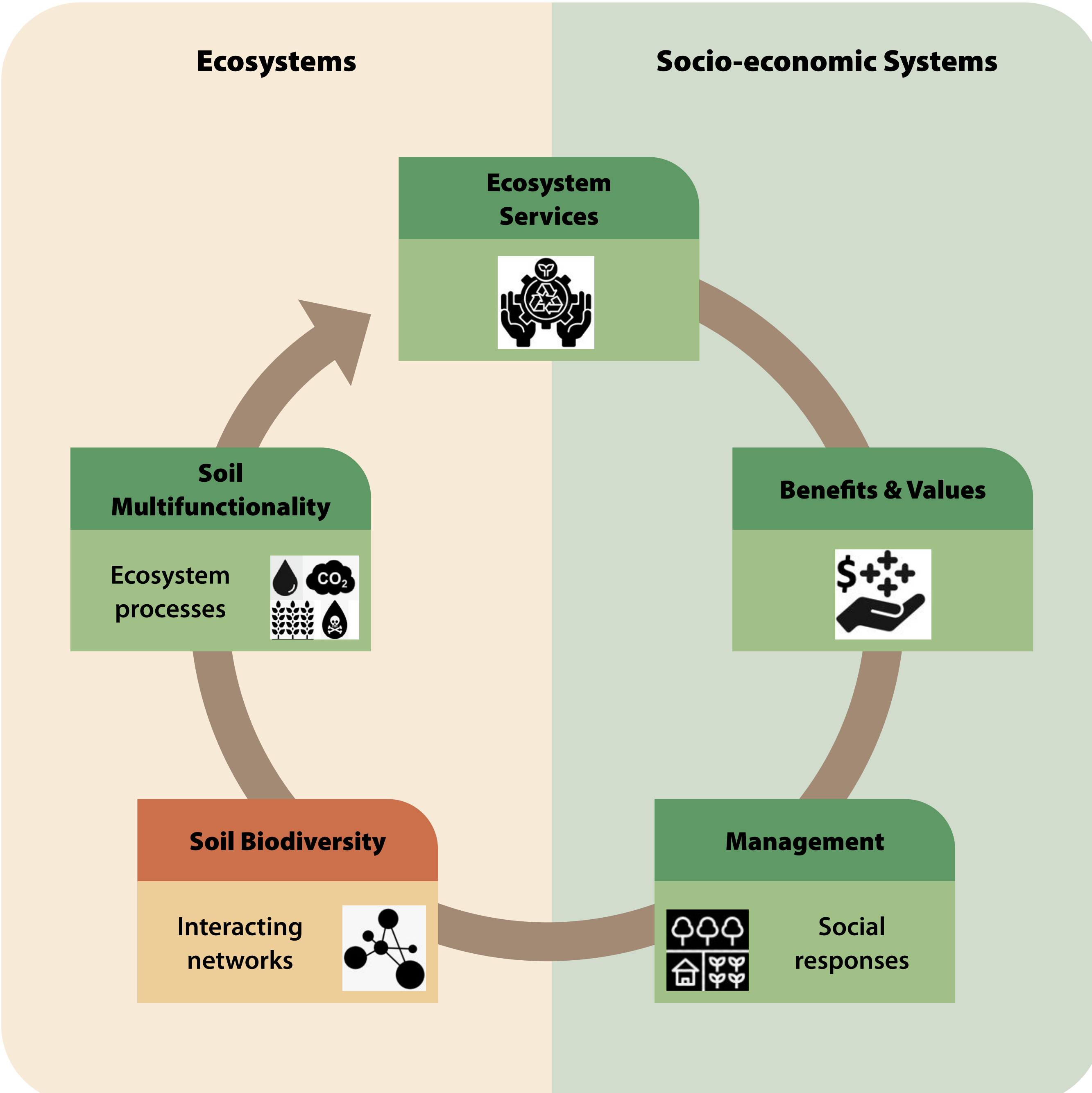

Figure 1.2. The central role of soil biodiversity in support of soil multi-functionality, ecosystem services, and the benefits and value provided to humanity. Ecosystems and socio-economic systems are intertwined, and feedbacks can either positively or negatively impact soil biodiversity and its essential role. *Credit: Eoin Brodie*

Biodiversity can be broadly defined as the variety of living organisms present in a place. From this perspective, soils represent one of the most biodiverse habitats on earth in part because it consists of a large number of distinctly different macro- and micro-habitats. Heterogeneity stems from vertical stratification of soil into layers with distinct characteristics as well as heterogeneity in the size and composition of the individual particles and aggregates that collectively make up soils. There is also large variation in physical and chemical properties of soils across California due to its diverse geological history and climate. Key soil properties that govern which soil organisms are found in a given habitat include pH (or acidity), redox potential (oxygen content), organic matter inputs from plants (energy), water, nutrients (e.g., nitrogen, phosphorus), and soil texture (i.e., how coarse or fine the soil particles are).

A single teaspoon of soil can contain dozens of microscopic worms (nematodes), miles of fungal hyphae and billions of bacterial cells. Scaling up, there are 57 billion nematodes on earth for every human being (van den Hoogen et al. 2019), and the global biomass of fungi and bacteria in soils amounts to 20 gigatons (Gt) of carbon (Bar-On, Phillips, and Milo 2018), compared with just 0.06 Gt for humans. As a result of the generations of accumulated soil life, soils currently store 1,500 Gt of carbon - twice as much as is found in the atmosphere. A single hectare of soil can contain up to 15 tons of soil life (Weil and Brady 2016), or 1.5 kg per square meter, including bacteria, fungi, nematodes, small arthropods, and earthworms (Figure 2.1).

Soil organisms range in size from nanometers (e.g., viral particles) to centimeters (e.g., earthworms). Broadly speaking, they can be classified into microorganisms (<10uM), microfauna (10 - 100uM), mesofauna (100uM-2mm), macrofauna (2-20mm) and megafauna (>20mm). As body size increases, the abundance of each group tends to decrease (Figure 2.2). A brief overview of the most common groups of soil organisms and their ecological roles is provided below. These include organisms that shred or decompose plant material, microbial grazers and predators, as well as plants which provide the base of the food web by transferring sunlight into food resources through photosynthesis[4] (Figure 2.3)

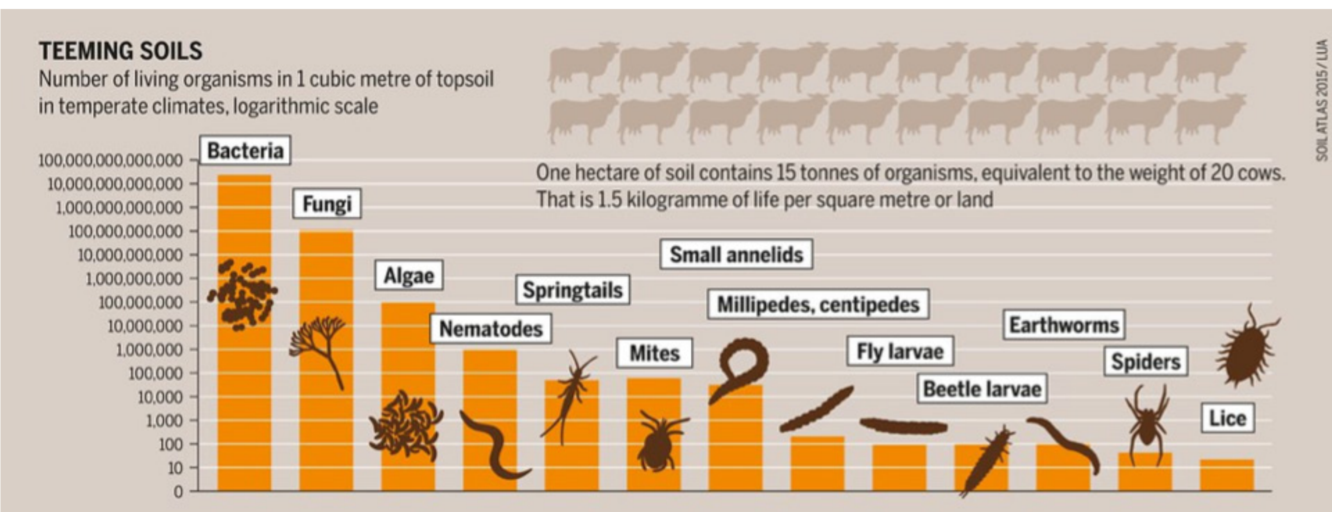

Figure 2.1. Numbers of different components of soil biodiversity found in a cubic meter of soil. *Credit: Bartz/Stockmar, CC BY-SA 3.0*

4 Additional information about the functions of soil biodiversity is found in Nielsen, Wall, and Six 2015, Nielsen 2019, and Orgiazzi et al. 2016.

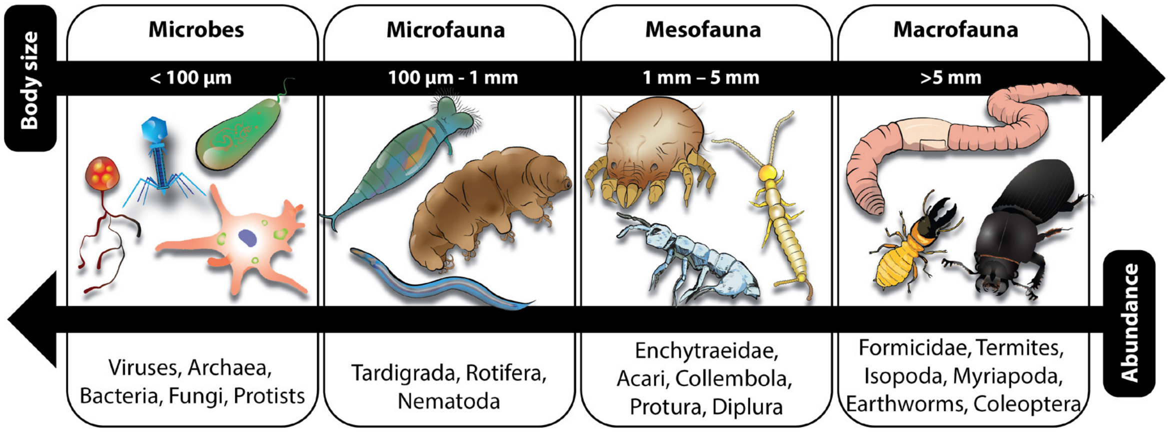

Figure 2.2. Size classification of soil organisms. As body size increases, abundance decreases. *Credit: Javier A. Ceja-Navarro. Modified from Global Soil Biodiversity Atlas (Orgiazzi et al. 2016); Credit: B Jakabek, Y Eglit, M Shaw, H Segers, L Galli, A Murray, RR Castro Solar, T Tsunoda, S Franzenburg, D Hope, C Abbe. Full-size DOI: 10.7717/peerj.9271/fig-2.*

REVIEW OF KEY GROUPS

MICROORGANISMS

VIRUSES

Highly abundant and diverse, soil viruses, also known as phages, can reach numbers as high as 109 per gram of soil (Williamson et al. 2017). They infect organisms from all trophic levels, influencing the growth of bacteria, fungi, and eukaryotic microfauna (protists and nematodes) and therefore indirectly affect the nutrient cycling processes mediated by their hosts (Suttle and Chan 1994; Ghabrial et al. 2015; Krstin et al. 2017; Seaton, Lee, and Rohozinski 1995). Viral lysis can directly liberate nutrients from microbial biomass and influence carbon (C) and nitrogen (N) cycling (C. P. D. Brussaard 2004). While data is lacking in soil, in the oceans it is estimated that viruses lyse up to 40% of marine microbial cells every day, with cascading influences on carbon and nutrient cycling as well as the climate (Suttle 2007; Danovaro et al. 2011; Weitz et al. 2015; Guidi et al. 2016; Roux et al. 2016). For example, changes in nitrite (NO2-) and nitrate (NO3-) concentrations are correlated with marine viral diversity as these molecules can drive host blooms and selection for viruses specific to the blooming hosts (Gregory et al. 2019; Bratbak, Jacobsen, and Heldal 1998). However, the environmental triggers or conditions inducing phage-mediated lysis in soil are still unknown, as well as the extent of their impacts on soil biota and nutrient cycling.

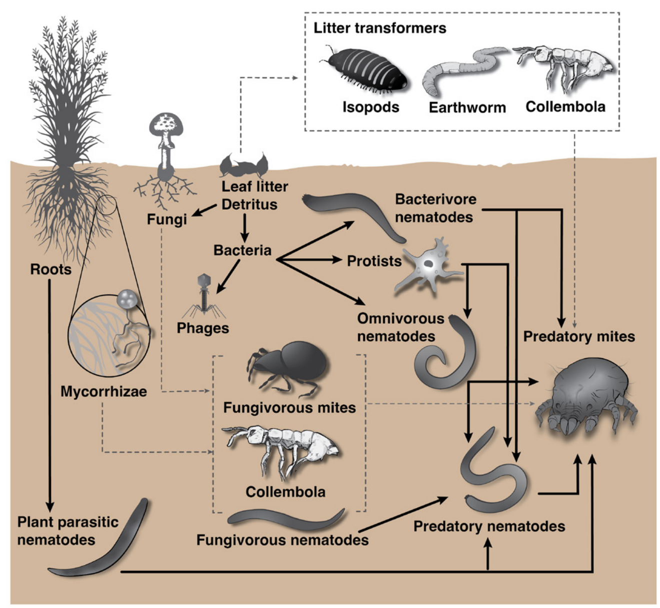

Figure 2.3. General groups of soil biodiversity and their ecological functions. *Credit: Javier A. Ceja-Navarr*

BACTERIA AND ARCHAEA

Bacteria and archaea are single-celled microorganisms with strikingly similar morphology. They both have similar cell shapes and lack a real nucleolus that encloses the DNA within the cell (they are prokaryotes). But despite these similarities, archaea and bacteria are taxonomically different.

Archaea, which are genetically closer to eukaryotes, were first discovered in extreme environments such as hypersaline lakes, soil around volcanic vents, or very acidic soils (Orgiazzi et al. 2016). Thus, archaea have been traditionally considered as extremophiles. However, recent studies show that archaeal species also exist in mesophilic environments and could play a key role in nitrogen cycling. In well-aerated cropping soils, archaea contribute to the oxidation of ammonia, dominating over bacterial nitrification in acidic soils (Prosser and Nicol 2012). In water saturated anaerobic soils, archaea are responsible for the formation of methane (CH_4), and thus play a key role in the global carbon cycle (L. Bräuer et al. 2020).

Bacteria are the most diverse domain of life on Earth, with the majority of species unidentified. Because of this, bacterial taxonomy is continuously revised, and some sources cite 30 bacterial phyla while others cite as many as 92 (Parks et al. 2018). A large body of literature published in the last decade cites the use of genetic markers to determine the most diverse and abundant bacterial phyla found in surface soils are proteobacteria, Firmicutes, Actinobacteria, Cyanobacteria and Acidobacteria (Orgiazzi et al. 2016). Soil physicochemical properties, namely pH, soil carbon, and oxygen availability, are major drivers of soil bacterial abundance and diversity across the globe (Fierer 2017; Delgado-Baquerizo et al. 2018). Bacteria exist in virtually any soil environment and perform a wide array of biochemical processes. Thus, the vast genetic diversity of this group is also matched by a large functional diversity. Soil bacteria are essential drivers of biochemical decomposition of organic matter, driving biogeochemical cycles and atmospheric composition under a wide range of environments, from boreal bogs to agricultural soils (Orgiazzi et al. 2016; Sokol et al. 2022). Bacteria are also involved in all steps of the nitrogen cycle, from N fixation to nitrification and denitrification, and are essential for ecosystem productivity (Philippot and Germon 2005).

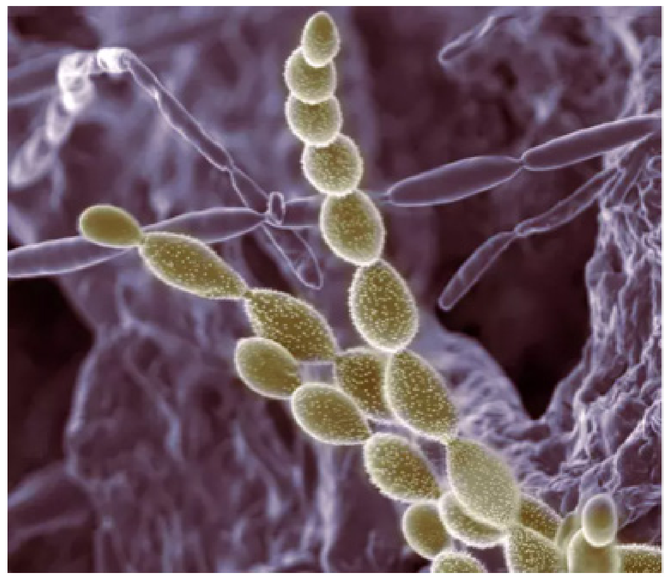

Figure 2.4. Soil bacteria in a ponderosa pine ecosystem via scanning electron microscopy. *Credit: Alice Dohnalkova, https://imaggeo.egu.eu/view/4213/, CC BY-3.0*

FUNGI

Fungi play a unique role in soils due to their filamentous nature. The biomass of many fungi are composed of threadlike filaments, known as hyphae, that are approximately 10 times narrower than a human hair. Collectively, the filaments of a single fungus can form a complex, branched network known as a mycelium. The mycelium allows fungi to simultaneously interact with the soil environment at a microscopic scale while also foraging across the many microhabitats in soils (Peay, Kennedy, and Bruns 2008). There are an estimated 6 million species of fungi globally (Baldrian et al. 2022), with approximately 100-200 species of fungi in an average handful of soil (Bar-On, Phillips, and Milo 2018). Fungi also play a diverse set of ecological roles in soils. First, the roots of almost all plants form a partnership with specific groups of fungi, known as mycorrhizal symbiosis (Brundrett and Tedersoo 2018). This symbiosis provides plants with additional nutrients (N, phosphorus (P)) and water than they could obtain on their own and thus greatly increase agricultural productivity (van der Heijden et al. 2015; Kakouridis et al. 2022). Second, fungi are the primary decomposers of plant tissues (de Boer et al. 2005). As such, they help control rates of nutrient release and carbon storage in soils. Some of the most important agricultural pathogens are fungi (e.g., stem rust of wheat) and may either spend a portion of their life cycle in soil or infect belowground plant parts. Other fungi that live inside of plants - known as endophytes - can act as bioprotectants, reducing the ability of pathogens to infect and cause damage to their hosts (Busby, Peay, and Newcombe 2016; Busby et al. 2017). Because of their key role in regulating nutrient cycles and effects on plant vitality, proper management of fungal diversity in soils is critical for California agriculture.

Figure 2.5. Fungi such as *Mycena californiensis* play an important role in the decomposition of woody material *Credit: Wikimedia, https://commons.wikimedia.org/wiki/File:Mycena_californiensis_72630.jpg. CC-SA 3.0*

PROTISTS

Although commonly classified as part of the microfauna, protists are also microorganisms whose sizes can range from a few microns to millimeters. The main groups of protists relevant to soil ecology based on abundance and functional diversity include the Amoebozoa, Cercozoa, Stramenopiles, Ciliophora, and Excavata (Bonkowski, Fiore-Donno, and Dumack 2019; Esteban, Finlay, and Warren 2015). Most of the Amoebozoa consume bacteria, but other nutrition strategies are common among this group; some consume fungi, and some predate other protists and small nematodes (Oliverio et al. 2020; Mahé et al. 2017). Most Cercozoa are bacterivores and active in soil, some can feed on fungi (Vampyrellida) or parasitize plants (Phytomyxea) (Adl et al. 2019). The Excavata include amoeboid organisms, most of which are bacterivores, but also include photosynthetic organisms (Jones 1997). Both the Stramenopiles and to a lesser extent the Chloroplastida are known to include photosynthetic protists (Murase and Frenzel 2008), while the Stramenopiles also include the Oomycota and the economically important plant pathogen genera, *Phytophthora* and *Pythium* spp. Finally, the Ciliophora tend to be highly diverse in terrestrial environments and exhibit bacterivory in wet soils (Saleem et al. 2012; Bates et al. 2013). Protists play critical roles in soil by stimulating the rate of organic matter decomposition (Clarholm 1985) and shaping bacterial dynamics (Clarholm 1989; Krome et al. 2009). Through their predatory activity, protists release nutrients from bacterial biomass and make nutrients available to plants and other organisms in their environment (Bonkowski 2004; Trap et al. 2016; Koller et al. 2013). While protists form the base of the bacterial food web and have a strong influence on carbon and nutrient cycling (Nielsen 2019), they are much less well- studied than other groups because they are difficult to extract and count from soil (although new molecular approaches are starting to become available).

Figure 2.6. An example of a protist in the group Ciliophora, which are known to eat bacteria. *Credit: Picturepest, https://www.flickr.com/photos/picksfromoutthere/13215594964/. CC BY 2.0*

Soil microfauna (under 0.1 mm) live inside water filled soil pore spaces. Their relatively larger size makes them recognizable under the microscope as small soil animals, although they are barely visible with the naked eye. As microfauna graze on bacteria and fungi, they influence carbon and nutrient cycling by increasing the rate of microbial turnover and can change the balance of prey species through selective feeding (Nielsen 2019). For example, bacteria-feeding nematode roundworms can eat up to 6.5 times their biomass per day (Lavelle and Spain 2001), and turnover of 20-130 kg N per year per hectare (Coleman et al. 1984). One of the best studied microfauna, nematodes, have diversified into nearly every life history strategy; nematode species may be bacterial-feeding, fungal-feeding, root feeding, omnivores, predators, or insect parasites. Nematodes may be particularly good bioindicators because they exploit many types of food sources, have diverse life history strategies and are abundant in soil. Indeed, nematodes are considered one of the most abundant multicellular animals on Earth, with numbers reaching millions per square meter of soil (Bongers 1999). Other microfauna groups include tardigrades and rotifers. Tardigrades, also called "water bears", eat bacteria, plants, and other microscopic organisms. While their ecological roles remain unclear, tardigrades can withstand periods of extreme drought in a resistant state for up to 200 years (Orgiazzi et al. 2016). Rotifers feed by filtering food particles from water and may consume bacteria, algae or other animals. Since most rotifers need to be identified alive, relatively little is known about their ecological influence.

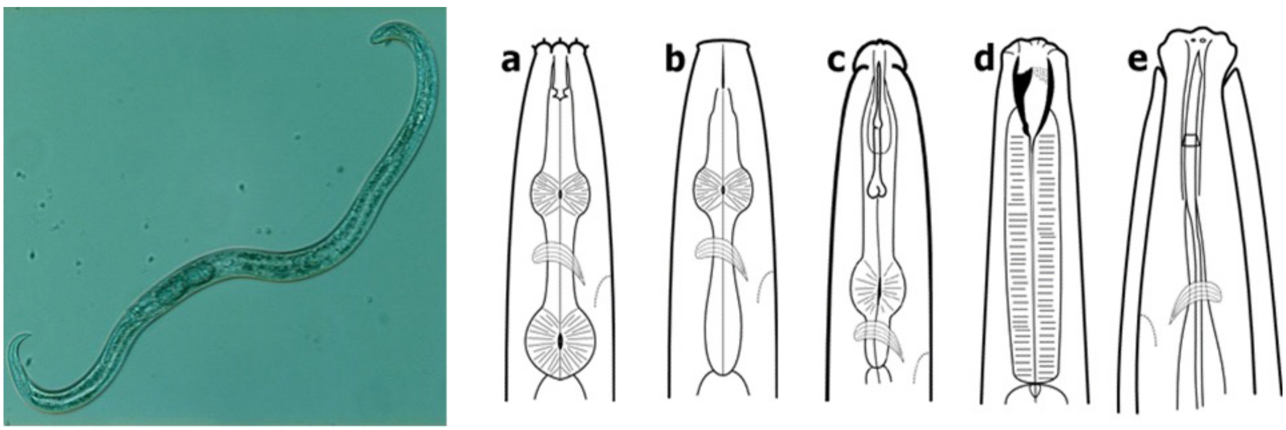

Figure 2.7. A fungal feeding nematode *(Aphelenchoides*) (left) and feeding habits of soil nematodes as identified by mouthparts (right): a: bacterial feeders; b: fungal feeders; c: plant feeders; d: predator/omnivore ingesters; e: predator/omnivore piercers. *Nematode photo credit: Amanda Hodson. Mouthparts diagram credit: Modified from Yeates et. al 2009, https://doi.org/10.1079/9781845933852.0001*

MESOFAUNA

While most microfauna live in water films around soil particles, larger mesofauna (0.1-2mm) live in the air spaces between soil pores. The most ecologically important groups of microfauna include microarthropods such as mites and springtails. Distant relatives of insects, springtails (Collembola) feed predominantly on microbes, particularly fungi and algae (Petersen and Luxton 1982) and can contribute to soil formation through the deposition of fecal pellets (Rusek, Úhelová, and Unar 1975). Free living soil mites contain approximately 40,000 known species and include three main groups that are known to contribute to ecosystem functioning (Nielsen 2019). The suborder Prostigmata includes a range of microbial grazers, decomposers, predators, and herbivores. The order Mesostigmata comprises mostly active predators that feed on nematodes and springtails, while turtle mites, in the suborder Oribatidae, contain groups which feed on decaying organic matter as well as others which graze on microbes. They are particularly important in carbon cycling because they fragment plant material into smaller pieces which are more easily processed by smaller members of the soil food web such as nematodes and microbes. The microfauna also includes the Enchytraeidae, small relatives of earthworms that feed on plant fragments in the soil. These are likely more important in cold, organic rich soils such as those found in England (Cole, Bardgett, and Ineson 2000) rather than California. Other microfauna which are present in lower abundances and have smaller or unknown contributions to ecosystem functioning include small relatives of scorpions (Pseudoscoprionida), as well as distant relatives of insects (Proturea, Dipulra, and Symphyla).

