# Why we need a National Living Soil Repository

Daniel K. Manter[a], Jorge A. Delgado[a,1], Harvey D. Blackburn[b], Daren Harmel[c], Adalberto A. Pérez de León[d,e], and C. Wayne Honeycutt[f]

Soils are the keystone of healthy and vibrant ecosystems, providing physical, chemical, and biological substrates and functions necessary to support life. In particular, it's the extensive and elaborate matrix of soil microorganisms and other life forms that contributes to soil health and utility.

But soils are under constant threat from heavy use, changing climate, and in some cases poor management (1, 2). In view of soil's key role and threatened status, we believe that there is a need for the scientific community to undertake coordinated research and development efforts that will lead to a unique asset: a National Living Soil Repository (Fig. 1).

Already local and national soil archives have been shown to be of great utility for studying, analyzing, and documenting long-term environmental and ecological trends. For example, the historical soil archive at Hubbard Brook helped researchers discover the link between fossil fuels and acidification of rain and snow (3); the Rothamsted Sample Archive in the United Kingdom has shown a steady increase in dioxins during the last century (4). And yet, a soil repository/archive designed to preserve native biological diversity does not currently exist.

Such an archive would provide the ability to acquire data on the current biological (e.g., soil health) state of soils around the country across soil types, cropping systems, and ecosystems and over time. Further, by maintaining soil archives and a catalog of their microbial communities, we will gain a better understanding of how soil organisms are distributed throughout the world, which could provide valuable insight into limitations on crop survival and production as new soils and/or locations are cultivated.

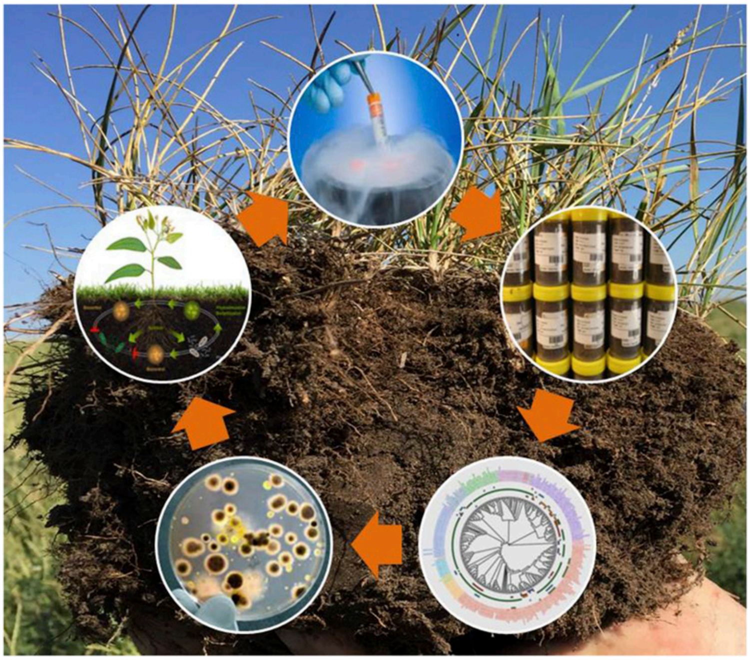

**Fig. 1. A National Living Soil Repository would store agricultural cryogenic and air-dried soil samples, analyze samples for microbial community composition, assess samples for microbial viability, and serve as a potential source of living organisms for various agricultural ecosystem services. Image courtesy of Jennifer Moore-Kucera (USDA Natural Resources Conservation Service) and Daniel Manter (USDA Agricultural Research Service).**

## Crucial Resource

It is becoming increasingly clear that soil microbes play significant roles in plant tolerance to stress (5). A repository of these microbes in their parent substrate would be critical for examining the influence of soil microbes on plant stress resilience in future environmental stress conditions. Given that soil microbes constitute such a significant portion of the genetic diversity in any ecosystem, they may be key to understanding how genetics, environment, and management practices interact to affect plant growth and ecosystem function—whether for future food production or ecosystem service benefits or to maximize soil productivity with reduced inputs for sustainable agriculture.

[a]Soil Management and Sugar Beet Research Unit, United States Department of Agriculture–Agricultural Research Service, Fort Collins, CO 80526; [b]National Laboratory for Genetic Resources Preservation, United States Department of Agriculture–Agricultural Research Service, Fort Collins, CO 80521; [c]Center for Agricultural Resources Research, United States Department of Agriculture–Agricultural Research Service, Fort Collins, CO 80526; [d]Knipling-Bushland US Livestock Insects Research Laboratory, United States Department of Agriculture–Agricultural Research Service, Kerrville, TX 78028; [e]Veterinary Pest Genomics Center, United States Department of Agriculture–Agricultural Research Service, Kerrville, TX 78028; and [f]Soil Health Institute, Morrisville, NC 27560

The authors declare no conflict of interest.