Figure 2.8. A fungal feeding mite in the genus Ameroseius (left) and a collembola, in the family Neanuridae, which also feeds mainly on fungi (right). *Credit (mite): Peter Mašán, https://doi.org/10.3897/zookeys.704.13304, CC BY-SA 4.0. Credit (collembola): Phillipe Garcelon, https://www.flickr.com/photos/philgar/49537574313/, CC BY 2.0.*

MACROFAUNA

Macrofauna comprises soil organisms larger than 2mm and smaller than 20mm. Macrofaunal diversity and abundance is typically highest in the topsoil and litter layer, although some earthworm taxa can dig deep galleries and live several meters belowground. Because of their larger size, macrofauna are easier to study, meaning much more is known about their diversity and functions in soil. Soil macrofauna play a regulatory role in the soil food web, and are crucial to soil formation, decomposition, nutrient cycling, biotic regulation, and for promoting plant growth (Briones 2018). For instance, centipedes, termites or ants can be predators and therefore essential for biological control and top-down regulation of the soil food web. Earthworms and isopods (pill bugs or roly pollies) are essential in regulating decomposition rates by shredding and comminuting soil detritus, therefore facilitating the work of soil microorganisms. Some groups of macrofauna like earthworms, ants and termites are also microbial grazers, regulating the abundance and diversity of soil microorganisms. Ants, termites, and earthworms are also well-known ecosystem engineers. By building nests and digging galleries they contribute to the soil-building process of bioturbation, increasing soil aeration and creating pathways for preferential water flow. Thus, either directly (by grazing and predation) or indirectly (by altering soil environmental conditions), macrofauna exert a strong role in decomposition and nutrient cycling.

Figure 2.9. An isopod, or roly poly, of the species *Porcellio pumicatus* which shreds decomposing plant material into smaller pieces making it accessible as food to smaller organisms. *Credit: Wikimedia, https://commons.wikimedia.org/wiki/File:Isopoda - Porcellio pumicatus.JPG, CC SA 3.0.*

MEGAFAUNA

Soil-disturbing vertebrates include small rodents, voles, moles, gophers, ground squirrels, snakes and lizards, and amphibians that spend the majority of their life in soil. These animals typically use burrows, and depending on the species, feed on plant roots and/or meso- and macrofauna. Their activity causes soil turnover and distribution impacting aeration, drainage and nutrient cycling. This disturbance results in elevated patches of increased ecosystem functioning and increased biodiversity (Mallen-Cooper, Nakagawa, and Eldridge 2019). In croplands, management and control of these megafauna mainly occurs through agricultural activities such as tillage.

Figure 2.10 Megafauna like Beldings ground squirrels *(Urocitellus beldingi)* facilitate soil aeration through burrowing. *Credit: Alan Vernon, https://flickr.com/photos/32541690@N02/4090107480, CC BY-NC-SA 2.0*

PLANTS

Plants are the main source of primary productivity in terrestrial ecosystems and roughly 50% of their biomass exists belowground as roots and other structures. Plants evolved in a world dominated by prokaryotic and eukaryotic microbes (Heckman et al. 2001) and through evolutionary time have established a physical and chemical dialogue with soil microbial dwellers via their roots (Lambers et al. 2009). Root structure varies substantially between species, from woody tap roots with lateral branches found in shrubs and perennials, to fibrous root systems found in grasses. The essential functions of plant water and nutrient uptake are closely related to species-specific plant root traits such as root diameter, root tissue density, or degree of branching. These functions are supplemented by associations with soil organisms. In exchange for carbohydrate-rich exudates from roots, plants form symbiotic relationships with mycorrhizal fungi or nitrogen-fixing root nodule forming bacteria (*Rhizobia* spp. and *Frankia* spp) to obtain essential nutrients and even water. These root exudates are the primary currency within the 'rhizosphere', the area around the root where a large biomass of microorganisms develops, using exudates as a food source (Nuccio et al. 2020). Because of this close association between plant roots and soil microorganisms, changes in root traits through choice of crop species or selective breeding can directly influence diversity in the soil (Haichar et al. 2008).

Plants can also exert influence on rhizosphere microorganisms by releasing specific chemical cues via root exudates (Zhalnina et al. 2018; Broeckling et al. 2008). These chemical signals act as a filter for microbial communities in a dynamic process associated with plant development (Chaparro, Badri, and Vivanco 2014). This filtering process – known as the "rhizosphere effect"--is defined as the influence of plant physiology on the physicochemical and biological properties of the root zone (Borruso et al. 2014; Pett-Ridge et al. 2021). This rhizosphere effect can modify the abundance, diversity, and composition of microbial communities across trophic levels, and it is frequently characterized by reduced diversity and more complex co-occurrence networks in the rhizosphere compared to bulk soil (Shi et al. 2016; Ceja-Navarro et al. 2021). These modifications to the rhizosphere community can influence processes such as N fixation, P solubilization, production of plant growth regulators, and disease protection (Tsurumaru et al. 2015; Chhabra et al. 2013; Majeed et al. 2015). As such, plant community diversity and composition influence the overall makeup of soil biological communities, from microbes to macrofauna, through cascading events that likely start with the plant's modulation of its own microbiome.

INTERACTIONS AND EMERGENT PROPERTIES OF SOIL BIOTA

An ecological community is defined as the sum of all the interacting species within a particular habitat (Box 2). The incredible taxonomic and functional diversity of soils (described above) make soil communities the product of a complex web of interactions. These interactions give rise to emergent properties of the community and soil ecosystem that are not predictable simply from the sum of their parts. Many of the effects of microfauna and mesofauna on ecosystem processes are indirect, mediated through their interactions with microbes. For example, bacterivore soil microfauna, such as protists and bacterial-feeding nematodes, can decrease microbial biomass through their feeding, but are paradoxically associated with increased microbial activity, nutrient cycling, and plant growth (Trap et al. 2016). It is thought that microfauna stimulate microbial turnover by their grazing, which then increases nitrogen mineralization and plant growth (Clarholm 1985; Bouwman and Zwart 1994). Similarly, mites and collembolans (termed microarthropods) consume organic matter but can also contribute directly to soil organic matter by depositing eggs, feces, or even their own dead bodies which may form nucleation points for soil aggregates to form (Maaß, Caruso, and Rillig 2015).

Box 2: Key Concepts in Soil Ecology

The structure of ecological communities are described primarily based on their diversity and composition.

Diversity (see Chapter 1) is broadly defined as the variety of living organisms found in a given sample or habitat. Diversity can be further partitioned into two components - alpha and beta diversity.

Alpha diversity refers to the diversity of species in a specific location (e.g., the number of species of bacteria in a handful of soil) while **beta diversity** refers to the differences in diversity between different locations. That is, how much taxonomic overlap is there in the composition of species found in two different soil communities.

Composition refers to the taxonomic mix of organisms found in that sample, for example which families, genera, or species of bacteria, fungi, or fauna are found there.

Soil biota's effects on ecosystem processes depend on their population densities and/ or abundance. For example, while low to moderate grazing of fungi by springtails stimulates microbial respiration and nutrient mineralization rates, high densities of springtails decrease microbial respiration, which could suppress aggregate formation (Teuben and Roelofsma 1990; Hanlon and Anderson 1979). Because of this complexity, while there is consensus that soil biodiversity contributes to ecosystem functioning, it has been very difficult to quantify these effects on an ecosystem scale to establish their consequences for agriculture.

The differences in diversity that arise between soils in agricultural systems are ultimately the result of the community assembly process. In soil, the community assembly process starts with the total pool of all soil biota that could be present. For any organism in this pool to become a member of a local soil community it must pass through a series of ecological sieves (Kraft et al. 2015). First, the organism must be capable of reaching that location (dispersal sieve). Second the organism must be able to grow successfully in the environmental conditions in that location (environmental sieve). Finally, the organism must be able to coexist with the other organisms that are already in that location (biotic sieve). The fact that these sieves are affected by human activity is both a threat and an opportunity. For example, human mediated dispersal may introduce unwanted pests into the regional species pool and facilitate their movement within California. At the same time, practices such as no-till or organic agriculture may create environmental conditions that favor desirable components of the soil biota.

How best to benefit from soil biological processes on a farm scale likely depends on many localized factors. For this reason, efforts to understand and manage soils holistically are often focused on the properties of local soil communities and comparison of soil communities in different habitats, climates, or management regimes. The following chapter outlines some specific ecosystem services associated with soil biota and threats to soil biodiversity in the California context.

ECOSYSTEM SERVICES PROVIDED BY BELOWGROUND BIODIVERSITY

As described in Chapter 2, soil organisms have diverse forms and functions that have direct and obvious effects on ecosystem-scale conditions through a network of complex interactions. Together, all soil organisms define key ecosystem processes through their interactions and mediate the responses of ecosystems to environmental change.

The numerous benefits provided by belowground biodiversity are rooted in the concept of multifunctionality - the idea that soil organisms contribute to many different ecosystem functions simultaneously (Manning et al. 2018). The complex interplay of ecological interactions such as symbiosis, predation, commensalism, and parasitism give rise to emergent properties that drive ecosystem functions at larger scales (Mastrangelo et al. 2014). The interconnected and emergent nature of these functions present both a challenge and an opportunity for ecosystem management, since practices intended to promote a specific function (such as carbon storage) may have an impact on multiple other functions (such as water infiltration and/or nutrient cycling). Because soil organisms affect multiple processes through their growth and activity, the concept of ecosystem multifunctionality can be used to generate a comprehensive understanding of the net effects of soil biodiversity loss.

When an economic or social value is ascribed to one of these ecosystem functions, it becomes an ecosystem service (Figure 1.2) (Bommarco, Kleijn, and Potts 2013). Valuation of these services is in turn, dependent on the goals of the system and the scale(s) of action and of interest (Nielsen, Wall, and Six 2015). For example, organic carbon has long been recognized as a desirable soil attribute that is linked to the potential for water and nutrient storage in agricultural soils. However, with the growing awareness of climate change, the ecosystem service of soil carbon formation and storage at the field scale has become more valuable as a potential climate change mitigation strategy at larger scales.

If the numerous, simultaneous challenges that society faces are to be overcome - climate change, land degradation, biodiversity loss and agricultural intensification - the immense value of ecosystem services driven by the multifunctionality of soils and belowground communities must be recognized. This value is supported by the ample evidence linking soil biodiversity, ecosystem functions, and the provision of agriculturally relevant ecosystem services (Wagg et al. 2021; Griffiths et al. 2000). It is also important to acknowledge that belowground biodiversity loss ultimately decreases multifunctionality and the provision of these vital ecosystem services.

LANDSCAPE

- Regional species pool from which beneficial organisms can disperse
- Improved broad-scale provision of services including regulation of soil erosion, nutrient dynamics and water
- Adaptability, resistance and resilience to environmental change

FARM

- Support local populations of beneficial organisms
- Improved species diversity including antagonists of plant pests and pathogens

FIELD

- Nutrient cycling and uptake
- Nutrient and water use efficiency
- Plant growth, health and stress tolerance
- Pest, pathogen and disease suppression
- Soil organic matter regulation
- Soil structure
- Water retention

Figure 3.1. A diagram showing the benefits of soil biodiversity at various scales. *Modified from (Nielsen, Wall, and Six 2015)*

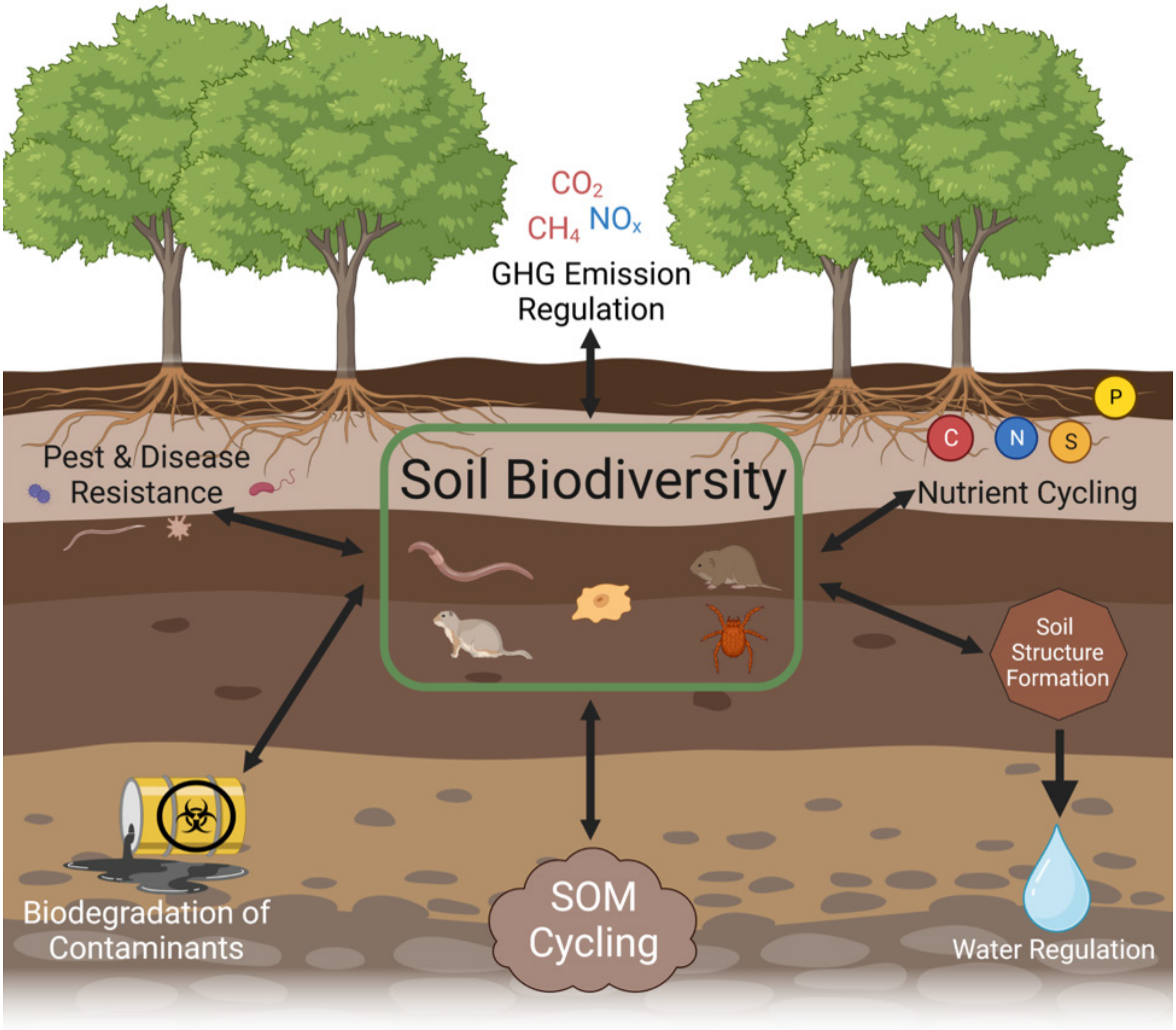

Figure 3.2. A simplified diagram illustrating the main interconnected key soil functions that are dependent on soil biodiversity. These include regulating GHG (Greenhouse Gas) emissions, SOM (soil organic matter) cycling, water regulation through soil structure formation, biodegradation of contaminants, nutrient cycling and pest and disease resistance. *Credit: Daniel Rath*

BIODIVERSITY AND WATER REGULATION

Soil biodiversity has a strong impact on water availability in agricultural soils. Water travels through soil in a densely interconnected network of pores that are formed through the collective action of microorganisms, micro-, meso- and macrofauna. Macrofauna such as earthworms, ants and termites can contribute to the formation of this pore network through burrow excavation and bioturbation (Chapter 2). Microorganisms such as bacteria and fungi drive the formation of soil structure through the secretion of sticky extracellular polymers and fungal hyphae that effectively "glue" soil particles together into aggregates (Six, Elliott, and Paustian 2000), leaving empty spaces between the aggregates that water can flow through. These organisms also collectively contribute to the formation of soil organic matter (SOM), which has a strong positive impact on soil water storage and infiltration (Lal 2020; Franzluebbers 2002; Rawls et al. 2003).

Given that California's rainfall mostly occurs outside of the summer growing season, this water stored in the soil pore network after infiltration (Falkenmark and Rockström 2010) has the potential to meet up to 20% of crop water demands (Devine and O'Geen 2019). Soil biodiversity can also contribute indirectly to agricultural water availability by promoting plant root growth and forming relationships among roots, mycorrhizal fungi and plant growth promoting bacteria (Acevedo et al. 2022). These relationships expand the effective soil area that plants can exploit for nutrients and water (Augé 2001) while increasing their ability to withstand drought stress (Rubin, van Groenigen, and Hungate 2017).

BIODIVERSITY AND ORGANIC CARBON STORAGE

Soil organic carbon (SOC), in the form of SOM, simultaneously represents an energy source that powers ecosystem functions, is a potential sink for carbon dioxide molecules from the atmosphere, and is an important structural component of soil (Kopittke et al. 2022). Decomposition of organic inputs is a concerted effort by the soil food web: macrofauna such as earthworms and ants shred plant residues into smaller pieces, which are further processed by fungi and bacteria to extract energy. Most soil organic inputs are re-released as carbon dioxide during the breakdown process, while the minority (between 3 – 33%) is retained in the soil as SOM (Cotrufo and Lavallee 2022). SOM undergoes continual cycling and transformation, being trapped into cellular structures and then released again through the process of cellular death and predation by organisms such as nematodes, protists, and springtails. Under the right environmental conditions, SOM can also be retained for centuries, locked within soil aggregates or associated with mineral surfaces that can protect it from decomposition (Lavallee, Soong, and Cotrufo 2020), although even this protected organic matter will eventually undergo further breakdown (Dynarski, Bossio, and Scow 2020). Whether organic carbon is completely decomposed or stored for any length of time in the soil is dependent on the interaction between soil organisms and factors such as plant communities, water content, temperature, oxygen content and pH.

Diverse soil communities play a key role in soil organic carbon storage by increasing the decomposition of organic inputs (Delgado-Baquerizo et al. 2020) and can maintain higher residue decomposition rates under short term disturbances than less diverse communities (Griffiths et al. 2000). Diverse soil communities also increase the stability of plant biomass production, plant diversity, litter decomposition, and the assimilation of soil carbon (Wagg et al. 2021). At the same time, reductions in soil biodiversity can lead to decreased transformation of plant inputs into organic matter (Wagg et al. 2014). Increases in SOM in California soils through farming systems that promote organic inputs and ecosystem restoration have been shown to increase biodiversity-driven ecosystem services such as crop yield stability (Li et al. 2019), nutrient cycling, and carbon uptake (Morriën et al. 2017).

BIODIVERSITY AND GREENHOUSE GAS REGULATION

Interactions within the soil community play an important role in regulating the emission of potent greenhouse gasses (GHG) such as nitrous oxide, carbon dioxide and methane from soils. The addition of nitrogen fertilizer can stimulate nitrifying bacteria that convert ammonia into water-soluble and plant-available nitrate (Li et al. 2020), but can also stimulate denitrifying bacteria that convert that nitrate into gaseous nitrous oxide. Nitrous oxide is produced when the denitrification process, which converts soil nitrogen to nitrogen gas, is not completed due to the presence of oxygen (Rohe et al. 2021). The denitrification process is driven by a diverse set of heterotrophic microbes that include bacteria, archaea and fungi (Lazcano, Zhu-Barker, and Decock 2021). Carbon dioxide is released from soils during the decomposition of organic matter (see previous section); this carbon dioxide also serves as the raw material for photosynthesis by plants, autotrophic bacteria and archaea. Methanogenic archaea produce methane during organic matter decomposition under anaerobic conditions (Keiluweit et al. 2017); this methane can then be oxidized by methanotrophic bacteria and archaea when oxygen becomes available (Serrano-Silva et al. 2014).

Promoting the tightly coupled cycling of both C and N within the soil and plant biological community can reduce nutrient loss and GHG emissions. For example, under controlled conditions, crop inoculation with a diverse soil community can increase N uptake by 29%, P uptake by 110% and reduce N losses by leaching 51% while increasing yield by 22% (Bender and van der Heijden 2015). Increased abundance, diversity and activity of soil denitrifiers can result in lower N2O emissions (Tatti et al. 2013). Plant inoculation with mycorrhizal fungi can increase N and water uptake, reducing GHG emissions and modifying denitrifier activity (Lazcano, Barrios-Masias, and Jackson 2014). The increased addition of processed organic inputs such as compost and biochar can also reduce nitrous oxide emissions compared to raw manure and inorganic nitrogen fertilizer by impacting denitrifier populations (Lazcano, Zhu-Barker, and Decock 2021).

BIODIVERSITY AND PATHOGEN SUPPRESSION

While soil multifunctionality emerges from a network of interactions between soil organisms, not all of these interactions are mutually beneficial. In particular, soils are home to a diverse range of pathogenic organisms that obtain energy and nutrients through exploitation of other organisms. Invasive pathogens were estimated to cause an annual crop loss of $21 billion dollars in the United States in 2009 (Rossman 2009), but many pathogenic organisms are a natural component of healthy soils and play an important role in regulating the overall composition and abundance of soil biodiversity. Since high pathogen abundance can have negative consequences for crop production and quality, soil organisms and communities that suppress and compete with pathogens provide a valuable ecosystem service (Busby et al. 2017).

The capacity for certain soils to suppress disease has been recognized for many decades in agriculture. Suppression is defined as the ability of resident soil microbes to persistently reduce pathogen establishment or disease incidence and severity, and is one of the most effective forms of agricultural biocontrol (Schlatter et al. 2017). Suppressive soils have been identified for many important agricultural pathogens and are classified in two main types: generally suppressive soils and specific suppressive soils. In generally suppressive soils, some feature of the overall microbial community (diversity, biomass, composition) prevents pathogens from obtaining sufficient resources to establish and can be encouraged by agricultural practices such as adding organic matter to soils (Weller et al. 2002). In specific suppressive soils, individual organisms have a specific antagonistic relationship with a known pathogen (Weller et al. 2002). For example, fluorescent Pseudomonads have been shown to reduce take-all disease of wheat through production of the antibiotic 2,4-diacetylphloroglucinol (DAPG) (Schlatter et al. 2017). Regardless of the specific mechanism, disease suppression and pathogen protection of crop plants relies on the presence of a diverse microbial community. Adoption of agricultural practices to cultivate generally diverse communities or specifically known beneficial microbes thus present an ecologically friendly opportunity to reduce a major source of crop loss.

BIODIVERSITY LOSS AND ECOSYSTEM SERVICES

As discussed in previous sections, the diverse groups of organisms that inhabit soils are responsible for the provision of critical ecosystem services that underpin agricultural productivity. While it may be intuitive that diversity in this broadest sense is important for California agriculture, there is also a strong relationship between the species diversity within groups of organisms, and the functions that they carry out. As a result, agricultural practices or climate change effects (see subsection below on threats) that reduce the diversity of soil organisms may compromise important ecosystem services. The relationship between biodiversity and ecosystem functions is a rich area of both theoretical and empirical ecological research. Conceptually there are two primary mechanisms by which diversity can positively affect function that are most relevant to management of agricultural soils (Loreau et al. 2001). First, the more species present in a local community the greater the likelihood of functional redundancy among species. This redundancy increases the temporal stability of a community by minimizing the likelihood that critical functions are lost anytime the community is perturbed (Biggs et al. 2020). Second, the more species present in a local community the greater the likelihood that those taxa have complementary physiological capabilities that maximize the efficiency of any given ecosystem function (Tilman, Isbell, and Cowles 2014). Greater efficiency tends to maximize resource uptake and resulting biomass of the focal community, but this efficiency can have additional benefits such as resistance to establishment of invasive species (Levine 2000) or pathogens (see previous section). While the exact mechanisms are not always clear, the tendency of more diverse crop communities to maximize ecosystem functions relative to monocultures has been observed consistently across a diverse range of organisms (Cardinale et al. 2006).

THREATS TO CALIFORNIA'S BELOWGROUND BIODIVERSITY

Describing the impact of the last several decades of global change on soil biodiversity requires highlighting some ecological concepts: disturbance, resilience, and resistance (Shade et al. 2012). Disturbances are events that alter either the soil environment or soil biological communities. These events may be sudden, such as a single tillage event, or gradual, such as reduced soil moisture in an extended drought. The severity of these disturbances depends on the vulnerability of soil communities and the environmental context. As an example, a single fire event in a regularly burnt pine forest may not be a large disturbance since the impacted soil community is either adapted to, or resistant to fire. However, if that same fire occurs in an area that does not have the same degree of resistance, it can result in major, long-lasting shifts in the composition and function of soil communities (Dacal et al. 2022). A resilient soil biological community would be able to return to a pre-disturbance condition in a relatively short period of time, provided additional disturbances do not occur. Under extended, gradual disturbance however, community composition baselines may shift to enter a new stable state (Philippot, Griffiths, and Langenheder 2021).

This shifting of baselines is what has happened to Californian soil biodiversity under the disturbances introduced by human presence (Culman et al. 2010). Ecosystems experiencing impacts via agricultural management (i.e., tillage, fertilizers, and pesticide application) meant to increase agricultural productivity have shifted their taxonomic and functional biodiversity baselines to accommodate these disturbances. This has resulted in a loss of belowground biodiversity relative to less disturbed ecosystems, a shift from fungal to bacterial-based food webs, and the biological regulation of soil functions being replaced by regulation through chemical and mechanical inputs (Giller et al. 1997). Fortunately, these belowground biodiversity losses can be mitigated or partially reversed by farming practices that reduce these disturbances (Thiele-Bruhn et al. 2012).

Since many California soils are starting from a place of reduced biodiversity compared to less disturbed ecosystems, they may be even more susceptible to future disturbances. In the next few decades, soil biodiversity faces the immediate threat of extended drought, land use conversion, and agricultural disturbance. It is important to protect soil biodiversity in the face of these immediate threats to prepare soils to handle the more gradual threats of longer and more active fire seasons, decreased rainfall and increased temperatures due to climate change. In the case of agricultural systems, this means focusing on agricultural management that looks beyond just yield, considering impacts on multifunctionality and biodiversity through initiatives such as 30x30 and biodiversity-focused farming systems.

LAND USE CHANGE AND HABITAT FRAGMENTATION

Land use change, which encompasses both the conversion of natural to managed ecosystems and shifts in existing management practices (Nielsen, Wall, and Six 2015), is both a major threat to soil biodiversity in California, and a potential vehicle for its preservation. Landscape changes such as deforestation, urbanization and agricultural expansion have significant, long-term impacts on soil microbial communities (Buckley and Schmidt 2001; Jangid et al. 2011) due to habitat loss, habitat fragmentation and soil degradation. In agricultural land, the less diverse vegetation assemblages associated with land use conversion to monocrop agriculture can negatively impact soil biodiversity (Figuerola et al. 2015). In addition, agricultural techniques can lead to the loss of soil biodiversity through the removal of residues, soil erosion, soil compaction due to soil structure degradation, and repeated application of agrochemicals (Tibbett, Fraser, and Duddigan 2020). Conversion of undisturbed to cultivated land can induce a shift towards bacterial-dominated food webs and higher bacterial diversity due to the negative impact of disturbances on fungal hyphae networks, but also result in an increase in plant pathogens (Labouyrie et al. 2023). The shift to bacterially-dominated communities may also influence nutrient retention as bacterial-dominated food webs are considered less conservative with nutrients than fungal-dominated food webs (Liiri et al. 2012). Management systems that reduce pesticide inputs and disturbance while increasing organic carbon inputs (such as organic and regenerative systems) can have a positive impact on biodiversity in agricultural plots that have already experienced biodiversity loss (Bengtsson, Ahnström, and Weibull 2005; de Graaff et al. 2019; Turley et al. 2020; Guo et al. 2021).

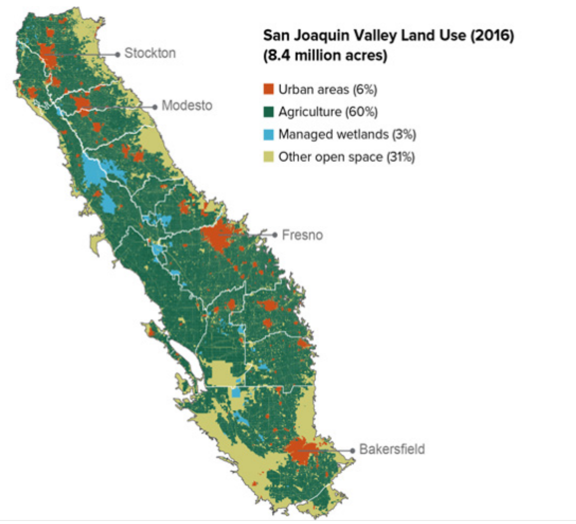

Figure 3.3 Agriculture occupies a significant portion of the California landscape. *Map developed by the Public Policy Institute of California using the CA Department of Water Resources 2016 land use layer.*

ARIDIFICATION / EXTREME DROUGHT

Current and expected changes in global precipitation and temperature will have disproportionately large impacts on ecosystems and their resident soil biota. More frequent and severe episodes of drought and warming are expanding the dryland area (aridification) of Western United States (US) landscapes and pose a significant threat to belowground biodiversity. Because soil moisture content is one of the primary constraints on soil biotic activity, changes in the amount, frequency, or seasonality of precipitation can alter the composition and function of belowground communities, especially in water-limited dryland ecosystems. Reduced precipitation has negative impacts on fungal biomass, collembolans, and enchytraeids (Blankinship, Niklaus, and Hungate 2011), and context-dependent effect on other soil fauna, such as nematodes (Sylvain et al. 2014) and protists (Stefan et al. 2014). These impacts may also be affected by simultaneous changes in temperature regimes, with increased temperatures potentially increasing microbial activity (Nottingham et al. 2019).

Figure 3.4. Reduced soil moisture, such as that experienced under droughts, can negatively impact soil microbial biomass and activity. *Credit: California Department of Food and Agriculture*

California's xeric soil moisture regime means that soil communities are adapted to dry summers and wet winters. However, during 2012–2016 California experienced the longest and most severe drought in a century, with reduction in winter rainfall and, thus, greater soil moisture shortages (Pancorbo, Quemada, and Roberts 2023). Forests with little snowpack experienced massive forest die-off (Asner et al. 2016) and severe wildfire seasons (van Mantgem et al. 2013). Drought conditions impacted surface water supplies, increased agricultural demand, increased groundwater extraction (resulting in land subsidence), increased non-cultivated croplands (Pancorbo, Quemada, and Roberts 2023), made habitat restoration efforts more tenable (Peterson, Pittelkow, and Lundy 2022) and had massive economic impacts on the ranching sector (Potter 2015). This reduction in soil moisture also potentially decreased microbial biomass and activity (Pérez Castro et al. 2019) and modified the production of CO_2 and N2O in deeper soil layers (Diamond et al. 2019).

ORGANIC MATTER DECLINE

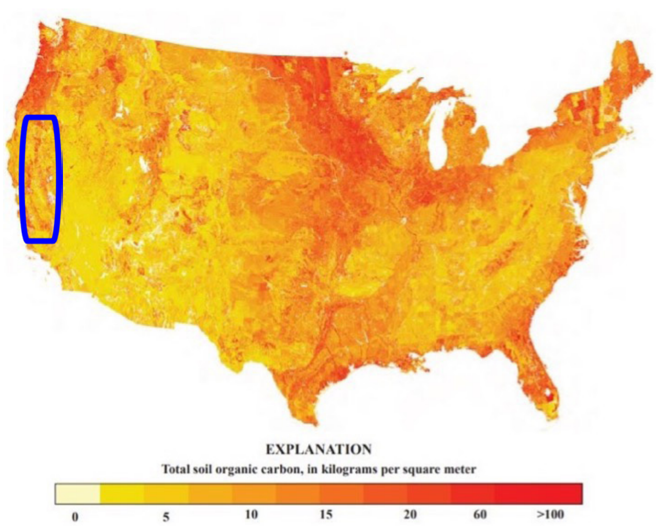

Figure 3.4. Organic carbon contents in the mineral soils of the San Joaquin Valley (outlined in blue) are relatively low compared to some Midwestern states. *Data from USDA NRCS SSURGO Database, 2009. https://www.usgs.gov/media/images/soil-organic-carbon-based-ssurgo-and-statgso2-databases*

The relationship between soil organic carbon (SOC) and belowground biodiversity is complex. Soil organisms are the main pathway for the formation and loss of SOC. Approximately half of the SOC in cropland and grassland soils is microbially derived (Wang et al. 2021). This microbially-derived carbon is a dynamic ecosystem component linked to soil functions like nutrient retention, enzyme activity, and soil stability (Singh and Gupta 2018; Serna-Chavez, Fierer, and van Bodegom 2013). Land use change to agriculture can decrease soil organic matter stocks (Sanderman, Hengl, and Fiske 2017) and shift the amount and type of organic matter inputs. Reductions in SOC due to agricultural management, reduced rainfall, and increasing aridification can reduce microbial biomass, belowground biodiversity, soil aggregate stability, and ecosystem services (Gardi, Jeffery, and Saltelli, 2013). Furthermore, the capacity of soils to retain water declines with a reduction in SOM (Libohova et al. 2018). This is a particular issue for drought-prone regions such as the California Central Valley, which are naturally lower in SOM than the deeper grassland soils of the American Midwest. The Central Valley's history of tilled agriculture has driven soil organic carbon losses in the past decade and is projected to continue losing carbon through 2100 under current practices (Sleeter et al. 2019). Given the strong positive relationship between SOC content and the abundance of soil organisms (Fang et. al 2019), continuous reductions in soil carbon content due to land use change represents a critical threat to biodiversity and its supported functions (Bastida et al. 2021). This link between soil carbon content and soil biodiversity is one of the main factors that drives potential synergy between programs such as the California Healthy Soil Program, and conservation efforts such as the 30x30 initiative.

SOIL SALINIZATION

Figure 3.5. Saline soils can have negative impacts on agriculture and belowground biodiversity due to high ionic concentrations. *Credit: India Water Portal via Flickr, CC BY 2.0*

Soil salinization, or high concentrations of soluble salts in the soil, presents a major global challenge to managed and natural ecosystems (Poffenbarger et al. 2017; Yu et al. 2020; Shahariar et al. 2021). The global area of salinized soils is rapidly expanding (Abbas et al. 2013) and is expected to further increase over the coming decades due to climate change (Hassani, Azapagic, and Shokri 2021). Soil salinization alters the composition, distribution, and activity of soil organisms through its toxic effects of ions and low osmotic potentials on microbial cells. Increasing salinity results in restricted water availability, causing the drying and lysis of microbial cells (Yuan et al. 2007). Specifically, studies have reported that salinity stress can shift the community structure of soil organisms, particularly bacterial and fungal communities (Yan et al. 2015; Rath, Maheshwari, and Rousk 2017; Rath and Rousk 2015; Yang et al. 2018) and lead to changes in SOC, GHG, CO_2, CH_4, and N_2O emissions (Shahariar et al. 2021; She et al. 2021). While natural processes account for most saline soils worldwide, 25% of the world's irrigated area experiences negative impacts from soil salinity due to poor quality of irrigation water and inefficient agricultural water management (Chang et al. 2019). This issue is likely to become worse with climate change and decreased precipitation.

California's soils contain salts due to their inherent geology and hydrology. The San Joaquin Valley's unique mix of marine sedimentary parent material high in gypsum and calcite, a low-permeability clay layer in many soil profiles, low precipitation and high evapotranspiration due to a semi-arid climate, is naturally conducive to salt accumulation. This issue is only exacerbated with high levels of fertilizer application and irrigation with saline groundwater. Up to 4.5 million acres of irrigated cropland in California (more than half of the agricultural land) is affected to some degree by soil salinization, especially in the Imperial Valley and the Western San Joaquin Valley regions. These combined processes that buildup salt in the soils not only threaten agricultural productivity, but also negatively impair the metabolic capabilities of soil organisms and, thus, the ecosystem functions provided by soil biodiversity.

SOIL POLLUTION

Soil pollution can take many forms including heavy metal contamination (Alloway 2013), waste materials (O'Connor et al. 2022), antibiotics (Gothwal and Shashidhar 2015), synthetic and organic compounds (Semple et al. 2007) and microplastics. Negative impacts to both humans and soil organisms can manifest through direct exposure or bioaccumulation (Khan et al. 2015) and will depend on a number of factors: the class of pollutant, soil type, the class of organism, and the concentration of that pollutant. For example, earthworms may be more sensitive to the presence of PAHs (polycyclic aromatic hydrocarbons) (Rodriguez-Campos et al. 2014), while heavy metals may negatively impact fungi and nematodes (Gutiérrez et al. 2016).

Figure 3.6. Heavy metal pollution, such as those found in mine tailings, can persist in soils for decades. *Credit: Wikimedia, commons.wikimedia.org/wiki/File:New_Idria_Mercury_Mine_Polluted_Water_2013.jpg, CC BY-SA 3.0*

Sources of soil pollutants include industrial processes, agrochemicals, human waste disposal, or soil amendments. The ever-increasing scope of industrial activities means that new soil pollutants are constantly emerging. One of these, microplastics, is produced by decomposition of larger plastic waste. Microplastics are nearly ubiquitous in natural ecosystems and are present in large quantities in soil, but its effects on the majority of soil biota are unknown (de Souza Machado et al. 2018). High levels of microplastics can increase earthworm mortality (Huerta Lwanga et al. 2016) and reduce springtail mobility (Kim and An 2020).

While some California soil communities (such as in the Salinas Valley) are adapted to naturally high levels of heavy metals, the addition of heavy metals or organic compounds to unadapted soils in California agriculture also represent a potential threat to belowground biodiversity (Ruuskanen et al. 2023). Chemicals such as fumigants can have long-term impacts on nematode community structure in California almond orchards, by reducing fungal populations that serve as a food source (Hodson et al. 2019). The addition of copper-based pesticides can also have negative effects on populations of earthworms, collembola, and microbial activity (Karimi et al. 2021), while organic pesticides such as glyphosate can reduce mycorrhizal fungi colonization and plant growth (Helander et al. 2018). Management systems (such as organic and regenerative systems) that reduce pesticide application can, in turn, have positive effects on soil biodiversity (Bengtsson, Ahnström, and Weibull 2005, Brühl et al. 2022).

COMPOUND THREATS TO BELOWGROUND BIODIVERSITY

The complexities of soil biodiversity and its connectedness to soil multifunctionality makes it impossible to identify a single specific organism responsible for an ecosystem service because these services emerge from interactions between multiple organisms. In much the same way, it is challenging to identify and tackle a single threat to soil biodiversity because these threats are interconnected and compounded. Compounded disturbances can magnify the individual impacts of threats, creating synergistically negative impacts. For example, in California, changes in rainfall patterns lead to aridification and the need to irrigate using groundwater. Since the groundwater in several regions contains high levels of dissolved minerals, this irrigation can increase soil salinity causing metabolic stress to soil organisms. Increased salinity necessitates the use of even more irrigation to flush salts from the soil or can drive conversion towards more marginal land. Cultivation of this marginal land can then drive the depletion of organic matter, a building block of microbial cells. Through these processes, even if some soil organisms could persist in more saline soils, the depletion of organic matter may threaten their persistence. Attempting to address a single threat to soil biodiversity, such as organic matter decline, does not take the interconnected nature of these threats into account and is hampered by the scarcity of literature on how compound disturbances impact belowground biodiversity. This highlights the need for holistic, systemic changes in the way that humans interact with soils in an attempt to address these threats.

INTRODUCTION

The enormous variety of life present in the soil (Chapter 2), and the many functions they are responsible for (Chapter 3), mean there are a number of ways to measure and characterize soil biodiversity. As discussed in Chapter 1, to describe soil biodiversity, one might measure the abundance of particular groups, determine taxonomic identity, measure potential to carry out specific functions, or measure processes it is carrying out. Soil biodiversity spans diverse scales, from variation at the level of genes, to individuals and populations, to communities and ecosystems (Figure 4.1).

Figure 4.1. Different scales at which soil biodiversity is measured (adapted from Geist 2011).

Scientists do not yet know enough about soil biodiversity – particularly microbial diversity, with only a small percentage of organisms identified – to be able to conduct a comprehensive census of all biodiversity in soil. As an alternative, biodiversity *indicators* are used to assess soil biodiversity. Indicators are defined as "quantitative measures … that provide information on the status and trends of biodiversity and its components" (Pereira et al. 2013). These indicators, which are usually measured as a group rather than individually, provide information that is measurable and communicable (Pulleman 2012). To be useful for measuring soil health, indicators must be sensitive to change, easily measured, interpretable at both scientific and policy levels, and be relevant to the functions of interest. Indicators, by design, are also simpler and less costly to employ in terms of time and money than conducting more detailed surveys.

Fortunately, a large body of existing information provides the base to identify soil biodiversity indicators relevant for California working lands. This chapter aims to provide a conceptual overview of *soil biodiversity indicators* including recent advances in their development. Suggested methods to measure indicators representing a variety of taxa and functions are also provided.

OVERVIEW OF SOIL BIODIVERSITY INDICATORS

Soil biodiversity is much more than a catalog of organisms. The organisms found in soil collectively form a community whose biomass (living and dead), biomolecular composition, functions, and interactions are critical to soil processes, soil health and the provision of ecosystem services. Indicators can be organized into five major categories – abundance, identity, functional traits, interactions, and processes – that together make up a catalog of soil biodiversity indicators. Each indicator category is described in detail below. Table 4.1 provides additional information on specific indicators, methods for measuring them, and references for each of the indicator categories (Figure 4.2).

Abundance (Quantitative Biomass)

- Imaging (e.g., Microscopy)
- Biochemical (e.g., CFE, PLFA, DNA)

Identity

- Imaging (e.g., Microscopy)
- Biochemical (e.g., microbial groups via PLFA, metabarcording of genetic diversity via DNA)

Functional Traits

- Imaging (e.g., morphology via microscopy)
- Biochemical (e.g., genes -> genomes -> traits via DNA)

Interactions

- Networks - co-occurrence analysis via DNA
- Foodwebs - predator-prey analysis via imaging or DNA

Processes

- Physical (e.g., aggregation, WHC)
- Chemical (e.g., pH, nutrients)
- Biological (e.g., mineralization, pathogen inhibition)

Figure 4.2. Categories of biodiversity indicators in soil ecosystems. Under each indicator category are listed some examples of methods (marked as bullets) used to measure these indicators.

ABUNDANCE

The abundance (biomass) of lifeforms in soil is in fact a reflection of the suitability of soil for life, and can be compared across similar soils in similar climates or before and after adoption of a new management approach. Total biomass can be measured biochemically (e.g., chloroform fumigation extraction or total phospholipid content) or by direct imaging of the organisms (e.g., microscopic counts). The abundance of particular groups of organisms can also be quantified after they are sorted based on unique physical features (e.g., mouthparts) or biochemicals (e.g., specific lipids, chitin).

IDENTITY

The current gold standard to determine composition for microorganisms (bacteria, archaea, fungi, protists) is deoxyribonucleic acid (DNA) sequencing via metabarcoding using specific marker genes like the 16S ribosomal ribonucleic acid (rRNA) gene for bacteria and archaea, the internal transcribed spacer (ITS) region for fungi and 18S rRNA for protists and nematodes. Extracted DNA from soil samples is analyzed by comparison to reference databases for taxonomic assignment. Community composition and diversity indices can also be analyzed using different statistical techniques. The cost of DNA sequencing a soil sample is not very different from that of common soil tests and will continue to decrease as technology improves and markets for these tests grow. Microbial community "fingerprints" are another output of DNA sequencing and can be used to compare soils with the help of advanced statistical techniques (e.g., multivariate statistics).

Larger organisms, such as micro- and meso-fauna, are usually trapped or extracted from soil, analyzed for physical characteristics via microscopy, and then compared to taxonomic keys for identification (see Figure 4.3). These whole organism methods sometimes pose challenges in terms of scalability, reproducibility, time required, and availability of expertise. Approaches using DNA metabarcoding on bulk soil samples are being developed to identify microfauna (nematodes, tardigrades, rotifers) (Watts et. al 2019) and macrofauna (Porter et al, 2019; Kawanobe et al. 2021).

Some methods, such as those used for biomass, can also provide information on identity. For example, some phospholipid fatty acids (PLFA) biomarkers unique to specific taxa estimate biomass and provide information on the identity of some groups. However, in some cases, different organisms (e.g., actinomycetes and sulfate-reducing bacteria) possess the same biomarker (Frostegård, Tunlid, and Bååth 2011) and thus are not reliable for identification.

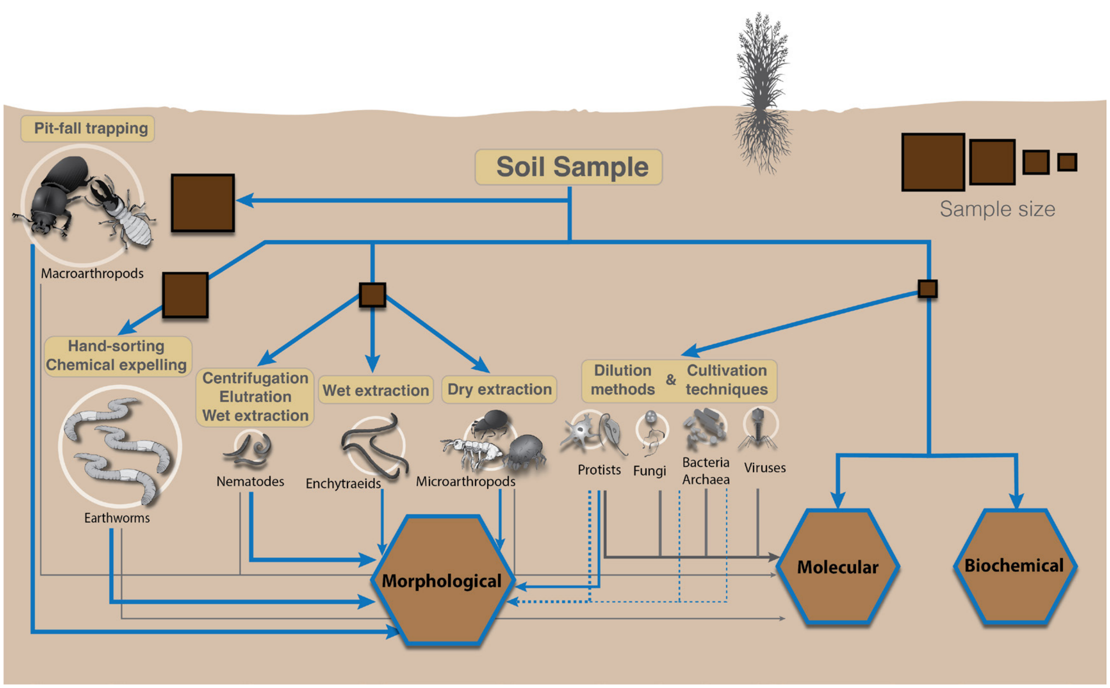

Figure 4.3. Overview of extraction methods for measuring soil fauna. *Credit: Javier A. Ceja-Navarro, adapted from Geisen et al. 2019.*

FUNCTIONAL TRAITS

The functional traits of soil organisms refer to their potential to engage in soil processes, including organic matter decomposition, nutrient cycling, and soil structure formation. As described in Chapter 2, soil microarthropods shred and consume plant litter, while nematodes contribute to nutrient cycling through their feeding on soil organic matter, microorganisms and roots. Protists can consume bacteria, while bacteria, archaea and fungi are critical to carbon and nutrient cycling. The vast diversity within and across these broad groups means that it is less meaningful to simply consider their potential to perform a single function. For this reason, functional trait measurements, combined with identity measurements, are particularly useful in soil assessments.