Any opinions, findings, conclusions, or recommendations expressed in this work are those of the authors and have not been endorsed by the views of the National Academy of Sciences.

[1]To whom correspondence should be addressed. Email: Jorge.Delgado@ars.usda.gov.

We propose that soil biology should also be included, because it contributes directly to both the genetic component contributing to plant function and the environment through modulation of the soil environment.

It's not clear how many small and/or private soil collections are in existence around the world, but two of the largest public national soil archives at the James Hutton Institute (Aberdeen, Scotland) and CSIRO National Soil Archive (Black Mountain, Australia) do not focus on the preservation and storage of soils in a manner that maintains microbial viability. A National Living Soil Repository, which aims to not only store soil but also maintain microbial viability, offers a unique opportunity for microbial biochemistry mining and DNA mining.

Although genome sizes vary greatly among soil bacteria, a typical bacterial cell has approximately 4,000 genes (6), resulting in more than $10^{12}$ bacterial genes in a single gram of soil alone. Through careful cataloging, annotation, and preservation, this genetic reservoir could be accessed much like current plant and animal germplasm repositories, hence providing the genetic material for addressing new challenges in agriculture.

There has been much debate on the effect of storage conditions on bacterial community composition. Lauber et al. (7) suggest that short-term storage has little impact on DNA-based assessments of community structure. However, soil preservation should focus not only on storing soil microbial DNA but also on the functional state and viability of soil microbes. In this regard, Clark and Hirsch (8) analyzed air-dried soil samples collected in 1843 from the Broadbalk field experiment on crop nutrition and found that DNA could still be extracted from these soils although microbial viability was greatly reduced. Furthermore, air drying may shift the microbial activity toward more drought-resistant groups, such as Firmicutes (9).

**A living soil repository would preserve genetic diversity as a national asset and serve as a research tool to document baseline changes in soil health, genetic variability, gene function, and population diversity over time.**

Thus, future research should investigate soil sample stabilization methods (e.g., flash-freezing, lyophilization, etc.) that not only preserve the soil microbial community in its natural state but also its recoverability at a later date. And because only 1% of microbes can be cultured with the use of current techniques (e.g., those discussed in ref. 6), it is important to preserve the soil microbial community in its native state for future research as new technologies for culturing and/or in situ assessment continue to be developed.

## A Multitude of Uses

The techniques used to measure soil microbial community structure and function are constantly evolving. Next-generation sequencing has had a major impact on our ability to quantify the structure and function of soil microbes. As these technologies continue to advance, soil archives that include both living samples and the appropriate metadata can be utilized to address long-term changes in genetic structure. A soil archive that preserves viable soil microbes is uniquely suited for identifying changes in individual microbial strain functions and genetic composition over time—especially because many analyses are currently not routinely performed because of both cost and technological limitations.

For example, plasmids are the genetic source of many important bacterial functions (e.g., antibiotic resistance and pathogenicity), and conjugative transfer of plasmids has been observed in soils (10). However, the majority of soil microbial community studies currently ignores plasmids and focuses only on analyses of genomic DNA and often a single gene, such as the ribosomal RNA gene. Even more recently, research in the nascent field of epigenetics has shown that plant markers, such as DNA methylation, are influenced by environmental factors (11). Yet, we are unaware of any studies that have assessed the role of epigenetics in a system with the complexity of organisms found in soil. Rapid advances in technology may eventually prove to be useful for the analysis of epigenetics in bacterial populations.

Natural soil microbes have been shown to play key roles in engineered industrial processes such as bioenergy (12) and bioremediation (13), as well as contributing to agricultural productivity by modulating disease (14), increasing plant tolerance to abiotic stress (15), and nutrient cycling (16). A living soil repository would preserve genetic diversity as a national asset and serve as a research tool to document baseline changes in soil health, genetic variability, gene function, and population diversity over time.

Recent reports call soil health a national priority and cite an urgent need for soil health-enhancing options for production management and agricultural-practice risk reduction across our agroecosystems (17, 18). Developing the capacity to store samples across key agroecological systems, soil orders, cropping systems, and various combinations of weather, soil types, and crops will allow us to preserve the current soil health status while conducting research that advances soil management practices, promotes soil biological diversity, and helps ensure that we don't lose the biological diversity of productive soil. We must also determine the changes in soil biology for some agricultural systems by identifying, for example, antibiotic-resistant bacteria in animal-production feedlots and antimicrobial-resistance genes found in some manure-treated soil systems.