> **Box 3. Nitrous Oxide Production**
>
> Nitrous oxide (N2O) is a potent greenhouse gas and a vehicle for nitrogen (N) loss in agroecosystems. The amount of N2O emitted is negatively correlated with the abundance of the N2O reductase gene (and the organisms that possess those genes) that reduces N2O to nitrogen gas N2 (You et al. 2022).

For microorganisms, only measuring taxonomic identity does not provide an adequate estimate of functional traits because many functions are carried out by a multitude of different organisms and one organism may be involved in a variety of functions. Instead, functional genes that target enzymes involved in different processes (e.g., nitrogen cycling, organic matter decomposition) can be targeted to estimate the potential of microbes to perform that function (functional potential) (e.g., Box 3). More recently, metagenomic analysis (see below) has been used to characterize a soil's functional potential.

With recent developments in metagenomics, entire genomes (e.g., beyond just 16S and ITS genes) can be reconstructed from soil communities, providing new insights into the full capabilities and lifestyle of soil organisms (i.e., their traits), and create opportunities to distill this complex information into more generalizable patterns that mechanistically link biodiversity and soil functions (Malik et al. 2020). Metagenomic sequencing is the analysis of random (untargeted) DNA sequences obtained from a mass of soil. While metagenomic approaches are not currently mainstream products available for soil health assessment, technologies to acquire, analyze, and interpret soil metagenomic information are becoming more cost effective and accessible. However, results from these analyses are not yet ready to translate into practical applications for practitioners. This is an important goal for future research (see Chapter 8).

Direct observation of soil micro- or mesofauna is also used to characterize functional traits. For example, nematode body size, maximum body length, maximum body width, stylet length, esophagus length and intestinal length can be used to classify nematodes into their functional groups which can give insight into a soil's functional potential (Bongers and Ferris, 1999).

INTERACTIONS

Soil biodiversity also includes the network of interactions (e.g., predation, competition, cooperation, symbiosis, etc.) among soil organisms, rather than just a simple inventory of organisms (Chapter 2). Ecosystems are complex and interconnected systems that consist of living organisms, their habitats, and the interactions between them. These interactions are what contribute to the *multifunctionality* of agroecosystems which refers to the capacity of an ecosystem to provide multiple services simultaneously, such as nutrient cycling, pollutant removal, disease regulation, climate regulation, and food production (Wagg et al. 2014). Multifunctionality is strongly related to how well-connected soil organisms are, as well as how many types of organisms are present (Jiao et al. 2021). Biological network analysis (Berberan et al. 2012; Matchado et al. 2021) is one of several modeling tools used to measure interactions, working with DNA sequences which provide the raw material used in these analyses. However, inferring network properties using patterns of co-occurrence of organisms remains difficult, requiring numerous samples to be analyzed within or across sites. These methods need further development before they will be feasible for general soil health assessments.

PROCESSES

Soil processes are regulated by the abundance of soil organisms, their identity, their capabilities (i.e., their functional biodiversity), and the network of their interactions. Because one major goal of soil biodiversity assessment is to establish its connection to overall soil function, measures of biodiversity are usually combined with measurements of soil processes, the substrates on which the processes operate, and their rates of transformation. These processes range across a gradient of complexity and include soil aggregate stability, soil organic matter content, particulate organic matter, in situ cellulose decomposition rates, active carbon, soil respiration rate, extractable proteins, and nitrification potential. Which processes to focus on will depend on the particular goal and use case. Measurements, often collected over time, are commonly performed on soil, water and/or gas samples collected from the field and analyzed in the lab. Some measurements are conducted directly in the field using sensors or remote sensing.

Data integration and synthesis to build biodiversity-to-function relationships: Quantifying soil biodiversity and linking it to function requires a data infrastructure that can connect different measurements (process, identity, abundance, etc.) performed on the same soil sample. Open access systems already exist to connect information from the same physical samples and to make that information relatable to measurements made in geographic proximity (e.g., https://www.geosamples.org/). These systems have existing standards for data reporting and could be leveraged as part of a soil biodiversity-to-function data informatics system to maximize gained knowledge and impact for State programs such CDFA's Healthy Soils Initiative (https://www.cdfa.ca.gov/healthysoils/).

Level	Indicator	Potential Methods
Abundance *(often with conversion factor)*	**Counts**: Cells, organisms, CFUs **Cellular Constituents:** Carbon, lipids, DNA, necromass, metabolites	Soil faunal counts, most probable number, direct counts (microscopy), colonization rates (mycorrhizae), phospholipid fatty acid analysis (PLFA), fatty acid methyl ester (FAME) analysis, total DNA, quantitative polymerase chain reaction (PCR) of taxonomic or functional genes, plating and CFU (colony forming units) counts, turbidity, flow cytometry, ergosterol, Microbial biomass carbon/ nitrogen/etc (MBC/N)
Identity	**Genotype Identification:** 16S/18S signature, ITS signature **Phenotype Identification:** Morphology, biochemical signature (lipids), culture-based methods	Plating and colony identification, nematode anatomy or morphology, microscopy identification (fungi, bacteria), flow cytometry, PLFA/NLFA (neutral lipid fatty acid)/FAME, quantitative PCR, FISH (fluorescence in situ hybridization)
Functional Traits	**Genetic Analysis:** Functional traits **Phenotype Analysis:** Morphology, proteome	Functional gene analysis, metagenomics, metaproteomics, metatranscriptomics, metabolomics, nematode anatomy or morphology
Interactions *(including measurements and derived data)*	Co-occurrence patterns, food web relationships	Network analysis of organism (taxonomic, functional group), co-occurrence patterns, food web modeling, process modeling, biochemical indicators (quorum sensing signals, antibiotics, signaling molecules)
Processes	Biogeochemical transformations, metabolites, growth rates	Enzyme assays, Potentially mineralizable nitrogen (PMN), Potentially mineralizable carbon (PMC), Respiration, Substrate induced respiration (SIR), Bioassays, qCO_2 (the microbial metabolic quotient, or respiration-to-biomass ratio), Biolog - Microbial Identification & Characterization, isotope analysis

Table 4.1: A list of soil biodiversity indicators by indicator category and suggested methods. For more detail on particular methods see the following resources: Orgiazzi et al. (2016); Delgado-Baquerizo et al. (2020): Bispo et al. (2009); Tas et al. (2021); Turbe et al. 2010) and see Table 2. *[Note: It is beyond the scope of this Committee to provide detailed methods for each indicator.]*

CHAPTER 5 | ASSESSING SOIL BIODIVERSITY: A REVIEW OF PREVIOUS EFFORTS

This chapter briefly summarizes previous institutional or project-based *efforts* to identify soil biodiversity indicators, some of which have identified methodological frameworks to select and help interpret them (Turbé et al. 2010).

Soil biodiversity is recognized by a number of initiatives as an important focus for protection, monitoring and policy. Recently, the protection of soil biodiversity was brought to the forefront when delegates at the COP 15 meeting released a 2020-2030 plan of action: *International Initiative for the Conservation and Sustainable Use of Soil Biodiversity*[5]. The plan calls for additional investment and efforts to monitor and assess soil biodiversity worldwide. The Global Soil Biodiversity Atlas, published by the European Commission, was the first comprehensive compendium of soil biodiversity indicators and discussion of their importance in monitoring and managing terrestrial ecosystems (Orgiazzi et al. 2016).

Organizations focusing on biodiversity as part of broader biodiversity initiatives include the FAO Global Soil Partnership and its Intergovernmental Technical Panel on Soils (ITPS), the Convention on Biological Diversity, the Global Soil Biodiversity Initiative (GSBI), the Soil Biodiversity Observation Network (SoilBON), and the US Soil Ecology Society[6] (SES). Recent publications have highlighted the importance and state of knowledge of soil biodiversity, the threats it faces, particularly in agricultural soils, and potential solutions to address these threats (FAO, et al. 2020.; Kendzior et al. 2022; Orgiazzi et al. 2016).

In the United States, however, soil biodiversity assessment and identification of indicators has received less attention. While numerous local-scale studies have been conducted, there has been no comprehensive initiative to assess soil biodiversity at the national level. Some locations in the US have been included in multinational efforts such as the National Science Foundation National Ecological Observatory Network (National Earth Observatory Network 2020), the Earth Microbiome Project (Shaffer et al. 2022), and the GSBI (Orgiazzi et al. 2016). The National Science and Technology Council (NSTC) announced a national strategic plan for microbiome research that supports assessment and protection of soil biodiversity (Microbiome Interagency Working Group, 2018), and the GSBI announced in 2022 the "first soil biodiversity assessment across the United States has begun in association with the global SoilBON," which includes all forms of soil biodiversity from microorganisms to macrofauna. The Natural Resources Conservation Service (NRCS) also recommends a set of biological indicators[7] as part of its soil quality indicators.

International efforts to develop indicators for soil biodiversity have gained significant momentum in recent years. Recognizing the crucial role of soil biodiversity in maintaining ecosystem health and functioning, organizations such as the United Nations (UN) Convention on Biological Diversity (CBD) and the GSBI have actively promoted the development and implementation of indicators to monitor soil biodiversity. These indicators aim to capture the diversity, abundance, and functional traits of soil organisms, including bacteria, fungi, nematodes, and earthworms, among others. Through collaborative research and data synthesis, scientists and policymakers are working towards standardizing and harmonizing soil biodiversity indicators to facilitate global assessments of soil health and guide sustainable land management practices. Recent studies, such as those by Orgiazzi et al. (2016) and Delgado-Baquerizo et al. (2020), have made significant contributions to the development of these indicators, providing valuable insights into the relationships between soil biodiversity, ecosystem processes, and environmental factors.

While infeasible to conduct a comprehensive review of all projects that have considered indicators of soil biodiversity in this report, an excellent compilation of soil biodiversity efforts going back almost 50 years can be found in Turbé et al. (2010). Table 5.1 summarizes some of the larger and more recent programs and projects that target soil biodiversity indicators for assessment of soil health or for monitoring soil quality in working lands.

5 https://www.cbd.int/doc/c/feeb/435c/2202483bb42af12650b184d5/cop-15-l-16-en.pdf
6 See semi-comprehensive list here: https://www.globalsoilbiodiversity.org/soilrelated-organizations
7 NRCS list of biological indicators and soil functions: https://www.nrcs.usda.gov/sites/default/files/2022-10/biological_indicators_overview.pdf

Program	Goals or Focus	Region	Indicators	Reference
Biological Indicator System for Soil Quality (BISQ)	Indicator system for biological soil quality that can be applied both in diagnostic and in prognostic approaches, and can be measured within a soil-monitoring network.	Netherlands	Abundance and diversity of earthworms, nematodes, micro-arthropods plus microbial activity and biomass used to assess five ecosystem services.	Schouten et al. 1997
Biological Soil Classification Scheme (BBSK)	Used to assess the function of soil as a habitat for soil organisms, assuming that similar soils should have a similar soil fauna.	Germany	Diversity and abundance of meso- and macro-fauna; distance-to-target indicator relative to a reference site.	Ruf et al. 2003
ENVironmental ASsessment of Soil for mOnitoring (ENVASSO)	Propose a well-defined set of indicators for each of a number of major EU soil threats, based on sound science.	EU	Microbial biomass, microbial activity (e.g., enzyme assays), basal respiration, earthworm and other soil fauna (micro and macro) abundance, DNA and PLFA analysis.	Bispo et al. 2009
SoilBON	Global soil biodiversity and ecosystem function monitoring framework to be part of international biodiversity strategies.	EU	"Essential biodiversity variables" (EBVs): soil respiration, soil enzyme activity, nutrient turnover and genetic diversity.	Guerra et al. 2021 https://joint-research-centre.ec.europa.eu/jrc-news/measuring-underground-world-soil-biodiversity-monitoring-and-indicator-system-2021-01-15_en
BIOSIS	A decision-making support tool to select soil biological indicators to specific soil functions: nutrient cycling, carbon management, disease suppression and water regulation.	Netherlands	From a broad variety of biodiversity indicators, specific ones selected based on the particular question or concern.	https://biosisplatform.eu/
Land Use/Cover Area frame statistical Survey Soil (LUCAS Soil)	The largest harmonized open-access dataset of topsoil properties available for the EU at the global scale, developed as an expandable resource.	EU	Bacterial 16S data and Fungal ITS DNA sequences for 885 samples collected as part of LUCAS 2018 Soil survey (Biodiversity module).	Orgiazzi et al. 2018
Identification of bioindicators – biological tools for sustainable	Standardization of soil biodiversity indicators and measures	France	List of indicators with respective methods and standards – links with the soil functions.	Bougon et al. 2021; Bouchez et al. 2016; Bispo et al. 2009

Table 5.1. Summary of selected programs and projects that identify soil biodiversity indicators.

Though not focused specifically on soil biodiversity, two US programs developing approaches and indicators for soil health assessment have included several soil biodiversity indicators in their frameworks:

1. Comprehensive Assessment of Soil Health (CASH) at the Cornell Soil Health Testing Lab.
2. North American Project to Evaluate Soil Health Indicators at the Soil Health Institute. The list of Indicators can be found in Norris et. al 2018. This includes biodiversity indicators such as DNA sequencing and PLFA.

BIOSIS: Example of a Decision-making Tool Linking Soil Biodiversity Indicators to Soil Health Assessment

In response to the need to identify soil biodiversity indicators that explicitly address different aspects of soil health, Professor Rachel Creamer and her team at Wageningen University, developed the BIOSIS platform, a decision-making support tool to select soil biological indicators to specific soil functions: nutrient cycling, carbon management, disease suppression and water regulation. These services were identified in the 2006 EU soil strategy as the four key soil functions that are crucial for land management and are the focus of the Soil Health Law to be adopted by the European Commission in the second quarter of 2023.

BIOSIS is based on a conceptual framework which connects soil biota to these functions through the contribution to different soil processes (Zwetsloot et al. 2021). Thus, BIOSIS follows a hierarchical structure (see Figure 5.1) in which a larger ecosystem function (e.g., water purification) is linked to sub-functions (e.g., water infiltration, water storage, etc.), then to soil processes (e.g., macropore formation, aggregation, etc.). These soil processes are then linked to groups of soil organisms responsible for their regulation (i.e., earthworms, ants, etc.). Then, a set of measurements (or indicators) associated with each biological actor are identified. These indicators are numerous and highly redundant, as shown in Figure 5.1.

Following the work of Ritz et al. (2009), BIOSIS uses a 'logical sieve' to prioritize and select a subset of measurements that are the most feasible, applicable and pertinent for different scenarios. This approach uses co-occurrence networks to identify the most important biological actors in a specific scenario and weighs in the technical difficulty of the indicators used to measure those actors. The tool takes the specific objectives and context of the user into consideration, thereby producing a unique list of ranked biological methods for the user to base its final selection on. The BIOSIS tool will be used in wide-scale monitoring across the EU as part of the Soil Health Law.

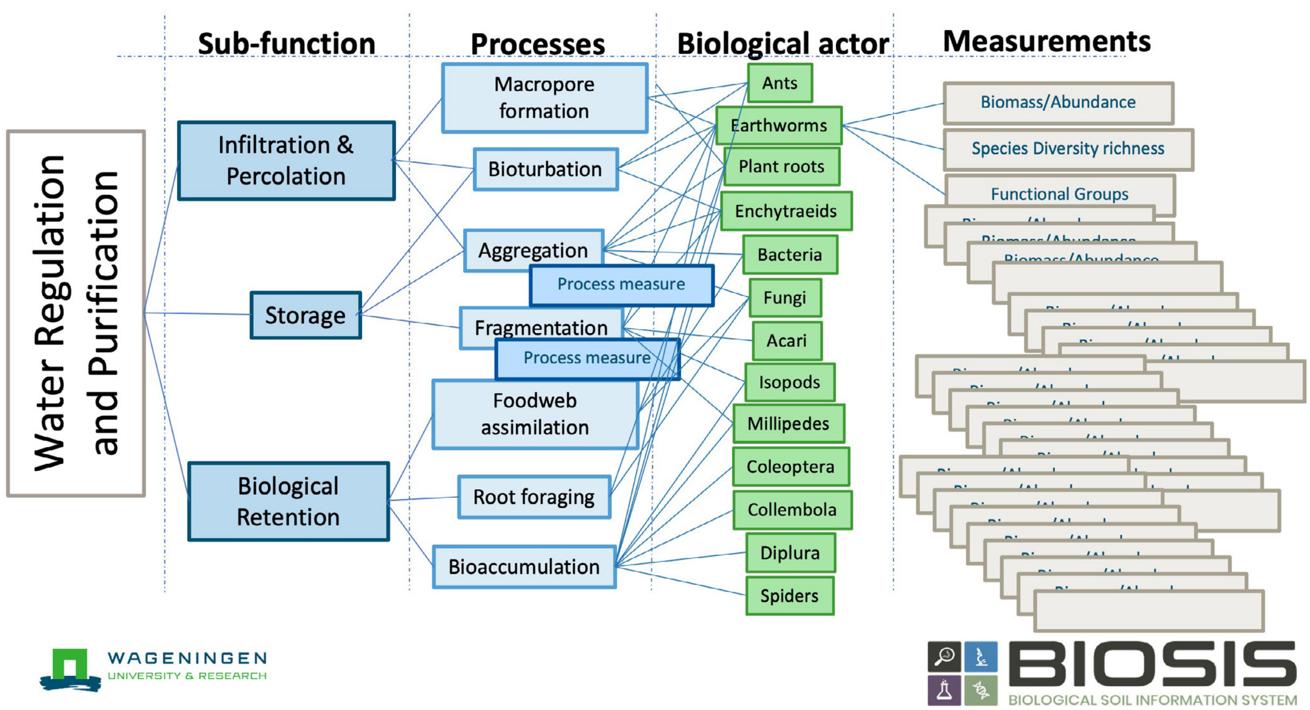

Figure 5.1. An illustration of the logical sieve process used to select potential measurements in the BIOSIS model. The ecosystem function of Water Regulation and Purification is linked to sub-functions, underlying processes, and the biological actors responsible. Potential measurements associated with those biological actors are then identified. *Citation: Dr. Rachel Creamer presentation to the BBAC on August 19, 2022, with figure adapted from R.E. Creamer et. al (2022) The life of soils: Integrating the who and how of multifunctionality* https://doi.org/10.1016/j.soilbio.2022.108561

CHAPTER 6 | CRITERIA FOR SELECTION OF INDICATORS

Having clear criteria to describe indicators and indicator frameworks can ultimately facilitate the distillation of very complex and disparate knowledge into indicators suitable to assess belowground biodiversity and their function. Below are general criteria to select indicators of belowground biodiversity, and the frameworks that can be used to understand them as a basis to establish case studies relevant to growers, policy makers, researchers, and the public (Chapter 7).

Notably, an indicator often exists in a framework, defined here as a conceptual structure that provides rationale and justification for a methodological approach to evaluate soil biodiversity. Such a structure can integrate indicators at different levels (taxonomic and functional) and scales (pedon to landscape) and make links to relevant ecosystem services. The selection of indicators will depend on the purposes of the assessment and the user. Different stakeholders have different information needs, and therefore prioritize diverse indicators to answer their particular interests or requirements.

The BBAC identified the following core criteria to select biodiversity indicators based on scientific and policy relevance and current biodiversity frameworks used to monitor biodiversity on working and natural landscapes. Attributes important for applicability, relevance to various audiences and goals (policy makers, farmers, scientists) and the California context were emphasized.

CORE CRITERIA OF A USEFUL INDICATOR

Four fundamental criteria emerge. An indicator should be:

1. Meaningful and targeted to the goal(s) of the assessment,
2. Relevant to the scale and biology of the organisms,
3. Feasible to measure and easy to interpret at both scientific and policy levels, and
4. Have an established and standardized sampling plan and/or methodology.

1. **Meaningful and Targeted to the Goal(s)**
    I. *Meaningful:* Indicators must consider the different goals to measure biodiversity that will impact what kind of indicators are selected. An indicator can be:
        a. *Utilitarian* with a direct commercial use (i.e., edible mushroom diversity and abundance).
        b. *Practical* (e.g., antibiotics) or have potential scientific value (e.g., global biodiversity mapping) for the benefit of future generations.
        c. Considered for its *intrinsic value* (i.e., spiritual significance, beauty, symbolism).
        d. *Functional* in its contributions to ecosystem processes, structure, and integrity (Swift, Izac, and Noordwijk 2004).
            a. Functional indicators can also relate to a variety of important functions or goals such as:
                i. Ecology: ecological resilience, ecosystem functioning.
                ii. Conservation: protection and enhancement of rare/ threatened species.
                iii. Biological control: diversity of beneficial/ pest/ potential pest organisms.
                iv. Cultural value: source of food, rituals and art, symbolic value, popular appeal.

2. **Relevant to the Scale and Biology of Organisms**

   I. *Naturally occurring:* Biodiversity indicators must be theoretically naturally occurring under the different environmental conditions/ ecosystems that are of relevance.

   II. *Spatio-temporal adequacy:* The suite of indicators and the sampling scheme should be able to capture the biology of the organisms in time and space as well as the critical scales and dimensions of biodiversity of interest.

3. **Feasible to Measure and Easy to Interpret at Both Scientific and Policy Levels**

   I. *Sensitive and relevant*: Indicators should be sensitive to changes in management/ policy and allow for comparisons with a baseline situation to capture progress towards targets.

   II. *Understandable:* Indicators should be easily understood and interpreted with clear classification and terminology. A range of values for the indicator should be available and validated in a variety of conditions. Indicators may reflect a single parameter (i.e., species densities) or combine variables into indices.

   III. *Measurable and cost-effective:* Indicators should use established methods, be cost-effective and measurable at reasonable throughput.

   IV. *Manageable:* The number of indicators selected for assessing soil biodiversity should be limited in the number and effort required to remain actionable. The goal is to select a minimum set of indicators that adequately characterize soil biodiversity.

4. **Have an Established and Standardized Sampling Plan and/or Methodology**

   I. *Standardized:* Parameters should be standardized to ensure comparability of data, and use quantitative and repeatable protocols of sampling and estimation. This includes potential for harmonization at the national and international levels and use of standard operating procedures.

   II. *Accurate:* The value of the indicators should be precise and robust, not error prone or subjective (i.e., minimize inter-laboratory variability). The functional traits of soil organisms must be considered, and methodologies should enable representation of both the complexity and the high temporal and spatial variability that characterize soil communities.

In practice, no single indicator will comply with all the criteria above, and the development of sets of complementary indicators in frameworks, including both biotic and abiotic parameters, should be the focus. Generally, a compromise between biological and socioeconomic constraints (e.g., effectiveness, cost) must be found and aligned with the assessment goals. It is important to consider the particular context before selecting any indicators. The following chapter offers four example use-case scenarios wherein these criteria are applied to stress the importance of considering context and goals when selecting indicators for assessing soil biodiversity.

POTENTIAL INDICATORS AND COST ESTIMATES

Cost estimates for soil analyses can vary depending on factors such as the specific laboratory or service provider, the geographic location, the number of samples, and the specific methods used. It is important to note that the following cost estimates are generalized ranges and may vary significantly. It is always advisable to contact specific laboratories or service providers for accurate pricing information as well as to discuss any specific requirements or additional services needed.

1. **Phospholipid fatty acid (PLFA) analysis:** Cost can range from $50 to $200 per sample, depending on the number of samples analyzed.
2. **DNA sequencing (metagenomics):** Cost can vary widely based on the sequencing platform, coverage depth, and bioinformatics analysis required. Typical ranges are from $100 to $1,000+ per sample, depending on the type of analysis and the number of samples analyzed.
    a. Data storage and processing are often the bottlenecks when analyzing sequence data. **Bioinformatics support** is often required and can cost between $120 – $240/hour. Projects may require up to 100 hours of analysis depending on the depth of sequencing, number of samples, and questions asked.
3. **Soil respiration measurement:** Cost can vary depending on the equipment and method used. Simple soil respiration measurements using portable devices or chambers can range from $5 to $50 per measurement, while more sophisticated automated systems or continuous monitoring setups can range from several hundred to several thousand dollars.
4. **Enzyme analysis:** Cost can range from $20 to $100 per sample, depending on the specific enzymes analyzed and the number of samples.

CHAPTER 7 | POTENTIAL EXAMPLE USE CASES/ SCENARIOS AND SUGGESTED INDICATORS

Soil is bursting with life of all shapes and sizes (Chapter 2), is critical for the sustainability and resilience of agriculture in California and faces multiple threats (Chapter 3). Previous efforts to characterize soil biodiversity have produced numerous potential indicators and frameworks for biodiversity assessment (Chapters 4 and 5) that require thoughtful consideration to understand how assessing soil biodiversity could help support and preserve soil biodiversity and achieve desired outcomes (Chapter 6).

This chapter outlines an *indicator selection framework* (ISF) that combines categories of biodiversity indicators in soil ecosystems (Chapter 4) with the criteria for selection provided in Chapter 6. This framework is applied to four example use cases to demonstrate how the ISF is used to select appropriate indicators that are most relevant and meaningful for different scenarios, goals, and audiences. The intention is to show stylized examples of how problem statements, goals, considerations, and indicators could be defined for a specific example use case.

The ISF could serve as a model for future efforts to specifically determine how to assess soil biodiversity in California working lands. The ISF can be adapted to multiple situations where impacts on soil biodiversity may be expected, including on-farm decision-making, State policy evaluation, and the effects of policy interventions (i.e., impact assessment).

INDICATOR SELECTION FRAMEWORK

One of the major challenges in measuring soil biodiversity is the difficulty to select and interpret indicators. As outlined in Chapter 4, myriad indicators already exist that represent different aspects of soil biodiversity (Table 4.1). Frameworks developed to help select appropriate soil biodiversity indicators have been applied in monitoring efforts across the world (Chapter 5).

Furthermore, it is challenging to identify the most relevant and cost-effective indicators for a specific scenario, or "use-case", without fully outlining that use case. The ISF is meant to be used in two parts. The first is to formulate clear goals for the use case assessment by clearly stating the problem to be addressed and/or key questions to answer, and identifying the intended audience of the assessment. **Ideally, these important decisions would be made through a process involving multiple stakeholders, including farmers, non-governmental organizations (NGOs), tribal members, State officials, academia, and others.**

After the use case is clearly outlined, the second part is to convene individuals with experience assessing soil biodiversity, to consider potential indicators and methods that address the problem, goals, and intended audience. It is recommended to use a table or matrix to organize these considerations. A template is provided below (Figure 7.1).

In the assessment of human health, expert assessment is used to provide recommendations because context matters - the same approach should be used to help select soil biodiversity indicators. The explicit inclusion of expert consultation in the ISF process is meant to accommodate the complexity of soil life and the rapidly evolving field of soil biodiversity. With rapid change happening in the field, some of the most promising indicators currently applied only in research settings will soon become more accessible in the future. Expert opinion also helps in narrowing the large number of possible indicators and different options for methods for each of these indicators, which vary considerably in cost, sampling and processing requirements, and interpretability.

The experts subsequently narrow down the list of potential indicators using the selection criteria outlined in Chapter 6) to arrive at final recommendations for indicator selection for that specific use case. Stakeholders can then review these recommendations and provide feedback.

HOW TO APPLY THE INDICATOR SELECTION FRAMEWORK

Part one: Answer questions about your specific use case/ scenario:

- **Define problem being addressed:** What are the larger issues or concerns you want to address in using soil biodiversity indicators?
- **Identify goals of assessment:** What outcomes and/or end products do you want to come from soil biodiversity assessment?
- **Identify intended audience:** Who are you targeting with the outcomes or end products?

Part two: Select indicators that address these needs:

- **Convene individuals familiar with biodiversity measurements to apply criteria to potential indicators:** How do your potential indicators address each of the selection criteria?
- **Evaluate and arrive at final indicator selection for use case:** Which indicator(s) best meet the criteria, are cost-effective and most relevant to your use case?

Summary of the ISF Process

Part 1

a. Define problem being addressed
b. Identify goals of assessment
c. Identify intended audience

Part 2

d. Convene experts to apply criteria to potential indicators
e. Evaluate and arrive at final indicator selection

Output: Selected indicators and application

EXAMPLE USE CASES

To illustrate the ISF process, four example scenarios or "use cases" for soil biodiversity assessment are presented below, developed through Committee discussion and with CDFA staff input.

The four example use cases are:

1. **General assessment of California soil biodiversity:** Creating an inventory of California state biodiversity under different land uses, including agriculture.
2. **Assess impacts of the CDFA Healthy Soils Program (HSP) on soil biodiversity:** Monitoring soil biodiversity as part of the HSP.
3. **Assist growers to manage the functions of healthy soils:** Providing information that supports adaptive management for growers and ranchers.
4. **Enliven soil biodiversity for growers, gardeners, ranchers, and consumers:** Engaging the general public on soil biodiversity.

These example use cases for the ISF serve to illustrate how selection criteria are applied in indicator selection as customized to specific goals and constraints.

As previously noted, Figure 7.1 presents a template to show the type of information involved in indicator selection for a particular use case. The process of evaluating indicators and methods in light of the selection criteria is not easily distilled into a workflow, as it relies on familiarity with a range of indicators, active discussion, and expert opinion. The example figure attempts to make the process of comparing and rating different possible indicators (given the use case and criteria for selection) as transparent as possible (Appendix A). Those indicators that score highly across most or all the selection criteria are those recommended for each of the four use cases.

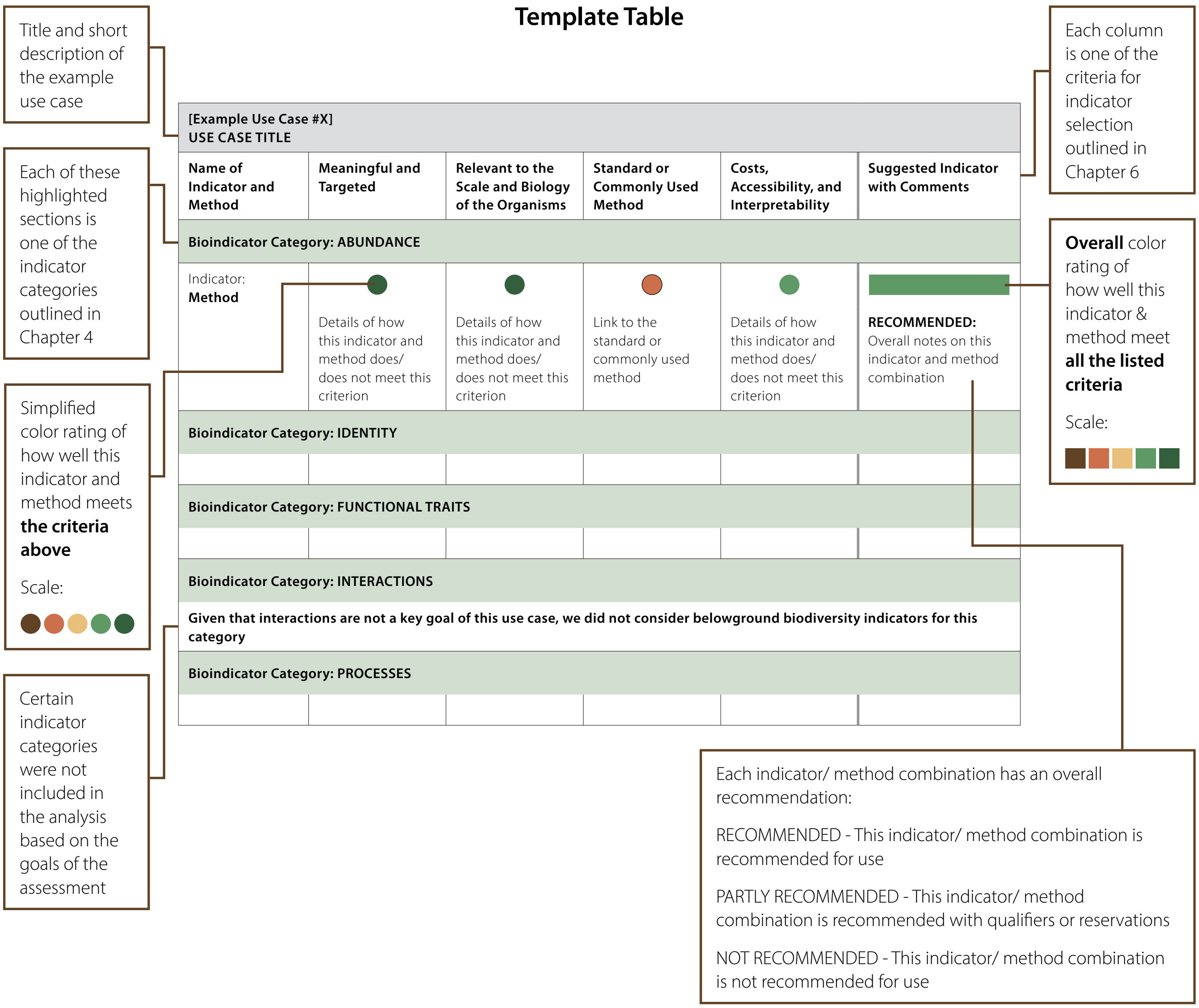

Figure 7.1 Template table used to consider potential soil biodiversity indicators and methods for a given use case, as part of the ISF.

EXAMPLE USE CASE #1: GENERAL ASSESSMENT OF CALIFORNIA SOIL BIODIVERSITY

Define problem being addressed: Conservation of biodiversity is increasingly prioritized in California, but soil biodiversity has been overlooked. Active management on working lands has high potential to support soil biodiversity, but only if soil biodiversity baselines are established.

Identify goals of assessment:

- To inventory the soil biological communities present across California working lands, (e.g., across different types of soil or farming systems) in order to identify hotspots of biodiversity and establish soil biodiversity baselines.
- To enable changes in soil biodiversity (e.g., due to management practices or climate shifts) to be assessed and quantified, and to evaluate state-wide soil biodiversity trends in working lands.

Identify intended audience: California policymakers, State and federal agency staff, researchers, and the general public.

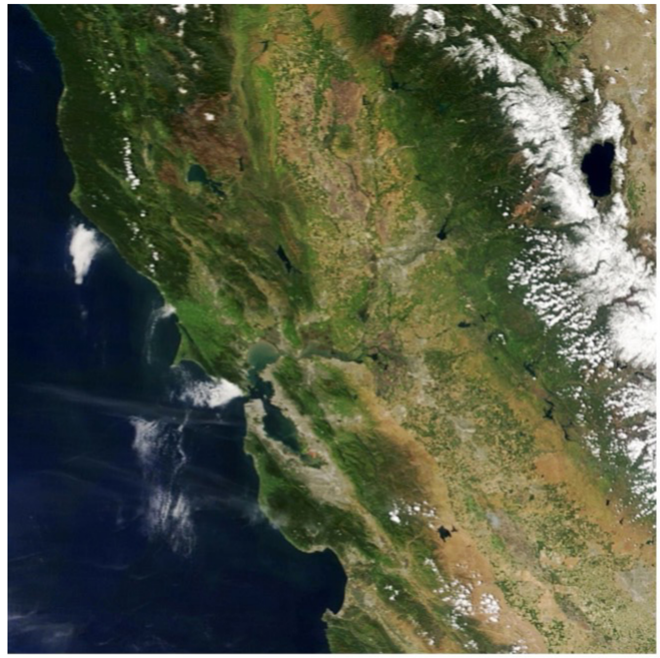

Figure 7.2. Satellite image of the Central Valley of California. *Credit: Blz 2049, CC0, via Wikimedia Commons*

Apply criteria to potential indicators:

See Appendix A, Table A1.

Example Use Case #1 Table Excerpt

[EXAMPLE USE CASE #1] General Assessment of California Soil Biodiversity					
Indicator and Method	**Meaningful and Targeted**	**Relevant to the Scale and Biology of the Organisms**	**Standard or Commonly Used Method**	**Costs, Accessibility, and Interpretability**	**Suggested Indicator with Comments**
Bioindicator Category: ABUNDANCE					
Microbial Biomass: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity Requires uncertain conversion factor.	Currently not optimal for soil fauna Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**RECOMMENDED** Valuable information on identity and total biomass. Provides separate information from DNA.
Microbial Biomass: **DNA (total)**	Quantitative biomass estimate - information on identity only with further tests Requires uncertain conversion factor.	Depending on amount of soil extracted, is not ideal for measuring abundance of macrofauna	ISO 11063:2020(en) Soil quality — Direct extraction of soil DNA	Analysis is rapid and cheap to perform after DNA is extracted	**RECOMMENDED** due to potential to combine with analysis of identity.
Microbial Biomass: **CFE (Chloroform Fumigation-Extraction)**	Quantitative biomass C estimate, no information on identity. Requires uncertain conversion factor.	Only relevant for microorganisms	ISO 14240-2:1997(en) Soil quality — Determination of soil microbial biomass — Part 2: Fumigation-extraction method	Widely used method Data is straightforward to process and analyze	**NOT RECOMMENDED** due to no information on identity - a priority goal of this example case.

Suggested indicators and application:

For this example use case, recommended indicators should be applicable across wide areas and should represent a more slowly-changing aspect of soil communities. They should provide broad characterization of soil communities, ideally with estimates of absolute abundance and not just relative abundance. **DNA metabarcoding of soil biological groups is the primary recommendation for this use case.** It is an increasingly affordable, scalable and information rich indicator of soil biodiversity that gives estimates of diversity and allows for a detailed characterization of soil communities across multiple taxa (including bacteria, archaea and eukaryotes). Global databases allow comparison to many studies, allowing California soil biodiversity and patterns of change to be compared to other regions and continents. Metabarcoding databases can also be subjected to new analyses over time. While metagenomic sequencing would also provide both functional potential and identity/ composition, the cost and complexity of both analysis and interpretation make it less feasible. **However, since DNA metabarcoding does not provide absolute abundances, we recommend a complementary indicator, PLFA also be measured.** PLFA provides a less detailed description of relative abundance of major groups of soil organisms (bacteria, fungi, protists), but can give estimates of absolute abundance. PLFA is a standard method that requires specific laboratory equipment; commercially available analyses are available. PLFA results can also be compared with global studies on the relationship between land management, soil properties, global change, and soil biodiversity (Panettieri et al. 2020).

A third potential indicator, **microscopy of nematodes,** provides rich information on the abundance, identity and functional potential of a group of soil fauna that acts as an indicator taxa for general soil biodiversity. Standard sampling, extraction and identification methods exist, and tools for interpreting nematode community data are available. One challenge of microscopy of nematodes is the time and expertise required to identify organisms.

EXAMPLE USE CASE #2: ASSESS IMPACTS OF THE CDFA HEALTHY SOILS PROGRAM ON SOIL BIODIVERSITY

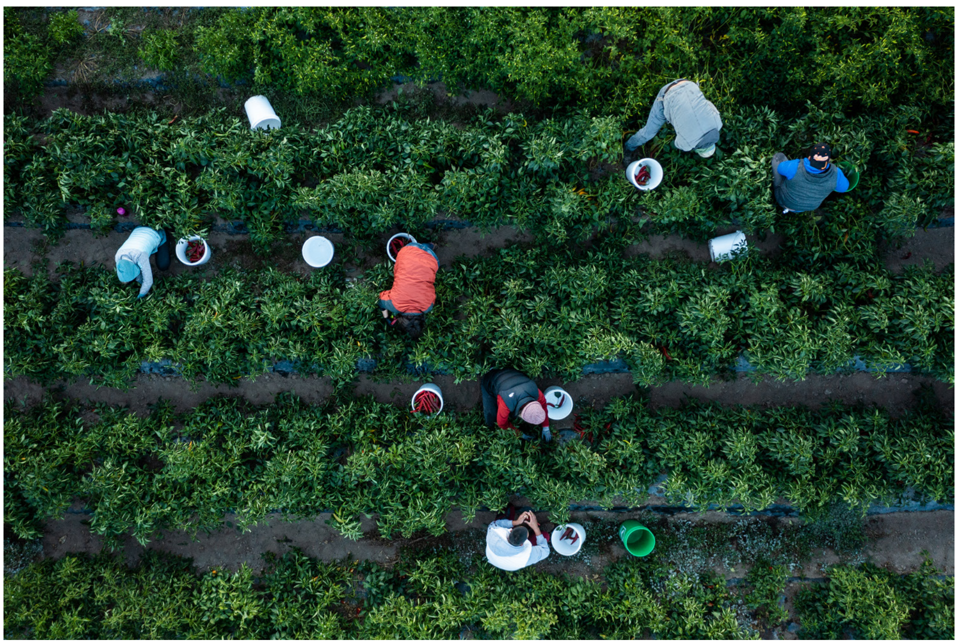

Figure 7.3. California farms can play a major role in improving soil health and mitigating climate change through the Healthy Soils Program. *Credit: CA Grown*

Define problem being addressed: While recipients of HSP Incentive Program Grants collect information on soil organic matter to infer changes in soil carbon, the supported practices can also affect soil biodiversity. Healthy Soils Demonstration Program projects[8] are not required to collect information on soil biodiversity or soil functions (beyond soil carbon and greenhouse gas fluxes).

8 https://www.cdfa.ca.gov/oefi/healthysoils/DemonstrationProjects.html. CDFA's Healthy Soils Demonstration Program aims to improve soil health, sequester carbon and reduce atmospheric GHGs funding on-farm demonstration projects that collect data and/or showcase conservation management practices that mitigate GHG emissions and increase soil health, and creating a platform promoting widespread adoption of conservation management practices throughout the state.

Identify goals of assessment:

- To assess the impacts of management practices on soil biodiversity in Healthy Soils Demonstration Program projects (including positive, negative, and unintended/ unanticipated impacts).
- To complement measurements of soil organic matter for indicating increases in ecosystem carbon stocks and greenhouse gas reductions.

Identify intended audience: Recipients of Healthy Soils Demonstration Program project grants, CDFA staff.

Apply criteria to potential indicators:

See Appendix A, Table A2.

Suggested indicators and application:

It is important that soil biodiversity indicators be sensitive to changes in soil management at the time scale of a Healthy Soils Demonstration Program project and be applicable to both crop and rangelands. Also, indicators should be related to soil organic matter to be complementary to current required measurements, as well as other measurements related to soil water dynamics that are currently under consideration. Another important criterion is use of methods that do not require extensive processing and analysis time to draw conclusions. Finally, it is important to represent as much of the soil community as possible in as few indicators as possible to assess general impacts on soil organisms.

Given the goals outlined in the use case and these design requirements, we recommend DNA metabarcoding combined with the measurement of total soil carbon via equivalent soil mass as the most accessible and scalable approach to quantifying soil biodiversity. To ensure that these measurements are complementary, sampling for DNA metabarcoding and total soil carbon should take place at the same time point. In order to relate changes in soil biodiversity to changes in outcomes (in this case, soil carbon storage), it is important to pair biodiversity measurements with complementary process measurements. In this case, total soil carbon data is sensitive to changes in management, has widely used and accurate methods, and is currently collected by the HSP. Total soil carbon can also be used to calculate changes in carbon stocks using the equivalent soil mass (ESM) method. This method is preferred to the widely used fixed depth stock calculation due to the ESM method's potential to reduce error associated with changes in bulk density (Wendt and Hauser 2013).

Well-developed and widely used methods for DNA metabarcoding are available, and many commercial labs are set up for each step in the process, even if methods would have to be adapted for these environmental samples. For instance, to capture as much of the soil community as possible, DNA from much larger sample volumes would need to be extracted to ensure larger organisms are represented, not just primarily microbes. Sampling over time at one site before and during implementation of a new practice could help draw conclusions about the impact of the change in management. Comparisons across different cropping systems or soil types could be challenging until more extensive information about typical values of indicators are available for these contexts. Other lower priority indicators like PLFA are highly sensitive to changes in management and thus are useful for assessment, but do not provide information on specific taxa, and labs capable of PLFA measurements are limited. Microscopy of nematode communities can provide insight into other soil taxa and structure of the food web (e.g., based on the abundance of bacterial and fungal-feeding nematodes) but does not provide specific quantitative information on this wider diversity of taxa that DNA metabarcoding can provide.

EXAMPLE USE CASES # 3A AND 3B: ASSIST GROWERS TO MANAGE THE FUNCTIONS OF HEALTHY SOILS USING INFORMATION ON SOIL BIODIVERSITY AND PROCESSES

Define problem being addressed: As soil health increases, ecosystem services from soil such as nutrient cycling, pest/disease regulation, and water capture and storage can increase. To reap the benefits of these services, growers need tools to assess them, and adapt their management accordingly. Two of these functions from healthy soils are addressed below:

a. *How can nutrient management be adjusted as soil health increases, including reducing synthetic nitrogen inputs?* Soil biodiversity drives the transformation and availability of nutrients to crops and underpins biological mineralization as a source of plant nutrient supply, especially in organic or low-external input farming systems. While traditional measures and indicators of soil nutrient availability (such as nitrate and ammonium levels) are widely used and easy to interpret, they can be uninformative or even misleading in healthier soils with more active soil biota and higher levels of organic matter (Bowles et al., 2015; Grandy et al., 2022). Measurement of indicators of biodiversity associated with nutrient availability would ideally complement chemical measurements such as organic matter content, pH, nitrate and ammonium content, and micronutrient content of soil.

Figure 7.4. Soil biodiversity can serve as indicators of agronomic processes in farming systems. *Credit: CDFA*

b. *How can information on soil biodiversity and processes assist growers to manage soil pests and diseases?* Reduced soil biodiversity can contribute to the incidence of soil-borne pests and diseases due to the absence of beneficial organisms that regulate or suppress pest populations. For example, herbivore nematode populations that feed on crop roots are suppressed by predator nematodes (Ferris 2010), however, predator nematode populations are reduced in agricultural intensification (Pothula et. al 2019). Chemical pest control methods are costly, and impact non-target organisms, leading to further reductions in biodiversity and, over time, a resurgence of the pest problem. Soil biodiversity assessments can give insight into the abundance of pests and disease-causing organisms, while simultaneously providing insight into the soil's potential to mitigate disease pressure through biological processes such as antibiosis, competition for resources, and predation.

Identify goals of assessment (#3a):

- To support management of soil fertility from organic inputs and soil organic matter to ensure sufficient crop nutrient availability while avoiding harmful nutrient losses to the environment.

Identify intended audience (#3a): Growers, extension specialists, technical assistance providers, land managers.

Apply criteria to potential indicators (#3a):

See Appendix A, Table A3.

Suggested indicators and application (#3a):

Soil biodiversity indicators can be effective when nutrient availability is difficult to measure by other means, such as nitrogen and phosphorus availability from organic matter, or carbon available for supporting microbial processes (Fierer, Wood, and Bueno de Mesquita 2021). For this use case, **the abundance of soil organisms and the soil processes they drive are considered the highest priority bioindicator categories.** An important focus is the soil processes that release nutrients from organic matter rather than on the amount of nutrients present at one time in soil. What is important is to assess the amount of organic matter present in the soil that is likely to be transformed and made available to plants through microbial activity. For instance, if **autoclave-citrate extractable soil protein** or **total soil nitrogen** is high, there is a greater amount of organic nitrogen available that could be made available to plants. This could complement a soil chemical test, indicating that even if soil nitrate values are intermediate or even low, then the soil could still supply sufficient nitrogen for crop production. A producer could then interpret this result in light of their experience and potentially add fewer external inputs of nitrogen than they would based on the results of a typical soil test alone.

Recommended measurements focusing on the **abundance of microbes (i.e., PLFA, chloroform fumigation extraction or total DNA)** and other key soil taxa like nematodes could provide additional insight in the capacity of the soil biota to transform organic matter into plant available nitrogen. The seasonal variability in these indicators would require that sampling considers factors such as timing, location, number of replicates, and depth. Interpretation of results may change depending on crop and growth stage, so standardizing by cropping system and timing of sampling may be necessary. Focusing on the period just before peak crop nitrogen demand would be the most informative for crop production, although other timing might be necessary to allow for fertility management. Into the future, combined measurements of soil biodiversity (e.g., abundance and process measures recommended in Table A3) and nutrient indicators from a plot over time can provide a strong record of how a particular soil responds to management and provide insight for nutrient management.

Identify goals of assessment (#3b):

- To support the management of healthy soils that suppress pests and diseases in agricultural cropping systems.

Intended audience (#3b): Growers, extension specialists, technical assistance providers, land managers.

Apply criteria to potential indicators (#3b):

See Appendix A, Table A4.