Recent ecological system restoration efforts have shown that the soil microbial community is a key component in plant survival and successful restoration efforts (19). Furthermore, the application of soil inocula not only promotes ecosystem restoration but can also alter the fate of plant community development toward different target communities, varying from grassland to heathland vegetation (20). For example, Wubs et al. (20) applied about 1 liter of donor soil per square meter to each site.

Although such a method is not possible with the use of smaller samples that might be stored in repositories, soil slurries obtained from smaller samples, such as those that may be stored in a soil bank, can be applied to soils, and these slurries reduce herbivore feeding (21); similarly, such slurries applied to soils have been shown to promote drought tolerance in plants (22). For these reasons, we propose that research efforts investigate how to optimize the augmentation and/or amplification of a soil slurry approach, where such slurries can serve as a future means of promoting soil health and productivity in degraded systems.

## Refining a Research Tool

Because maintaining biological integrity and activity has not been the primary focus of previous soil archives, several research questions must first be addressed to identify storage methods that maintain microbial diversity and its genetic information in a natural state. We, as a research community, will have to investigate and devise strategies for sampling, handling, and preserving. Spatial and temporal variations are important aspects to be considered when designing sampling approaches and capturing biological diversity in a given soil system (23).

We must determine the best ways to effectively and economically establish a living soil repository. One strategy is to establish a repository based on a tiered approach that provides the samples, data, and services to the scientific and public community, while organizers remain cognizant of external factors, such as funding levels and stability, and how they affect the capacity of the soil archive.

High-priority "tier 1" samples could be acquired from long-term agricultural and natural field sites [e.g., United States Department of Agriculture (USDA) Long-Term Agroecosystem Research sites, National Science Foundation National Ecological Observatory Network sites, etc.] that currently have a wealth of related data and information (e.g., management, climate, etc.) and/or systematic national surveys of particular relevance to soil health (e.g., National Soil Health Assessment, Soil Health Institute 2017). "Tier 2" samples could be acquired from sites that possess unique characteristics that are not already represented in the tier 1-based living soil repository (e.g., soil and cropping types, disease suppressive soils, etc.). Future "tier 3" samples, the lowest priority, could be acquired from small-scale research plots or trials or natural environments. This tiered or phased approach would be one way to develop a living soil repository that addresses high-value samples while remaining cost conscious.

A national repository of soil biological samples complete with corresponding metadata about management practices and history of the original samples, including site, weather, and other chemical and physical soil properties, would help determine what samples should be made a high priority. This might contribute to the development of new industries focused on developing new management practices (e.g., soil biological amendments) that could improve soil health and productivity, which could contribute to land reclamation, soil and water conservation, and other benefits. It could also contribute to the development and/or improvement of models to assess soil health. The soil biological and metadata should be connected to current and/or new chemical, physical, and biological database systems (e.g., GRACEnet, NUOnet, and BIOnet) and new analytical software (e.g., myPhyloDB), with all databases being open access so that researchers and members of the public can more easily develop and calibrate new tools and products and contribute to the improvement of modeling tools used for assessing agricultural productivity, conducting environmental assessments, conducting research, and teaching.

Although the soil amounts that can be stored will be finite, research efforts should be conducted to evaluate ways to amplify soil community members in a nonbiased manner. These efforts should include both DNA samples (i.e., an unlimited supply for subsequent analyses) and cultivable microbes (i.e., the source of biological material). Particularly for the latter, research is needed to develop novel culturing techniques that can be used to target the widest array of microbial taxa as possible—and ideally, "amplify" the entire living biological complexity of a soil in a nonbiased manner. Until such research is conducted and the methods and results evaluated, we propose that some soil, however, should always be retained until unbiased methods are developed to "regrow" the extant soil community members.

**For all these reasons, a living soil repository is an investment in our future—one that enhances the scientific community's ability to advance soil health research and agricultural sustainability.**

Soils, and the living biodiversity contained in soils, are vital to functioning terrestrial ecosystems. Although there is much debate about the long-term fate of soil biodiversity, it is increasingly clear that the genetic diversity in soils is highly dynamic and a key contributor of the genetic material necessary for ecosystem functioning. A national living soil repository would help preserve genetic diversity as a national asset and would serve as a research tool to document baseline changes in genetic variability, gene function, and population diversity over time.

As envisioned, such a living soil repository will not only preserve soils and their living biological communities in their current state but also (*i*) enable opportunities to use revived soil samples for future agricultural enhancement needs and (*ii*) enable research on the changes in soils over time and under varied management practices, allowing for the investigation of interactions between soil microbial composition and changes in agricultural productivity.