Suggested indicators and application (#3b):

While the targeted identification of pests and disease-causing organisms is an essential and routine agricultural operation, it is far more difficult to identify what enables a soil to suppress disease. In many cases, a lack of disease incidences does not mean the total absence of a pathogen or pest, but rather that those pathogen populations are being controlled by some aspect of soil biodiversity. **For this use case, the identification of potential pathogens and interactions that could cause the suppression of pathogen populations is the highest priority.** A variety of pests and pathogens may cause soil-borne plant diseases, including nematodes, fungi, bacteria, and protists such as Phytophthora and Pythium species. **The ideal diagnosis method is often pathogen-specific, and can include methods such as microscopy, visual diagnosis, culturing, and PCR.** Numerous diagnostic laboratories are set up to provide this information. DNA metabarcoding can also potentially identify plant pathogens and provide the foundation for co-occurrence network analyses to identify potential leads for disease suppression and control. Other methods such as PLFA and total DNA can provide estimates of abundance of broad groups but may not be effective to identify pathogen abundance.

For plant-parasitic nematode pests, recommended measurements should focus on nematode abundance and functional potential. Nematode analyses are valuable since they can simultaneously identify the abundance of plant parasitic nematodes, and higher-level predatory nematodes that may control pathogenic populations. Nematode populations can be classified into different feeding groups based upon structure of the mouthparts which distinguish those feeding on plants from those that consume bacteria, fungi, are omnivores, and from other nematodes. Nematode community structure is also a potential indicator of soil disease suppressiveness as the ratio of predators and omnivores to herbivores provides insight into how those herbivore populations may be regulated (Ferris, Bongers, and Goede 2001; Du Preez et al. 2022).

EXAMPLE USE CASE #4: ENLIVENING SOIL BIODIVERSITY FOR GROWERS, GARDENERS, RANCHERS, AND CONSUMERS

Define problem being addressed: Soils are among the most diverse biomes on earth. However, the vast majority of soil biodiversity is difficult to experience directly with human senses. The majority of life in soil is microscopic. For gardeners, growers, and ranchers, this can make it hard to develop relational bonds with soil biodiversity, in contrast with other biodiversity, like birds or other aboveground charismatic taxa. However, there is no doubt that a healthy soil with abundant biodiversity has odor, texture, feel and appearance characteristics that become more evident with experience.

Figure 7.5. Boosting awareness of soil biodiversity among both producers and consumers is key to preservation efforts.

Identify goals of assessment:

- Increase the awareness of and appreciation for soil biodiversity and its role in sustaining agriculture.

Identify intended audience: California farmers, ranchers, gardeners, consumers, and other agricultural participants.

Apply criteria for selection:

See Appendix A, Table A5.

Suggested indicators and application:

To help highlight how soil biodiversity impacts their everyday lives, individuals must directly experience indicators with their senses. While this limits the soil biological community that can be represented, indicators still exist which broadly relate to soil health. In this use case especially, indicators must be easy to sample and process, ideally directly in the field, and should be readily interpretable. Because there are so many easy to measure indicators that can reflect processes of interest, only a small number are represented here.

Earthworms and other macrofauna are well-known, charismatic and can be easily sampled according to standard methodologies and are linked to soil structure formation and nutrient cycling. Their populations also generally respond to soil health management (e.g., reduced soil disturbance and continual living cover). In California, most earthworms found in agricultural soils are invasive, but they are still linked to soil functioning. Although they are smaller and more difficult to observe directly, some microarthropods such as mites and collembola can be easily collected from the soil surface using pitfall traps and distinguished from other soil biodiversity using inexpensive digital magnifying scopes. These provide a good example of charismatic creatures that most producers have never seen before. Academic or commercial labs can assist producers with assessing smaller members of the soil biological community such as nematodes and microbes.

Since nematodes play roles as bacterivores, fungivores and predators, evaluating the soil nematode community can offer growers an inspiring look at the structure of the soil food web. Photos of free-living nematodes found at a site and shared with producers commonly proves very beneficial for assessments of research and extension projects. Nematode identification is also supported by online resources such as Nemaplex and interpretation of nematode faunal structures is effectively demonstrated by online tools like Nematode Indicator Joint Analysis, or NINJA. Growers are also increasingly using commercial soil microbiome tests to assess nutrient cycling, disease risk, and management effects, although the methods used are often proprietary and questions remain about the scalability of these tests given the small soil sample size.

Beyond looking at soil organisms directly, in-field soil health assessments (e.g., NRCS Cropland In-Field Soil Health Assessment Worksheet) have readily observable indicators that relate to prospects to support soil biodiversity such as soil respiration or the presence of biopores. Community science projects around the world have also used common items made of organic materials, like tea bags and even cotton underwear (e.g., Soil Your Undies), as indicators of soil biological activity and decomposition following burial and retrieval after one to several months. These tests offer readily observable evidence of microbial activity in soil that can be compared across sites.

CONCLUSION

Measuring and monitoring soil biodiversity is an essential step to conserve belowground organisms and the ecosystem services they provide to producers and all the people of California. These example use cases show there are no "one-size-fits-all" biodiversity indicators since goals and intended audiences vary depending on the application.

The ISF, at the intersection of bioindicator categories and selection criteria, helps identify a short list of possible soil biodiversity indicators pertinent to a specific use case. Moving from these example use cases to actionable plans to assess soil biodiversity in different scenarios will first require clear problem and goal identification with a diverse group of stakeholders. Domain experts can then assist by applying criteria outlined in Chapter 6 to the goal and to create a set of recommended indicators with transparent pros and cons of different options.

The following presents a set of **Targeted Recommendations** in response to the BBAC's charge to provide recommendations on soil biodiversity indicators associated with soil health and agricultural sustainability in California working lands to the CDFA. Taken together, the targeted recommendations can serve as a "soil biodiversity road map", providing clear, step-by-step guidance for policymakers and diverse stakeholders to advance efforts to conserve biodiversity and enhance soil health.

The following also highlights **Broader Recommendations and Opportunities** identified during BBAC discussions to further advance and leverage activities pertaining to soil biodiversity. Finally, this chapter provides a list of **Suggestions for Future Research and Initiatives** to advance knowledge and inform policy connected to soil biodiversity in California.

Recognizing that some recommendations are beyond the scope of CDFA alone to implement, the following identifies other local, state, federal and global entities with whom partnerships can be pursued to collaborate on moving these recommendations forward.

TARGETED RECOMMENDATIONS

1. **Use soil biodiversity as a key metric to assess, preserve, and prioritize soil health and help meet climate and sustainability goals in California agroecosystems. Integrate soil biodiversity assessment into CDFA's HSP.**

Rationale: Soil biodiversity is central to soil health and increasingly recognized for the important role it plays (and tools it can provide) in supporting essential ecosystem services that are pivotal for climate-smart, resilient, and regenerative food systems. Including soil biodiversity assessments HSP projects will provide valuable information for monitoring and improving healthy soils and climate-smart agriculture.

- Relevant to CDFA Strategic Plan 2019 - 2022[9]:
    - Goal 1, Item F: Promote and Protect - Make use of best available science in the development of policies, statutes, and regulations.
- Relevant to three CDFA Ag Vision 2023 Strategic Priorities[10]:
    - #1. Foster climate-smart, resilient, and regenerative food systems.
    - #3. Drive next-generation talent and tools.
    - #5. Collaborate on smarter regulations.

2. **Use and refine the preliminary Indicator Selection Framework to assess soil biodiversity under a range of applications and conditions.**

Rationale: The sheer number of potential soil biodiversity indicators makes it challenging to identify those most relevant and cost-effective for a specific scenario without first identifying the audience and objectives. The preliminary ISF was created to guide this effort and provide a decision-making support tool to guide soil biodiversity assessments (e.g., "soil biodiversity road map").

9 https://www.cdfa.ca.gov/exec/public_affairs/pdf/CDFA_StrategicPlan2019-22.pdf
10 https://www.cdfa.ca.gov/agvision/docs/AgVision_2023_Plan.pdf

Relevant parties: CDFA, California State agencies, farming industry, academia, private industry.

Recommendations for implementation:

a. Run a pilot program within the HSP, selecting a subset of projects that relate soil biodiversity indicators with complementary measurements of soil health.

   i. Complete the ISF for the specific use case.

      1. Refer to the four example use cases (Chapter 7) developed for different applications and conditions.

      2. Apply the criteria for selection (Chapter 6) to identify appropriate soil biodiversity indicators for the specific goals and/or information needs.

      3. Select relevant methods. Additional methods not listed in the example use cases may be considered if selected according to the criteria.

      4. Conduct cost assessment of recommended/selected methods.

      5. Finalize indicator(s) and method(s) selection and assess soil biodiversity for the specific scenario.

   ii. Interpret data and communicate the relationship of indicators with management practices and relevance to soil health.

      1. Evaluate quantitative relationships between biodiversity indicators and existing metrics of soil health (e.g., change in organic matter content).

      2. Obtain feedback from participants in the HSP.

b. Update or modify the preliminary ISF based on the results and feedback of HSP participants, CDFA staff and EFA SAP to extend the ISF use beyond the HSP pilot program.

3. **Develop an Adaptive Management Framework, expand data management infrastructure, and increase capacity to support soil biodiversity assessments.**

Rationale: More expertise is needed in this rapidly evolving and expanding field. Monitoring soil biodiversity indicators may generate complex data that requires specialized analysis, integration with other datasets and data archiving, while future agricultural practices and diagnostic capabilities will increasingly rely on knowledge of soil biodiversity. The need to create actionable knowledge by translating biodiversity assessment into management practices for soil health and policy recommendations requires an Adaptive Management Framework (Box 4).

Box 4. Adaptive Management Framework Definition

An adaptive management framework in the context of agriculture refers to a systematic approach that allows farmers and agricultural practitioners to dynamically respond to changing conditions and uncertainties in their farming systems. It involves a cyclical process of planning, implementing, monitoring, evaluating, and adjusting management practices to optimize productivity, sustainability, and resilience.

- Relevant to CDFA Strategic Plan 2019 - 2022:
  - Goal Five, Item A: Invest in Employee Development - Explore innovative training opportunities to further enhance the skills of CDFA employees.
- Relevant to CDFA Technology Roadmap (April 2022) Sections:
  - 5.2 Data management.
  - 5.3 Infrastructure services.
  - 5.5 IT Staffing, Training, and Tools.

Relevant parties: CDFA, California State agencies, federal agencies, NGOs, private industry, academia;

Recommendations for implementation:

a. Explore creation of an Adaptive Management Framework to integrate soil biodiversity assessment with agricultural management decisions.

    i. Synthesize data from CDFA programs where biodiversity and soil health indicators have been acquired to develop quantitative relationships needed to inform management decisions.

    ii. Identify collaboration opportunities to create a formal Adaptive Management Framework.

b. Expand data management infrastructure to integrate soil biodiversity data.

    i. Consider inclusion of soil biodiversity indicator data in CDFA's database for soil carbon (under development) to allow for integration with other environmental information.

    ii. Preserve data accessibility according to standard FAIR principles (Findable, Accessible, Interoperable, and Reusable) and privacy. (Carroll et al. 2021).

c. Establish additional expertise in soil biology and informatics to support soil biodiversity activities in CDFA and other State agencies.

    i. Identify or recruit a member of the EFA SAP to provide expertise in soil biodiversity.

    ii. Identify or create a position in CDFA to lead and coordinate soil biodiversity activities in the State.

BROADER RECOMMENDATIONS AND OPPORTUNITIES

1. **Optimize regional, statewide and global partnerships to promote California soil biodiversity through education, outreach, and cooperation.**

Rationale: Assessment of soil biodiversity and the preliminary ISF is of broad relevance for State, federal and global programs responsible for protecting biodiversity on natural and working lands. Public engagement with diverse stakeholders is needed to promote appreciation and understanding of the economic, societal and ecological importance of soil biodiversity and health, and create support for adoption of policies to protect soil health.

- Relevant to three CDFA Ag Vision 2023 Strategic Priorities:
    - #1 Foster climate-smart, resilient, and regenerative food systems.
    - #3 Drive next-generation talent and tools.
    - #5 Collaborate on smarter regulations.
- Relevant to CDFA Strategic Plan 2023:
    - Goal 1 Promote and Protect:
        - A. Strengthen CDFA's public outreach and awareness efforts.
        - C. Optimize local and global partnerships to promote California projects through education and cooperation (including Strategy 3: Continue international collaboration).
        - F. Make use of best available science in the development of policies, statutes, and regulations.
    - Goal 3 Education and Engagement:
        - A. Provide outreach and education to industry, stakeholders, academia, and the general public to discuss issues, build partnerships, and take action.

Relevant parties: California State agencies, federal agencies, commodity boards, private industry, NGOs, academia.

Recommendations for implementation:

a. Identify linkages among efforts developing soil biodiversity assessment in CDFA Healthy Soil Demonstration Projects, Incentive Program, Block Grant Pilot Program (as relevant) as part of the California Healthy Soil Initiative.

b. Launch an inclusive soil biodiversity campaign to provide outreach and education to industry, stakeholders, academia, and broad groups of the general public to discuss issues, build partnerships, and take action.

c. Link soil biodiversity assessment on working lands to the California Biodiversity Network 30x30 Partnership:

    i. Participate in the *Systematic Conservation Planning Roundtable*[11] to consider recommending the inclusion of soil biodiversity indicators in the development of data, analyses, and tools to improve land management, habitat restoration, and land acquisition.

    ii. Participate in the *Climate-Biodiversity Sentinel Site Network Roundtable* to consider opportunities to include CDFA Healthy Soil plots in the State sentinel site network[12].

d. Incorporate soil biodiversity outreach and education in organic and regenerative agriculture programs such as the Sustainable Agricultural Lands Conservation Program, Urban Greening, Farm to School, and Green Schoolyards, 4H, Future Farmers of America.

e. Incorporate soil biodiversity as a theme in the annual California Plant and Soil Conference, and other statewide meetings.

f. Collaborate on soil health initiatives with private sector, commodity boards, grower collectives and others working on soil health.

g. Collaborate with NGOs and consult and/or collaborate with Tribal entities.

h. Link to initiatives outside of California that address soil biodiversity, such as the Soil Health Institute and NRCS Dynamic Soil Properties Initiative.

i. Collaborate and coordinate with global efforts on soil biodiversity. For example GSBI, FAO's Global Soil Partnership, and BIOSIS in the Netherlands and European Union collaborative projects such as Soil Health Benchmarks. Host webinars with other agencies/institutions with similar goals.

2. **Build State capacity within the public and private sector to provide services and training for soil biodiversity analysis and assessment.**

Rationale: The increased focus on soil biodiversity assessment in California will require additional expertise and service providers to support activities. There is a strong need to develop more expertise and incentivize both the public and private sector to provide leadership and capacity for soil biodiversity analysis.

- Relevant to CDFA Strategic Plan 2019-2022:
    - Goal 1, Item E: Promote and Protect - *Provide a comprehensive prevention, response and surveillance system,* Strategy 1: Expand analytical testing capability.
- Relevant to CDFA Ag Vision 2023 Strategic priority:
    - #3. Drive next-generation talent and tools.

11 List of California Biodiversity Network Roundtables: https://cabiodiversitynetwork.org/roundtables/

12 February 15, 2022 Report: How a Network of Dedicated Climate-Biodiversity Sentinel Sites can Support 30x30 Implementation https://cabiodiversitynetwork.org/wp-content/uploads/2022/04/CBN-Sentinel-Site-Supplemental-Report.pdf

Relevant parties: CDFA, California State agencies, public and private sector, NGOs.

Recommendations for implementation:

a. Conduct a California state-wide survey of needs for services for measuring soil biodiversity.

b. Develop capacity of California service providers for soil biodiversity assessment such as training/incentives for commercial labs and designating and scaling up a centralized diagnostics lab (e.g., at UC or California State University (CSU) through strategic partnership).

c. Develop soil biodiversity expertise for farm advisor/extension specialist training through collaboration with UC, CSU, UC Agriculture and Natural Resources (UC ANR), other agencies and NGOs.

SUGGESTIONS FOR FUTURE RESEARCH AND INITIATIVES

The following are ideas and opportunities (sorted by theme) identified by the BBAC for future research and initiatives to advance knowledge and inform policy connected to soil biodiversity in California. **These are not recommendations but instead, are general ideas that can be used to develop future studies and research programs.** Advancement of any idea will likely require funding or matching funds from sources outside of CDFA such as USDA National Institute of Food and Agriculture (NIFA), NRCS, Foundation for Food and Agriculture Research (FFAR), or other agencies with applicable programs, commodity boards, private sector, etc. Some suggested projects have direct links with climate smart outcomes (adaptation and mitigation in particular carbon sequestration) and thus, may be eligible for funds from a variety of carbon sequestration and GHG related programs and legislation.

Rationale: Numerous opportunities for future research to build on and expand soil biodiversity assessment were identified during meetings of the BBAC. CDFA can help facilitate/coordinate this research by bringing together researchers and the healthy soil trials.

- Relevant to CDFA Strategic Plan 2023:
    - Goal 1, Item F: Promote and Protect - Make use of best available science in the development of policies, statutes, and regulations.
- Relevant to two of CDFA Ag Vision 2023 Strategic Priorities:
    - #1. Foster climate-smart, resilient, and regenerative food systems.
    - #5. Collaborate on smarter regulations.

Relevant parties: CDFA, California State agencies, federal agencies, NGOs, academia.

Create a monitoring program to determine the status of and trends of soil biodiversity in California working lands.

a. Compile existing data of soil biodiversity measurements in California natural and working lands. Collect data from existing publications, gray literature studies, and publicly available data, and include complementary indicators of soil processes/ functions (e.g., 'soil health' biological indicators) that are part of CASH focused on processes or outcomes of processes. This will be valuable in identifying regionally relevant criteria for selecting targets or ranges to identify biodiversity indicators of 'healthy soils.'

b. Develop California-relevant predictive models that incorporate soil biodiversity to predict response to changing conditions including those due to environmental change and management practices, and the consequences for ecosystem services (e.g., carbon storage, water retention). Predictive models can be process-based, take advantage of artificial intelligence technologies, or be combinations of these. Such models will be important to provide tools to advise policy and practitioners.

c. Develop a soil biodiversity health score (i.e., "score card") tailored to California agroecosystems leveraging existing data from various programs and the literature synthesized as suggested under item (b).

d. Explore how soil biodiversity may be integrated into the Soil Survey.

Investigate relationships between soil biodiversity and soil health in California working lands.

e. Carry out meta-analyses of existing literature of how management practices impact the relationship between soil health metrics, soil biodiversity and soilborne pathogen control.

f. Implement well-designed field-based studies controlling for key variables that influence soil biodiversity in order to quantitatively connect a change in management practices to improvements in soil biodiversity and soil health. Widely available remote sensing information and other data layers can be assimilated into models or data analysis frameworks to identify paired farms. For example, identifying farms with similar crops, soils, and climate but that vary in productivity would allow soil biodiversity baselines to be related to production outcomes. This information could be used to initiate new demonstration projects and could form the basis of a state-wide network of healthy soil adaptive management studies.

g. As in (f), a systematic field study of the relationship of soil biodiversity indicators and presence/severity of soilborne diseases is needed and would benefit from a paired farm approach.

h. Systematic assessments of soil biodiversity and relationships to soil health will likely support the identification of keystone species, consortia of species, or community types that support soil health and various ecosystem services. Identifying these will lead to new biodiversity indicators with explicit connections to soil function, and targets for soil biodiversity conservation.

i. Investigate the role of soil biodiversity in practices and evaluation of organic and regenerative agricultural systems.

Establish causal relationships between soil biodiversity and human health.

j. Identify linkages between soil biodiversity and soil-borne diseases (e.g., Valley Fever) (Wall, Nielsen, and Six 2015).

k. Soil management practices can influence crop micronutrients and the anti-inflammatory and antioxidant phytochemical contents of crops (Montgomery and Biklé, 2021, Reganold et. al 2010). However, while it is certain that soil biodiversity is involved, there is limited data to identify generalizable connections to human health. There are significant opportunities within California to connect research on soil health, soil biodiversity, and micronutrient and phytochemical content of crops and human health outcomes.

l. Soil biodiversity is a source of genetic, medicinal and biochemical resources (Thiele-Bruhn, 2021) and the degradation of soil biodiversity reduces our natural exposure to these benefits and our ability to harness them in support of human health. The management of working lands may therefore be a key tool in the conservation of these resources.

Investigate impacts of climate and land use change on soil biodiversity and identify roles of soil biodiversity in mitigation and adaptation to climate change.

m. Atmospheric CO_2 concentrations are now 50% higher than before the industrial revolution, increasing plant growth but also requirements for nitrogen and other nutrients, leading to lower crop nutrient contents. A meta-analysis of the relationship between elevated CO2, crop nutritional quality and soil biodiversity can help determine whether soil biodiversity can help mitigate this trend.

n. The impacts of land use change on soil biodiversity are rarely considered in management decisions. Land-use change can be bi-directional (e.g., conversion from rangeland to crops, conversion to tree crops and vineyards, or reversion back to less managed systems). There is a need to quantify the effect of these changes on soil biodiversity and the associated ecosystem services – especially across time.

o. More research is needed on the relationship between climate change driven changes to water use/availability and soil biodiversity (e.g., drought and flooding impacts on abundance and biodiversity of soil biota and impacts of soil biodiversity on soil physical properties related to improved infiltration and aquifer recharge).

p. There is a need to evaluate how more frequent and intense fires in working lands will impact soil biodiversity and function. This information can help identify management practices that restore soil biodiversity.

q. Evaluating soil biodiversity as part of the implementation of the Sustainable Groundwater Management Act (SGMA), e.g., via the Multi-benefit Land Repurposing Program, is recommended. Working with Groundwater Sustainability Agencies, researchers should determine the consequences of large-scale changes in management on soil biodiversity, and related ecosystems services.

r. The growing state-wide focus on nature-based solutions to atmospheric CO2 drawdown includes enhancing the land carbon sink through reforestation, and approaches to increased soil carbon storage through enhanced mineral weathering. It will be important to determine the impacts of these carbon management practices on soil biodiversity. There is also an opportunity to understand how soil biodiversity may support and improve these practices.

Conduct research on soil biodiversity indicators.

s. Earthworms are useful indicators of soil biodiversity and soil health in several frameworks in European agriculture. Native and non-native earthworms are both present in California. Their incidence and relevance as ecosystem engineers in California working lands is not well known and is a research opportunity.

t. Conduct surveys of arbuscular mycorrhizal fungi in cropland and rangeland soils. They are important to soil processes, crop resilience to climate disturbance, and sensitivity to conventional land management practices (i.e., tillage and inorganic fertilizer).

u. Nematodes, including their biomass, feeding groups, and food web complexity are valuable indicators of soil biodiversity. Enhance existing databases on the functional characteristics and eco-physiological attributes of nematodes by connecting those traits to genetic biomarkers in order to enhance the value of DNA metabarcoding.

v. DNA metabarcoding, or so called “shotgun” metagenomic approaches, are becoming more accessible and affordable and are increasingly used to obtain an inventory of organisms in soil. There is an opportunity to develop standard methods that simultaneously quantify biodiversity across all living organisms in soil, and data analysis methods to assess their patterns of co-occurrence. These approaches represent the next wave of biodiversity indicators.

APPENDIX A: EXAMPLE USE CASE TABLES

HOW TO USE THE EXAMPLE USE CASE TABLES WITHIN THE INDICATOR SELECTION FRAMEWORK

The example use case tables are an important step in the Indicator Selection Framework (ISF). The tables are used after defining the problem, goal, and intended audience, and then considering important criteria for selecting indicators. The BBAC, in concert with CDFA staff, applied these initial steps in the ISF to four example use cases. The BBAC then designed a table that could transparently compare different possible indicators across the criteria given the specific goal of the use case and criteria for selection (Template Table). Indicators that scored highly across all the selection criteria led to recommendations for indicators for the four examples. **It is essential that the selection of appropriate soil biodiversity indicators are closely linked to the goals of the use case.**

EXAMPLE INDICATOR SELECTION FRAMEWORK AND EXAMPLE USE CASE TABLE

a. Define problem being addressed: Here we list the issues or concerns that underlie the importance of this use case.

b. Identify goals of assessment: Here we outline the outcomes and/or end products that we would like to achieve by assessing soil biodiversity.

c. Identify intended audience: Here we list the individuals that we are targeting with the outcomes or end products.

d. Apply criteria to potential indicators: Here we list more detailed outlines of the criteria for indicator selection using the list provided in Chapter 6. The primary and secondary goals were outlined through discussion and based on the assessment goals outlined above.

CRITERIA FOR SELECTION:

1. Meaningful and targeted to the goal(s)
    a. Primary Goals (central to stated objective):
        i. Criteria considerations specific to this use case
    b. Secondary Goals (not central to stated objective, but extremely valuable information)
        i. Criteria considerations specific to this use case
2. Be relevant to the scale and biology of the organisms
    a. Criteria considerations specific to this use case
3. Be feasible to measure and easy to interpret at both scientific and policy levels
    a. Criteria considerations specific to this use case
4. Have a standardized sampling and/or methodology
    a. Criteria considerations specific to this use case

Key to the Example Use Case Table: The tables are divided into five sections corresponding to the biodiversity indicator categories described in Chapter 4. Not every indicator category will be relevant in all use cases. Within each category, each row represents an indicator and a proposed method for measuring this indicator. The same indicator (e.g., abundance of soil microbes) can have several possible methods for measuring it (e.g., total microbial PLFA versus total microbial DNA). Relevant details about how each indicator/ method combination meets or does not meet the four criteria for selection are included, and each criterion has been assigned a simplified suitability rating. Links to ISO standards have been included when available, or to most commonly used methods when not available. The final column provides an overall rating of the indicator with comments specific to the use case.

Template Table

Title and short description of the example use case

Each of these highlighted sections is one of the indicator categories outlined in Chapter 4

Simplified color rating of how well this indicator and method meets **the criteria above**

Scale:

Certain indicator categories were not included in the analysis based on the goals of the assessment

Each column is one of the criteria for indicator selection outlined in Chapter 6

Overall color rating of how well this indicator & method meet **all the listed criteria**

Scale:

[Example Use Case #X] USE CASE TITLE					
Name of Indicator and Method	**Meaningful and Targeted**	**Relevant to the Scale and Biology of the Organisms**	**Standard or Commonly Used Method**	**Costs, Accessibility, and Interpretability**	**Suggested Indicator with Comments**
Bioindicator Category: ABUNDANCE					
Indicator: **Method**	Details of how this indicator and method does/ does not meet this criterion	Details of how this indicator and method does/ does not meet this criterion	Link to the standard or commonly used method	Details of how this indicator and method does/ does not meet this criterion	**RECOMMENDED:** Overall notes on this indicator and method combination
Bioindicator Category: IDENTITY					
Bioindicator Category: FUNCTIONAL TRAITS					
Bioindicator Category: INTERACTIONS					
Given that interactions are not a key goal of this use case, we did not consider belowground biodiversity indicators for this category					
Bioindicator Category: PROCESSES					

Each indicator/ method combination has an overall recommendation:

RECOMMENDED - This indicator/ method combination is recommended for use

PARTLY RECOMMENDED - This indicator/ method combination is recommended with qualifiers or reservations

NOT RECOMMENDED - This indicator/ method combination is not recommended for use

EXAMPLE USE CASE #1:
General Assessment of California Soil Biodiversity

Define problem being addressed: Conservation of biodiversity is increasingly prioritized in California, but soil biodiversity has been overlooked. Active management on working lands has high potential to support soil biodiversity, but only if soil biodiversity baselines are established.

Identify goals of assessment:

- To inventory the soil biological communities present across California working lands (e.g., across different types of soil or farming systems) in order to identify hotspots of biodiversity and establish soil biodiversity baselines.
- To enable changes in soil biodiversity (e.g., due to management practices or climate shifts) to be assessed and quantified, and to evaluate state-wide soil biodiversity trends in working lands.

Identify intended audience: California policymakers, State and federal agency staff, researchers, researchers, and the general public

APPLY CRITERIA TO POTENTIAL INDICATORS:

1. **Meaningful and targeted to the goal(s)**
    a. *Primary Goals (central to stated objective):*
        i. Need an indicator of both identity and abundance to establish baselines
    b. *Secondary Goals (not central to stated objective, but extremely valuable information):*
        i. An indicator of functional potential (requires outlining potential functions of interest)
        ii. An indicator that gives insight into biological relationships
2. **Be relevant to the scale and biology of the organisms**
    a. Need indicators that capture multiple size classes of organisms (nematodes, microorganisms, etc.)
    b. Need indicators that are not overly sensitive to seasonal variation to accommodate large scale sampling
3. **Be feasible to measure and easy to interpret at both scientific and policy levels**
    a. Need indicators that do not require unusually extensive processing and analysis to outline conclusions
    b. Need indicators that support ongoing global efforts and analyses (long-term dataset)
4. **Have a standardized sampling and/ or methodology**
    a. Need standard methods with suitable calibration controls to compare indicators within and across systems
    b. Assays for selected indicators need to be commercially available or existing methods with potential to scale

Least useful → **Most Useful**

[EXAMPLE USE CASE #1]
General Assessment of California Soil Biodiversity

Indicator and Method	Meaningful and Targeted	Relevant to the Scale and Biology of the Organisms	Standard or Commonly Used Method	Costs, Accessibility, and Interpretability	Suggested Indicator with Comments
Bioindicator Category: ABUNDANCE					
Microbial Biomass: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity Requires uncertain conversion factor.	Currently not optimal for soil fauna Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**RECOMMENDED** Valuable information on identity and total biomass. Provides separate information from DNA.
Microbial Biomass: **DNA (total)**	Quantitative biomass estimate - information on identity only with further tests Requires uncertain conversion factor.	Depending on amount of soil extracted, is not ideal for measuring abundance of macrofauna	ISO 11063:2020(en) Soil quality — Direct extraction of soil DNA	Analysis is rapid and cheap to perform after DNA is extracted	**RECOMMENDED** due to potential to combine with analysis of identity.
Microbial Biomass: **CFE (Chloroform Fumigation-Extraction)**	Quantitative biomass C estimate, no information on identity. Requires uncertain conversion factor.	Only relevant for microorganisms	ISO 14240-2:1997(en) Soil quality — Determination of soil microbial biomass — Part 2: Fumigation-extraction method	Widely used method Data is straightforward to process and analyze	**NOT RECOMMENDED** due to no information on identity - a priority goal of this example case.

Least useful				**Most Useful**

Bioindicator Category: IDENTITY					
Phenotype Identification: **PLFA**	Can estimate group identity with low resolution and varying levels of certainty.	Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Samples need to be analyzed/ frozen quickly after collection	**RECOMMENDED** Has the potential to provide low resolution information on identity and total biomass
Genotype Identification: **DNA metabarcoding**	Provides broad survey of taxonomic groups (Bacteria/Archaea; Eukaryotes) Caveat: Method uses PCR amplification with primer biases that may exclude or limit coverage of certain groups of organisms	Requires large sample volume for DNA extraction to ensure larger soil organisms (e.g. nematodes, earthworms) are represented.	No current ISO protocols for soil metabarcoding but well developed and widely used methods are available: Microorganisms Eukaryotes	Taxonomic or phylogenetic richness estimation analyses are mature. Large global databases are available. Software is readily available. Data requires specialized analysis and processing for interpretation	**RECOMMENDED** Currently DNA metabarcoding is the most accessible and scalable approach to quantifying soil biodiversity, with the potential to shed light on biodiversity interactions.
Phenotype Identification: **Microscopy (Nematodes)**	Nematodes are good indicator taxa and can provide insight into other taxonomic groups for soil biodiversity	Directly measures nematode diversity and abundance Nematode indices can provide indirect estimates of bacterial and fungal diversity	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Nematode indices are a valuable tool for interpretation. Online resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Analysis performed by commercial, government, university labs Analytical tools available on-line, data are easy to interpret	**PARTLY RECOMMENDED** as the method does not provide estimates of abundance for other soil taxa - a priority goal of this use case. However, it does serve as an indicator of both functional potential and interactions.

Least useful				Most Useful

Bioindicator Category: FUNCTIONAL TRAITS					
Phenotype: **Microscopy (Nematodes)**	Provides insight into breadth of functional relationships, diversity of taxa, temporal succession and metabolic rates	Directly measures nematode diversity and abundance Nematode indices can provide estimates of bacterial and fungal functional potential	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes	Analysis performed by commercial government, university labs Analytical tools available on-line, data are easy to interpret Supporting databases such as NINJA, NEMAPLEX are available	**PARTLY RECOMMENDED** Nematode analyses are good indicators for functional potential of soil communities, and can provide insight into metabolic footprints and nematode abundance. This indicator addresses Tier 2 goals.
Genotype: **Metagenomic Analysis**	Can provide in-depth analysis of functional gene abundance across a wide range of taxa Conducive to further analysis using newly developed methods	Requires large extraction volume to capture larger soil organisms Only gives estimates of relative abundance of taxa	No current ISO protocols for soil metagenome analysis but method development is ongoing Microbiome analysis	Significant amounts of data produced, requiring specialized data storage, analysis and processing Costs of sample processing and analysis are significant, requiring specialized equipment	**NOT RECOMMENDED** due to cost of data processing and lack of data on abundance, a core goal of this use case.

Least useful				Most Useful

Bioindicator Category: INTERACTIONS					
Co-Occurrence Patterns: **Taxonomic Network Analysis**	Tracking co-occurrence patterns across multiple samples can give insight into the environmental niches occupied by microorganisms	Captures microorganism co-occurrence patterns, but does not provide insight into the nature of interactions (symbiotic, predator-prey, etc.)	This is a data analysis step performed on metabarcoding data. No current ISO protocols exist for soil DNA metabarcoding or network analysis but well developed and widely used methods are available: Guidelines for DNA metabarcoding of microorganisms are available. Network Analysis guidelines are available.	Large global databases of taxonomic diversity are available for comparison. Network analysis and taxonomic analysis software is readily available Results can be difficult to interpret without technical expertise	**PARTLY RECOMMENDED** if DNA metabarcoding is measured in order to maximize the value of information collected.
Food Web Relationships: **Nematode Indices**	Can provide insight into soil food web structure and complexity, food web basal components, and predation footprints	Does not capture relationships among microorganisms	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Nematode indices are a valuable tool for interpretation. Online resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Analysis performed by commercial, government, university labs Analytical tools available on-line, data are easy to interpret	**PARTLY RECOMMENDED** Nematode analyses have the potential to offer targeted insight into both functional potential and biological interactions, both secondary goals.
Bioindicator Category: PROCESSES					
Given that process measurements are not a key goal of this use case, we did not consider belowground biodiversity indicators for this category					

EXAMPLE USE CASE #2:
Assess Impacts of the CDFA Healthy Soils Program on Soil Biodiversity

Define problem being addressed: While recipients of the HSP Incentive Program Grants collect information on soil organic matter to infer changes in soil carbon, the supported practices can also affect soil biodiversity. Healthy Soils Demonstration Program projects are not required to collect information on soil biodiversity or soil functions beyond soil carbon and greenhouse gas fluxes.

Identify goals of assessment:

- To assess the impacts of management practices on soil biodiversity in Healthy Soils Demonstration Program projects (including positive, negative, and unintended/ unanticipated impacts).
- To complement measurements of soil organic matter for indicating increases in ecosystem carbon stocks and greenhouse gas reductions.

Identify intended audience: California policymakers, Sate agency staff, researchers, and the general public

APPLY CRITERIA TO POTENTIAL INDICATORS:

1. **Meaningful and targeted to the goal(s)**
    a. *Primary Goals (central to stated objective):*
        i. Need indicators of both identity and abundance to establish baselines
        ii. Indicator needs to be sensitive to management
        iii. Need indicators that can be related to soil organic carbon storage processes
    b. *Secondary Goals (not central to stated objective, but extremely valuable information):*
        i. An indicator of functional potential (requires outlining potential functions of interest)
        ii. An indicator that gives insight into biological relationships
2. **Be relevant to the scale and biology of the organisms**
    a. Need indicators that capture multiple size classes of organism - e.g., nematodes to microorganisms
3. **Be feasible to measure and easy to interpret at both scientific and policy levels**
    a. Need indicators that do not require unusually extensive processing and analysis to outline conclusions
4. **Have a standardized sampling and/ or methodology**
    a. Need standard methods with suitable calibration controls to compare indicators within and across systems
    b. Assays for selected indicators need to be commercially available or existing methods with potential to scale

Least useful | | | | Most Useful

[EXAMPLE USE CASE #2] Assess Impacts of the CDFA Healthy Soils Program on Soil Biodiversity					
Indicator and Method	**Meaningful and Targeted**	**Relevant to the Scale and Biology of the Organisms**	**Standard or Commonly Used Method**	**Costs, Accessibility, and Interpretability**	**Suggested Indicator with Comments**
Bioindicator Category: ABUNDANCE					
Microbial Biomass: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity Requires uncertain conversion factor.	Currently not optimal for soil fauna Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**RECOMMENDED** Valuable information on identity and total biomass that is sensitive to management.
Microbial Biomass: **DNA (total)**	Quantitative biomass estimate - information on identity only with further tests Requires uncertain conversion factor.	Depending on amount of soil extracted, is not ideal for measuring abundance of macrofauna	ISO 11063:2020(en) Soil quality — Direct extraction of soil DNA	Analysis is rapid and cheap to perform after DNA is extracted	**PARTLY RECOMMENDED** due to the potential to combine with analysis of identity, but less sensitive to management
Microbial Biomass: **CFE (Chloroform Fumigation-Extraction)**	Quantitative biomass C estimate, no information on identity. Requires uncertain conversion factor.	Only relevant for microorganisms	ISO 14240-2:1997(en) Soil quality — Determination of soil microbial biomass — Part 2: Fumigation-extraction method	Widely used method Data is straightforward to process and analyze	**NOT RECOMMENDED** due to no information on identity.

Least useful				Most Useful

Bioindicator Category: IDENTITY

Microbial Biomass: **Phospholipid Fatty Acid Analysis (PLFA)**	Can estimate group identity with low resolution and varying levels of certainty.	Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Samples need to be analyzed/ frozen quickly after collection	**RECOMMENDED** Has the potential to provide low resolution information on identity alongside total biomass. Potential to indicate nutrient or water stress
Genotype Identification: **DNA metabarcoding**	Provides broad survey of taxonomic groups (Bacteria/Archaea; Eukaryotes) Caveat: Method uses PCR amplification with primer biases that may exclude or limit coverage of certain groups of organisms	Requires large sample volume for DNA extraction to ensure larger soil organisms (e.g. nematodes, earthworms) are represented.	No current ISO protocols for soil metabarcoding but well developed and widely used methods are available: Microorganisms Eukaryotes	Taxonomic or phylogenetic richness estimation analyses are mature. Large global databases are available. Software is readily available. Data requires specialized analysis and processing for interpretation	**RECOMMENDED** Currently DNA metabarcoding is the most accessible and scalable approach to quantifying soil biodiversity, with the potential to shed light on biodiversity interactions.
Phenotype Identification: **Microscopy (Nematodes)**	Nematodes are good indicator taxa and can provide insight into other taxonomic groups for soil biodiversity	Directly measures nematode diversity and abundance Nematode indices can provide indirect estimates of bacterial and fungal diversity	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes	Analysis performed by commercial government, university labs Analytical tools available on-line, data are easy to interpret Supporting databases such as NINJA, NEMAPLEX are available	**PARTLY RECOMMENDED** as the method does not provide direct estimates of abundance for other soil taxa - a priority goal of this use case. However it provides an inferred abundance of other taxa that are food or prey for the nematode feeding groups.

Least useful				Most Useful

Bioindicator Category: FUNCTIONAL TRAITS					
Phenotype Analysis: **Microscopy (Nematodes)**	Provides insight into breadth of functional relationships, diversity of taxa, temporal succession and metabolic rates	Directly measures nematode diversity and abundance Nematode indices can provide estimates of bacterial and fungal functional potential	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes	Analysis performed by commercial government, university labs Analytical tools available on-line, data are easy to interpret Supporting databases such as NINJA, NEMAPLEX are available	**PARTLY RECOMMENDED** Nematode analyses are good indicators for functional potential of soil communities, and can provide insight into metabolic footprints and nematode abundance. This indicator addresses Tier 2 goals.
Genetic Analysis: **Metagenomic Analysis**	Can provide in-depth analysis of functional gene abundance across a wide range of taxa Conducive to further analysis using newly developed methods	Requires large extraction volume to capture larger soil organisms Only gives estimates of relative abundance of taxa	No current ISO protocols for soil metagenome analysis but method development is ongoing Microbiome analysis methods are available online.	Significant amounts of data produced, requiring specialized data storage, analysis and processing Costs of sample processing and analysis are significant, requiring specialized equipment	**NOT RECOMMENDED** due to cost of data processing and lack of data on abundance, a primary goal of this use case.

Least useful				Most Useful

Bioindicator Category: INTERACTIONS					
Co-Occurrence Patterns: **Taxonomic Network Analysis**	Tracking co-occurrence patterns across multiple samples can give insight into the environmental niches occupied by microorganisms	Captures microorganism co-occurrence patterns, but does not provide insight into the nature of interactions (symbiotic, predator-prey, etc.)	This is a data analysis step performed on metabarcoding data. No current ISO protocols exist for soil DNA metabarcoding or network analysis but well developed and widely used methods are available: Guidelines for DNA metabarcoding of microorganisms are available. Network Analysis guidelines are available.	Large global databases of taxonomic diversity are available for comparison. Network analysis and taxonomic analysis software is readily available Results can be difficult to interpret without technical expertise	**PARTLY RECOMMENDED** if DNA metabarcoding is measured in order to maximize the value of information collected..
Food Web Relationships: **Nematode Indices**	Can provide insight into soil food web structure and complexity, food web basal components, and predation footprints	Does not capture relationships among microorganisms	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Nematode indices are a valuable tool for interpretation. Online resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Analytical tools available online, data are easy to interpret	**PARTLY RECOMMENDED** Nematode analyses have the potential to offer targeted insight into both functional potential and biological interactions, both Tier 2 goals.

Least useful				Most Useful

Bioindicator Category: PROCESSES					
Soil Organic Matter Content: **Loss on Ignition (LOI)**	Measures the amount of organic matter (OM) present, a potential indicator of soil carbon accumulation Requires uncertain conversion factor	Reflects a potential source of both organic carbon and nutrients to microbes, but requires further analysis to quantify amounts	No current ISO protocols for soil organic matter through the LOI method, but well developed and widely used methods are available. A widely used protocol is available from the Cornell Soil Health Lab.	Low cost, accessible in commercial labs. Widely measured. Difficult to detect small short-term increases in soil C	**PARTLY RECOMMENDED** due to potential correlations with soil water infiltration and nutrient availability
Carbon Storage: **Density Fractionation of Mineral Associated (MAOM) and Particulate Organic Matter (POM)**	Measures organic matter closely bound to mineral particles (MAOM), and lightweight organic matter fragments that are relatively undecomposed (POM)	MAOM may reflect carbon that is resistant to microbial breakdown and can be stored for longer periods, while POM may reflect carbon available for breakdown	No current ISO protocols for density fractionation Methods used may vary depending on labs	Limited # of labs Method is difficult and time consuming	**NOT RECOMMENDED** due to difficulty of measurement and need for standardized methods
Carbon Storage: **Total Carbon (TC) w/ Equivalent Soil Mass (ESM)**	Measures the total amount of carbon present in the soil (organic and inorganic) and allows for the calculation of carbon stocks	While total carbon measurement does not necessarily reflect microbially available carbon, it is sensitive to changes in management	ISO 10694:1995 (en) Soil quality — Determination of organic and total carbon after dry combustion (elementary analysis) Standardized and widely used method allowing for relatively easy comparison across locations	Low cost, accessible in commercial labs. Widely measured May not easily detect short-term changes in soil C	**RECOMMENDED** due to accuracy and reliability of method in tracking longer term soil C changes

Least useful				Most Useful

Greenhouse gas fluxes: **Soil respiration burst upon rewetting**	Respiration is a measure of the metabolic activity of the soil microbial community	Reflects both the abundance of soil microbes and the pool of active carbon, or carbon that is readily available for microbial decomposition.	No current ISO protocols for soil respiration burst upon rewetting, but well developed and widely used methods are available. A widely used protocol is available from the Cornell Soil Health Lab.	Low cost, accessible in commercial labs. Can be combined with microbial biomass (e.g. from CFE) to provide an estimate of microbial respiration per unit of biomass.	**RECOMMENDED** as a measure of soil carbon availability that is cheap to measure and responsive to management
Greenhouse gas fluxes: **COMET modeling**	The COMET model is widely used in land management planning to predict the impact of changes in agricultural practices on GHG emissions	The COMET model does not yet consider explicitly consider biological interactions in estimating carbon stocks, but can be effective when soil sampling is not feasible	While there are no ISO methods for modeling GHG emissions, a widely used CA-specific version of the COMET model is available through the COMET-Planner CDFA HSP	Model available online Optimized COMET model for California exists Preferred USDA method	**RECOMMENDED** due to ease of use and potential for generating data for model improvement

EXAMPLE USE CASE # 3A:
Assist Growers to Manage the Functions of Healthy Soils Using Information on Soil Biodiversity and Processes

Define problem being addressed: As soil health increases, ecosystem services from soil such as nutrient cycling, pest/ disease regulation, and water capture and storage can increase. To reap the benefits of these services, growers need tools to assess them, and adapt their management accordingly. We focus on two of these functions from healthy soils:

a. *How can nutrient management be adjusted as soil health increases, including reducing synthetic nitrogen inputs?* Soil biodiversity drives the transformation and availability of nutrients to crops and underpins biological mineralization as a source of plant nutrient supply, especially in organic or low-external input farming systems. While traditional measures and indicators of soil nutrient availability (such as nitrate and ammonium levels) are widely used and easy to interpret, they can be uninformative or even misleading in healthier soils with more active soil biota and higher levels of organic matter (Bowles et al., 2015; Grandy et al., 2022). Measurement of indicators of biodiversity associated with nutrient availability would ideally complement chemical measurements such as organic matter content, pH, nitrate and ammonium content, and micronutrient content of soil.

Identify goals of assessment (#3a):

- To support management of soil fertility from organic inputs and soil organic matter to ensure sufficient crop nutrient availability while avoiding harmful nutrient losses to the environment.

Identify intended audience (#3a): Growers, extension specialists, technical assistance providers, land managers

APPLY CRITERIA TO POTENTIAL INDICATORS (#3A):

1. **Meaningful and targeted to the goal(s)**
    a. *Primary Goals (central to stated objective):*
        i. Need indicators of processes related to soil fertility such as nitrogen release from organic matter
        ii. Need indicators of abundance that can be related to process measurements
        iii. Need indicators that are sensitive to management
    b. *Secondary Goals (not central to stated objective, but extremely valuable information):*
        i. Need indicators of functional potential that can be related to soil fertility processes
2. **Be relevant to the scale and biology of the organisms**
    a. Need indicators that can be related to carbon and nutrient release from the microbial biomass
3. **Be feasible to measure and easy to interpret at both scientific and policy levels**
    a. Need indicators that can be easily interpreted by growers
4. **Have a standardized sampling and/ or methodology**
    a. Assays for selected indicators need to be widely available or existing methods with potential to scale

Least useful				Most Useful

[EXAMPLE CASE STUDY #3A] **Assist Growers to Manage the Functions of Healthy Soils Using Information on Soil Biodiversity and Processes**					
Indicator and Method	**Meaningful and Targeted**	**Relevant to the Scale and Biology of the Organisms**	**Standard or Commonly Used Method**	**Costs, Accessibility, and Interpretability**	**Suggested Indicator with Comments**
Bioindicator Category: ABUNDANCE					
Microbial Biomass: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity Requires uncertain conversion factor.	Currently not optimal for soil fauna Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**RECOMMENDED** Valuable information on identity and total biomass. Provides separate information from DNA.
Microbial Biomass: **CFE (Chloroform Fumigation-Extraction)**	Quantitative biomass C estimate, no information on identity. Requires uncertain conversion factor.	Only relevant for microorganisms	ISO 14240-2:1997(en) Soil quality — Determination of soil microbial biomass — Part 2: Fumigation-extraction method	Widely used method Data is straightforward to process and analyze	**RECOMMENDED** since microbial biomass is linked to the overall capacity of the soil community to process organic nitrogen
Microbial Biomass: **DNA (total)**	Quantitative biomass estimate - information on identity only with further tests Requires uncertain conversion factor.	Depending on amount of soil extracted, is not ideal for measuring abundance of macrofauna	ISO 11063:2020(en) Soil quality — Direct extraction of soil DNA	Analysis is rapid and cheap to perform after DNA is extracted	**PARTLY RECOMMENDED,** if combined with analysis of identity.
Nematode Biomass: **Nematode Counts**	Nematodes enhance nitrogen mineralization, especially bacterial and fungal feeders	Only measures nematodes, but they are good indicator taxa	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes	Several Labs do this at reasonable cost, including CDFA diagnostic labs and commercial labs	**PARTLY RECOMMENDED,** if combined with analysis of functional potential, provides complementary information to estimates of microbial abundance and reflects the contribution to nitrogen mineralization of microbial predation by fauna.