For all these reasons, a living soil repository is an investment in our future—one that enhances the scientific community's ability to advance soil health research and agricultural sustainability.

## Disclaimer

The USDA prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc.) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD). To file a complaint of discrimination, write to USDA, Director, Office of Civil Rights, 1400 Independence Avenue, S.W., Washington, D.C. 20250-9410, or call (800) 795-3272 (voice) or (202) 720-6382 (TDD). USDA is an equal opportunity provider and employer.


1 Delgado JA, et al. (2011) Conservation practices to mitigate and adapt to climate change. *J Soil Water Conserv* 66:118A–129A.
2 Montgomery DR (2007) Soil erosion and agricultural sustainability. *Proc Natl Acad Sci USA* 104:13268–13272.
3 Likens GE, Bormann FH (1995) *Biogeochemistry of a Forested Ecosystem* (Springer, New York), 2nd Ed.
4 Karssies L, Wilson P (2014) Soil archives: Supporting research into soil changes. *IOP Conf Ser: Earth Environ Sci* 25:012021.
5 Prathap M, Ranjitha Kumari BD (2014) A critical review on plant growth promoting rhizobacteria. *J Plant Pathol Microbiol* 6:266.
6 Trevors JT (2010) One gram of soil: A microbial biochemical gene library. *Antonie Van Leeuwenhoek* 97:99–106.
7 Lauber CL, Zhou N, Gordon JI, Knight R, Fierer N (2010) Effect of storage conditions on the assessment of bacterial community structure in soil and human-associated samples. *FEMS Microbiol Lett* 307:80–86.
8 Clark IM, Hirsch PR (2008) Survival of bacterial DNA and culturable bacteria in archived soils from the Rothamsted Broadbalk experiment. *Soil Biol Biochem* 40:1090–1102.
9 Martí E, et al. (2011) Air-drying, cooling and freezing for sample storage affects the activity and the microbial communities from two Mediterranean soils. *Geomicrobiol J* 2:151–160.
10 Vilas-Bôas GFLT, Vilas-Bôas LA, Lereclus D, Arantes OMN (1998) *Bacillus thuringiensis* conjugation under environmental conditions. *FEMS Microbiol Ecol* 25:369–374.
11 Bowers EC, McCullough SD (2017) Linking the epigenome with exposure effects and susceptibility: The epigenetic seed and soil model. *Toxicol Sci* 155:302–314.
12 Hunter WJ, Manter DK (2014) Pre-treatment step with *Leuconostoc mesenteroides* or *L. pseudomesenteroides* strains removes furfural from *Zymomonas mobilis* ethanolic fermentation broth. *Bioresour Technol* 169:162–168.
13 Hunter WJ, Manter DK (2012) *Pseudomonas kuykendallii* sp. nov.: A novel γ-proteobacteria isolated from a hexazinone degrading bioreactor. *Curr Microbiol* 65:170–175.
14 Handelsman J, Stabb EV (1996) Biocontrol of soilborne plant pathogens. *Plant Cell* 8:1855–1869.
15 Yang J, Kloepper JW, Ryu C-M (2009) Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci* 14:1–4.
16 Aislabie J, Deslippe JR (2013) Soil microbes and their contribution to soil services. *Ecosystem Services in New Zealand: Conditions and Trends*, ed Dymond JR (Manaaki Whenua Press, Lincoln, New Zealand), pp 143–161.
17 Soil Health Institute 2017. Enriching soil, enhancing life: An action plan for soil health. soilhealthinstitute.org/wp-content/uploads/2017/05/Action-Plan-FINAL-for-flipbook-3.pdf. Accessed November 21, 2017.
18 USDA Natural Resources Conservation Service 2017. Soil health. https://www.nrcs.usda.gov/wps/portal/nrcs/main/national/soils/health/. Accessed November 21, 2017.
19 Harris J (2009) Soil microbial communities and restoration ecology: Facilitators or followers? *Science* 325:573–574.
20 Wubs ERJ, van der Putten WH, Bosch M, Bezemer TM (2016) Soil inoculation steers restoration of terrestrial ecosystems. *Nat Plants* 2:16107.
21 Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM (2013) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytol* 198:264–273.
22 Zolla G, Badri DV, Bakker MG, Manter DK, Vivanco JM (2013) Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. *Appl Soil Ecol* 68:1–9.
23 Knaebel DB (2007) Surface soil microbial sampling methods. *Manual of Environmental Microbiology*, eds Hurst CJ, et al. (American Society for Microbiology, Washington, DC), 3rd Ed, pp 597–607.