Least useful				Most Useful

Bioindicator Category: IDENTITY					
Given that identity measurements are not a key goal of this use case, we did not consider belowground biodiversity indicators for this category					
Bioindicator Category: FUNCTIONAL TRAITS					
Nitrogen mineralization from the microbial biomass: **Functional potential of nematodes (microscopy)**	Nematodes enhance nitrogen mineralization, especially bacterial and fungal feeders	Only measures nematodes, but they are good indicator taxa	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Online resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Analysis performed by commercial, government, university labs Analytical tools available online, data are easy to interpret	**PARTLY RECOMMENDED,** Nematode community assessments are one of the easiest ways to gain insight into functional potential of a key taxonomic group, and represent potential for nitrogen release from consumed microbial cells.
Bioindicator Category: INTERACTIONS					
Given that interaction measurements are not a key goal of this use case, we did not consider belowground biodiversity indicators for this category					
Bioindicator Category: PROCESSES					
Nitrogen mineralization: **Potentially mineralizable nitrogen (anaerobic incubation)**	Reflects the amount of soil nitrogen that microbes can easily turn into plant available forms of nitrogen	The organic nitrogen pool measured is operationally-defined by the measurement.	No current ISO protocols for potentially mineralizable nitrogen. Widely used method is outlined in Waring and Bremner (1964)	Low cost, but limited commercial lab availability.	**NOT RECOMMENDED,** given the low prevalence of this measurement in commercial labs.
Nitrogen mineralization: **Autoclave-citrate extractable (ACE) protein test**	Indicator of the amount of protein-like substances in soil, a large pool of organic nitrogen mineralizable by microbial activity, which can be made available for plant uptake	More targeted measurement of organic nitrogen than potentially-mineralizable nitrogen and reflects nitrogen fraction that can be readily broken down and then mineralized.	No current ISO protocols for ACE protein, but widely used methods are available. Method is outlined in Hurisso et al (2018)	Low cost, accessible in commercial labs. Efforts underway to increase interpretability.	**RECOMMENDED,** due to the accessibility of the measurement and the link with a fraction of organic nitrogen

Least useful				Most Useful

Nitrogen mineralization: **Potential activity of soil aminopeptidases**	Measures the potential activity of enzymes involved in organic nitrogen breakdown	Reflects both the abundance and activity of soil microbes.	No current ISO protocols for soil aminopeptidase activity. Methods vary widely across labs. Important to standardize both controls and assay conditions before using	Medium cost and not widely available in commercial labs.	**NOT RECOMMENDED,** due to the expense and accessibility of the methods, and widely varying protocols.
Nitrogen mineralization: **Total soil nitrogen**	The total amount of nitrogen in soil, though only a small portion is readily converted to plant available forms.	May be more predictive of soil fertility over long periods rather than during a single growing season.	ISO 13878:1998 Soil quality — Determination of total nitrogen content by dry combustion ("elemental analysis")	Low cost, accessible in commercial labs. Widely measured.	**RECOMMENDED,** even if it is not as tightly linked to within season nitrogen mineralization, it is a widely measured indicator of fertility that is readily accessible and comparable.
Carbon availability: **Soil respiration burst upon rewetting**	Respiration is a measure of the metabolic activity of the soil microbial community	Reflects both the abundance of soil microbes and the pool of active carbon, or carbon that is readily available for microbial decomposition.	ISO 16072:2002 Soil quality — Laboratory methods for determination of microbial soil respiration A widely used protocol is available from the Cornell Soil Health Lab.	Low cost, accessible in commercial labs. Efforts underway to increase interpretability. Can be combined with microbial biomass (e.g. from chloroform fumigation extraction) to provide an estimate of the metabolic quotient, microbial respiration per unit of biomass.	**PARTLY RECOMMENDED,** as a measure of soil carbon availability that is cheap to measure and responsive to management
Carbon availability: **Permanganate oxidizable carbon (POXC)**	Reflects the amount of "active carbon" in soil, which is important for microbial activity. Responsive to management.	Operationally defined fraction of soil organic matter.	No current ISO protocols for POXC measurement, but a widely used protocol is available from the Cornell Soil Health Lab.	Low cost, accessible in commercial labs. Efforts underway to increase interpretability.	**PARTLY RECOMMENDED,** as a measure of soil carbon availability that is cheap to measure and responsive to management

EXAMPLE USE CASE # 3B:
Assist Growers to Manage the Functions of Healthy Soils Using Information on Soil Biodiversity and Processes

Define problem being addressed: As soil health increases, ecosystem services from soil such as nutrient cycling, pest/ disease regulation, and water capture and storage can increase. To reap the benefits of these services, growers need tools to assess them, and adapt their management accordingly. We focus on two of these functions from healthy soils:

b. *How can information on soil biodiversity and processes assist growers in managing soil pests and diseases?* Reduced soil biodiversity can contribute to the incidence of soil-borne pests and diseases due to the absence of beneficial organisms that regulate or suppress pest populations. For example, herbivore nematode populations that feed on crop roots are suppressed by predator nematodes (Ferris 2010), however, predator nematode populations are reduced in agricultural intensification (Pothula et. al 2019). Chemical pest control methods are costly, and impact non-target organisms, leading to further reductions in biodiversity and, over time, a resurgence of the pest problem. Soil biodiversity assessments can give insight into the abundance of pests and disease-causing organisms, while simultaneously providing insight into the soil's potential to mitigate disease pressure through biological processes such as antibiosis, competition for resources, and predation.

Identify goals of assessment (#3b):

- To support the management of healthy soils that suppress pests and diseases in agricultural cropping systems.

Intended audience (#3b): Growers, extension specialists, technical assistance providers, land managers

APPLY CRITERIA TO POTENTIAL INDICATORS (#3B):

1. **Meaningful and targeted to the goal(s)**
    a. *Primary Goals (central to stated objective):*
        i. Need indicators to identify pests and disease-causing organisms and antagonistic organisms
        ii. Need indicators that highlight biological interactions that may suppress pests and diseases
        iii. Need indicators that are sensitive to management
2. **Be relevant to the scale and biology of the organisms**
    a. Need indicators that can identify common classes of pests and pathogens such as nematodes and microorganisms
3. **Be feasible to measure and easy to interpret at both scientific and policy levels**
    a. Need indicators that can be easily interpreted by growers
4. **Have a standardized sampling and/ or methodology**
    a. Assays for selected indicators need to be accurate and sensitive to pest and pathogen presence with low false positive rates

Least useful | | | | Most Useful

[EXAMPLE CASE STUDY #3B]
Assist Growers to Manage the Functions of Healthy Soils Using Information on Soil Biodiversity and Processes

Indicator and Method	Meaningful and Targeted	Relevant to the Scale and Biology of the Organisms	Standard or Commonly Used Method	Costs, Accessibility, and Interpretability	Suggested Indicator with Comments
Bioindicator Category: ABUNDANCE					
Microbial Biomass: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity Requires uncertain conversion factor.	Currently not optimal for nematodes Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**PARTLY RECOMMENDED:** PLFA does provide abundance information for broad groups, including those suppressive to disease causing organisms. It does not allow for positive identification of pests and disease causing organisms or nematodes.
Nematode Biomass: **Nematode Counts**	Abundance of plant parasitic nematodes of concern	Yes, plant parasitic nematodes are good indicators for disease and pest potential	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes	Several Labs do this at reasonable cost, including CDFA diagnostic labs and commercial labs	**RECOMMENDED:** Nematode counts are the most commonly utilized current method to provide estimates of problem populations
Microbial Biomass: **DNA (total)**	Quantitative biomass estimate - information on identity only with further tests Requires uncertain conversion factor.	Depending on amount of soil extracted, is not ideal for measuring abundance of macrofauna	ISO 11063:2020(en) Soil quality — Direct extraction of soil DNA	Analysis is rapid and cheap to perform after DNA is extracted	**NOT RECOMMENDED** due to the limited information on specific abundance of pests and pathogens

Least useful				Most Useful

Bioindicator Category: IDENTITY					
Phenotype Identification: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity Requires uncertain conversion factor.	Currently not optimal for nematodes Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**PARTLY RECOMMENDED:** While it does provide abundance information for broad groups, it does not allow for positive identification of pests and disease causing organisms or for nematodes
Phenotype Identification: **Microscopy (Nematodes)**	Nematodes are good indicator taxa and can provide estimates of metabolic activity and ecosystem services of different nematode functional groups, including herbivore and predator groups	Directly measures nematode diversity and abundance	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Soil sample size and collection should represent probable distribution of target organisms. Online resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Commercial labs routinely provide this service. Also available through CDFA diagnostic lab	**RECOMMENDED:** Nematode phenotype identification can aid in assessment of potential or current damage to plants, potential for biological regulation of pests and selection of cultivars that are non-hosts or resistant to the predominant pest species. Results can also provide insight into the presence of natural controls on pest species.
Genotype Identification: **DNA metabarcoding**	Provides broad survey of taxonomic groups (Bacteria/Archaea; Eukaryotes) Caveat: Method uses PCR amplification with primer biases that may exclude or limit coverage of certain groups of organisms	Requires large sample volume for DNA extraction to ensure larger soil organisms (e.g. nematodes, earthworms) are represented.	No current ISO protocols for soil metabarcoding but well developed and widely used methods are available: Microorganisms Eukaryotes	Taxonomic or phylogenetic richness estimation analyses are mature. Large global databases are available. Software is readily available. Data requires specialized analysis and processing for interpretation	**RECOMMENDED** DNA metabarcoding can identify the presence of some pest and disease causing organisms, as well as their antagonists and has the potential to shed light on biodiversity interactions when combined with network analysis

Least useful				Most Useful

Genotype Identification: **PCR**	● Method uses targeted PCR primers to amplify genetic sequences and detect the presence of specific pests and pathogens	● May require large soil sample volume for DNA extraction to ensure larger soil organisms (e.g. nematodes) are represented.	● National centers such as the USDA Plant Pathogen Confirmatory Diagnostics Laboratory are in charge of providing validated diagnostic controls as well as protocols, hands-on laboratory training, and troubleshooting.	● Several labs do this at reasonable cost, including CDFA diagnostic labs and commercial labs Provides accurate results	**RECOMMENDED:** PCR identification of pests and pathogens has several advantages, including high sensitivity, no need to culture organisms, rapid analysis and the potential for adaptation to a wide variety of organisms.
Phenotype Identification: **Microscopy, Culturing and Visual Diagnosis**	● These are some of the most widely used methods to identify the presence of pathogens and pests	● The method for diagnosing specific pathogens and pests will differ depending on the organism being considered	● Due to the wide variety of methods used, there is no single ISO standard used.	● Diagnostic labs such as the CDFA Plant Diagnostics Center will use a wide range of methods to diagnose plant diseases	**RECOMMENDED:** Phenotype identification via methods such microscopy, culturing and visual diagnosis are some of the most widely used and accurate ways to identify pathogen and pest presence.

Least useful				Most Useful

Bioindicator Category: FUNCTIONAL TRAITS					
Genotype: **Metagenomic Analysis**	Can provide in-depth analysis of functional gene abundance across a wide range of taxa Conducive to further analysis using newly developed methods	Requires large extraction volume to capture larger soil organisms Only gives estimates of relative abundance of taxa	No current ISO protocols for soil metagenome analysis but method development is ongoing Microbiome analysis	Significant amounts of data produced, requiring specialized data storage, analysis and processing Costs of sample processing and analysis are significant, requiring specialized equipment	**PARTLY RECOMMENDED:** Metagenomic analysis can provide some insight into the genetic mechanisms behind the action of pests and diseases, but cannot currently identify pests and pathogens with the accuracy of more targeted methods such as PCR. While the data generated may be conducive to the development of new methods, further research may be needed to make this method more viable.
Phenotype Analysis: **Microscopy (Nematodes)**	Abundance of functional groups provides assessments of magnitude of bacterivore, fungivore, herbivore and predator activity	Directly measures nematode diversity and abundance	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Analysis performed by commercial government, university labs Analytical tools available on-line, data are easy to interpret	**RECOMMENDED:** Nematode analyses can provide insight into predator and herbivore nematode abundance. This information needs to be combined with microscopy and PCR data to obtain accurate pest abundance and identity information.

Least useful | | | | **Most Useful**

Bioindicator Category: INTERACTIONS					
Co-Occurrence Patterns: **Taxonomic Network Analysis**	Tracking co-occurrence patterns across multiple samples can give insight into the relationships between disease causing and disease suppressing organisms	Captures microorganism co-occurrence patterns, but does not provide insight into the nature of interactions (symbiotic, predator-prey, etc.)	This is a data analysis step performed on metabarcoding data. No current ISO protocols exist for soil DNA metabarcoding or network analysis but well developed and widely used methods are available: Guidelines for DNA metabarcoding of microorganisms are available. Network Analysis guidelines are available.	Large global databases of taxonomic diversity are available for comparison. Network analysis and taxonomic analysis software is readily available Results can be difficult to interpret without technical expertise	**RECOMMENDED** if DNA metabarcoding is carried out. Information collected on co-occurrence patterns of disease causing organisms can provide leads into disease suppression and control.
Food Web Relationships: **Microscopy (Nematodes)**	Can provide insight the potential for biological control of plant parasitic nematodes by predator nematodes	Not easily performed in the field, requires soil sampling and sample submission	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Commercially available nematode assessments available through private and university laboratories at moderate cost.	**RECOMMENDED:** Nematode community assessments can provide insight into the potential for suppression and control of plant parasitic nematodes.
Bioindicator Category: PROCESSES					
Given that process measurements are not a key goal of this use case, we did not consider belowground biodiversity indicators for this category					

EXAMPLE USE CASE # 4:
Enlivening Soil Biodiversity for Growers, Gardeners, Ranchers, and Consumers

Define problem being addressed: Soils are among the most diverse biomes on earth. However, the vast majority of soil biodiversity is difficult to experience directly with human senses. The majority of life in soil is microscopic. For gardeners, growers, and ranchers, this can make it hard to develop relational bonds with soil biodiversity, in contrast with other biodiversity, like birds or other aboveground charismatic taxa. However, there is no doubt that a healthy soil with abundant biodiversity has odor, texture, feel and appearance characteristics that become more evident with experience.

Identify goals of assessment:

- Increase the awareness of and appreciation for soil biodiversity and its role in sustaining agriculture.

Identify intended audience: California farmers, ranchers, gardeners, and other agricultural participants.

APPLY CRITERIA FOR SELECTION:

1. **Meaningful and targeted to the goal(s)**
    a. *Primary Goals (central to stated objective):*
        i. Indicators need to demonstrate the activity of soil organisms in an easily understood form, rather than fully represent the soil biological community
2. **Be relevant to the scale and biology of the organisms**
    a. Indicators need to represent soil on a farm or ranch at a single point in time.
    b. Indicators should refer to easily visible organisms (such as meso or macrofauna)
    c. Indicators should relate back to important agronomic processes such as soil fertility or soil water storage
3. **Be feasible to measure and easy to interpret at both scientific and policy levels**
    a. Indicators should be extremely easy to interpret
    b. Indicators should engage in a variety of learning styles
    c. If possible, indicators should be able to be translated into pictures/media/art for greater impact
4. **Have a standardized sampling and/ or methodology**
    a. Indicators should be easy to sample and process, ideally in the field with minimal equipment

Least useful				Most Useful

[EXAMPLE USE CASE #4] Enlivening Soil Biodiversity for Growers, Gardeners, Ranchers, and Consumers					
Indicator and Method	**Meaningful and Targeted**	**Relevant to the Scale and Biology of the Organisms**	**Standard or Commonly Used Method**	**Costs, Accessibility, and Interpretability**	**Suggested Indicator with Comments**
Bioindicator Category: ABUNDANCE					
Macrofauna Abundance: **Earthworm counts**	Good indicators of soil health, earthworms also reflect processes of decomposition, soil aeration, infiltration, and nutrient cycling	Easily visible in the topsoil, if present in the field. Will only be relevant in locations where earthworms are present.	ISO-23611-1:2018 Soil quality — Sampling of soil invertebrates — Part 1: Hand-sorting and extraction of earthworms	Low cost, somewhat seasonal measurement; depends on soil moisture and organic matter.	**RECOMMENDED:** Earthworms are one of the most well-known members of the soil ecosystem, and play a vital role in aeration and decomposition in healthy soils. Non-native earthworms are also spreading in CA irrigated agricultural systems.
Mesofauna Abundance: **Collembola and mites**	Can indicate processes of organic matter decomposition, soil moisture and microbial activity.	Can be easily counted and identified with inexpensive magnifying scope.	ISO 23611-2:2006 Soil quality — Sampling of soil invertebrates — Part 2: Sampling and extraction of micro-arthropods (Collembola and Acarina) Pitfall traps are also a widely used method	Low cost, can be applied to most soils easily	**RECOMMENDED:** Collembola are one of the most charismatic soil biodiversity representatives and fairly easy to distinguish. Mites may be predators or decomposers and encompass a broad diversity of groups that is more difficult to separate.
Fauna Abundance: **Counts of beetles, ants, millipedes and fly larvae**	Provides a broad survey of taxonomic groups	Can be easily counted and identified with inexpensive magnifying scope	ISO 23611-5:2011 Soil quality — Sampling of soil invertebrates — Part 5: Sampling and extraction of soil macroinvertebrates A comprehensive soil macrofauna manual is also available from the FAO.	Low cost; requires a shovel, magnifying glass, alcohol and plastic containers for storage.	**RECOMMENDED:** Macrofauna groups can be easily related to soil porosity, organic matter decomposition

Least useful | | | | Most Useful

Microbial Biomass: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity	Currently not optimal for soil fauna Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**RECOMMENDED:** Valuable information on identity and total biomass. Can provide information on abundance of broad groups such as bacteria, fungi, etc.
Bioindicator Category: IDENTITY					
Phenotype Identification: **Phospholipid Fatty Acid Analysis (PLFA)**	Quantitative biomass estimate with some information on identity Requires uncertain conversion factor.	Currently not optimal for soil fauna Only relevant for microorganisms	ISO/TS 29843-2:2021(en) Soil quality — Determination of soil microbial diversity — Part 2: Method by phospholipid fatty acid analysis (PLFA) using the simple PLFA extraction method	Limited # of labs Does not require significant amounts of data processing Samples need to be analyzed quickly after collection	**RECOMMENDED:** Valuable information on identity and total biomass which can also be related back to nutrient and water stress using fatty acid ratios.
Genotype Identification: **DNA sequencing**	Relative abundance of different taxa.	Currently not optimal for soil fauna Only relevant for microorganisms	ISO 11063:2020 Soil quality — Direct extraction of soil DNA	Currently offered by several commercial labs Results in large datasets that require complex data processing and interpretation	**PARTLY RECOMMENDED:** Most commercial soil microbiome assessments are not yet at the stage where they can provide actionable data for producers, but they can serve to connect growers to their soil and provide limited insight into changes in soil biodiversity over time with multiple measurements

Least useful				Most Useful

Phenotype Analysis: **Microscopy (Nematodes)**	The abundance of bacterial and fungal feeding nematodes reflects the abundance of prey microbes.	Not easily performed in the field, requires soil sampling and sample submission	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Commercially available nematode community assessment available through private and university laboratories at moderate cost.	**RECOMMENDED:** Nematode community assessments are one of the easiest ways to gain insight into functional potential of a key taxonomic group
Phenotype analysis: **Identification of macro- and meso-fauna**	Provides a broad survey of taxonomic groups	Can be easily counted and identified with inexpensive magnifying scope	ISO 23611-5:2011 Soil quality — Sampling of soil invertebrates — Part 5: Sampling and extraction of soil macro-invertebrates A comprehensive soil macrofauna manual is also available from the FAO.	Low cost; requires a shovel, magnifying glass, alcohol and plastic containers for storage.	**RECOMMENDED:** Fauna group identification can demonstrate the wide variety of belowground organisms that exist in a field

Least useful				Most Useful

Bioindicator Category: FUNCTIONAL TRAITS					
Genetic Analysis: **Commercial soil microbiome analysis**	Used currently by many growers as indication of nutrient cycling, disease risk and management effects.	Only relevant for microorganisms and small soil sample size raises issues about scalability to farm level.	No ISO methods available. Methods are often proprietary and not scientifically published. Relative abundance of bacteria and fungi obtained through DNA sequencing is related to inferred functions such as carbon and nitrogen cycling using algorithms and databases such as PICRUST, FUNGuild, Tax4Fun, FAPROTAX	Interpretative reports provided but can be oversimplified. Moderate cost.	**PARTLY RECOMMENDED:** Most commercial soil microbiome assessments are not yet at the stage where they can provide actionable data for producers, but they can serve to connect growers to their soil and provide limited insight into changes in soil biodiversity over time with multiple measurements
Phenotype Analysis: **Microscopy (Nematodes)**	The abundance of bacterial and fungal feeding nematodes reflects the abundance of prey microbes.	Not easily performed in the field, requires soil sampling and sample submission	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Commercially available nematode community assessment available through private and university laboratories at moderate cost.	**RECOMMENDED:** Nematode community assessments are one of the easiest ways to gain insight into functional potential of a key taxonomic group

Least useful				Most Useful

Bioindicator Category: INTERACTIONS					
Food Web Relationships: **Microscopy (Nematodes)**	Can provide insight into soil food web structure and basal components, and predation footprints	Not easily performed in the field, requires soil sampling and sample submission	ISO 23611-4:2022(en) Soil quality — Sampling of soil invertebrates — Part 4: Sampling, extraction and identification of soil-inhabiting nematodes Resources such as Nemaplex.ucdavis.edu and NINJA can aid in analysis and interpretation	Commercially available nematode assessments available through private and university laboratories at moderate cost.	**RECOMMENDED:** Nematode community assessments can provide insight into both functional potential and biological interactions
Bioindicator Category: PROCESSES					
Decomposition rates: **Tea Bag Index**	Reflects decomposition rate and litter stabilization processes	Reflects soil biological activity, and is especially relevant to microbial decomposition	No ISO method available.Methods are outlined in Keuskamp et al (2013), with the potential to contribute to a global dataset through Teatime for Science	Inexpensive. Specific teabag brands (e.g. Lipton) are recommended for standardization	**RECOMMENDED:** Developed originally for leaf litter decomposition. There is potential to design a CA agriculture-specific approach
Decomposition rates: **Soil Your Undies**	Reflects decomposition rate of a common plant input (cellulose)	Reflects soil biological activity in an easily visualized form. Can show increased decomposition in soils that regularly receive plant inputs	No ISO methods exist, so there are many potential options	Inexpensive and simple. Has the potential to compare different soil types	**RECOMMENDED:** Simple, and visually appealing. Can give insight into a soil process relevant to growers - plant residue decomposition

Least useful				Most Useful

Decomposition rates: **The Bait-Lamina test**	● Based on the visual assessment of feeding on small portions of thin laminated bait inserted in the soil	● Measures biological activity such as arthropod feeding activity and response to agrochemical input in a more standardized form	● ISO 18311:2016 Soil quality — Method for testing effects of soil contaminants on the feeding activity of soil dwelling organisms — Bait-lamina test Methods are outlined in Kratz (1998)	● Inexpensive. Not widely used, and requires setup of bait substrate.	**PARTLY RECOMMENDED:** Requires more setup and monitoring than above methods, but can allow for comparisons of feeding activity among different baits and fields
Carbon mineralization: **Soil respiration**	● Reflects soil biological activity, decomposition and mineralization	● Measures CO2 released by heterotrophic organisms of all sizes	● ISO 16072:2002 Soil quality — Laboratory methods for determination of microbial soil respiration Several commercially available methods exist, including SOLVITA, Draeger Tube ®	● Relatively inexpensive and easy to interpret, as higher CO2 measured means more respiration and biological activity.	**RECOMMENDED:** Respiration is an easy to measure process indicator that is closely associated with soil moisture, temperature and soil organic matter.
Soil Structure Formation: **Biopore Assessment**	● Abundance of biopores is related to water infiltration, soil aeration and root growth	● Yes, indicative of earthworm activity and plant root growth	● No ISO method available Visually assessed using NRCS guidelines	● Inexpensive and easy to measure	**RECOMMENDED,** especially as a simple visual indicator that is part of the NRCS Cropland In-Field Soil Health Assessment

APPENDIX B: BIBLIOGRAPHY

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