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Perspectives Paper

How microbes can, and cannot, be used to assess soil health

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ABSTRACT

Healthy soils are critical to the health of ecosystems, economies, and human populations. Thus, it is widely acknowledged that soil health is important to quantify, both for assessment and as a tool to help guide management strategies. What is less clear is how soil health should actually be measured, especially considering that soil health is not exclusively a product of soil physical and chemical characteristics. Given their well-established importance to many aspects of soil health, microbes and microbial processes are often used as metrics of soil health with a range of different microbe-based metrics routinely used across the globe. Unfortunately, it is our opinion that many of these pre-existing microbial measurements are not easy to interpret and may not necessarily provide credible inferences about soil health status. Here we review the microbial indices used to assess or monitor soil health and discuss some of the broader issues associated with their use. We provide recommendations to more effectively guide and improve how microbial information could be used to yield relevant and actionable assessments of soil health.

1. Introduction

Soils are a valuable resource because they are linked to human health, agriculture-based economies, air and water quality, and food security. Yet, soil health is under threat across the globe. These threats include: climate change, salinization, erosion, compaction, nutrient depletion, contamination with toxic heavy metals or pesticides, human-assisted migration of soil-borne pests, and overgrazing (FAO, 2015). Many of these threats are long-lasting and often difficult to ameliorate. We rely on the dwindling supply of healthy soils for the ecosystem services they provide, from maintaining a potable drinking water supply to sequestering carbon from the atmosphere (McBratney et al., 2014). "Don't treat our soils like dirt" is not just a slogan on a bumper sticker, but a rallying call with broad economic, societal, and public health implications.

There have been many efforts to describe what makes a 'good' soil. Notions such as soil tilth, fertility, and quality have all articulated various aspects of the physical, chemical, and biological nature of how soils function. The concept of 'soil health' is the most recent attempt to define and measure a soil that supports positive agricultural and environmental outcomes (Kibblewhite et al., 2008; Lehmann et al., 2020; Norris et al., 2020). What conceptually distinguishes soil health is its emphasis on the integrated inclusion of soil biota and biotic processes (Doran and Zeiss, 2000). While the importance of soil biota has been recognized for longer than modern soil science has existed (Darwin, 1881), there are at least three features that make the concept of soil health newly relevant. First is the capacity to link empirical measurements to soil biology as there have been rapid advances in our ability to characterize and quantify soil communities and processes. Thus, there is strong motivation to include microbes, and other biota, in soil health assessments. Numerous microbial-based indicators of soil health have been proposed and some of these tests are already commercially available and used routinely (Table 1). Second, there is demand for indicators of soil health that apply outside of row crop agricultural systems that have traditionally been the main focus of long-standing efforts to quantify soil quality. For example, there is growing interest in being able to characterize soil health in rangelands, but soil health indicators that may be applicable in row crop agricultural systems may not necessarily be applicable in rangelands. Likewise, how specific values are interpreted will also be context dependent. Third, there is growth in the demand for rapid, management-relevant soil testing in the age of precision agriculture. Companies that make public commitments to

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Table 1
Description of some common microbial-based metrics of soil health, the methods often used to measure these metrics, and an overview of some of the assumptions and caveats associated with these metrics. We do not rank the metrics by their utility as the choice of metric strongly depends on the context in which it is applied, the specific methods used, and the care with which the metrics are interpreted. However, the abundance of caveats associated with these metrics does highlight that these metrics should always be interpreted with caution and analyses should only be conducted if the specific interpretation of the results is clear *a priori*. qPCR = quantitative polymerase chain reaction, PLFA = phospholipid fatty acid analysis, SIR = substrate induced respiration.

Microbial Metric	Description	Methods Used	Assumptions	Caveats
Microbial biomass	Amount of microbial biomass per gram soil, volume soil, or unit organic carbon	Direct microscopy, culturing, qPCR, chloroform fumigation, PLFA, SIR	Greater microbial biomass indicates a healthier soil	- No information on which particular taxa are present - More biomass is not necessarily optimal nor desirable - More biomass does not necessarily equate with more microbial activity - Results can vary depending on methods and soil properties
Fungal: bacterial ratio	Amount of fungi versus bacteria in a given soil, expressed as a ratio of biomass, cell numbers, or DNA amounts	Direct microscopy, qPCR, PLFA	Higher fungal:bacterial ratio indicates a more sustainable soil system	- Does not reflect current ecological understanding of complex, multi-trophic soil food webs - Fungi and bacteria often have overlapping niches and functions in soil - Fungal:bacterial ratios can vary for many reasons, making interpretation difficult
Microbial enzyme activities and ratios	Potential or actual activities of microbial extracellular enzymes per amount of soil per unit time. Enzymes typically include those associated with C, N, and P cycling.	Substrate incubation assays	Greater activity of a particular enzyme indicates the nutrient the enzyme is targeting is more limiting.	- Activities of enzymes associated with C/N/P metabolism do not always accurately predict the actual limiting nutrient - Higher enzyme activities can be interpreted as more nutrient availability or reduced nutrient availability - Enzymes typically measured represent a small subset of potentially important enzymes
Nitrifier abundance and composition	Abundance and taxonomy of nitrifiers, including ammonia-oxidizing archaea or bacteria and nitrite-oxidizing bacteria	Potential nitrification assays, qPCR, high-throughput marker gene sequencing	Greater nitrifier richness and abundances indicate greater losses of soil N via nitrification and nitrate leaching	- Nitrifier abundances may be related to factors other than N availability - Nitrifier abundances may not necessarily correlate with nitrification rates
Mycorrhizal abundance and composition	Abundance and composition of arbuscular mycorrhizal fungi	Root staining and microscopy, spore counts and microscopy, qPCR, high-throughput marker gene sequencing	Greater abundance and richness of mycorrhizae indicate greater benefit to plant growth	- Relationships between mycorrhizae and plants are dynamic, context dependent, and move along the mutualism-parasitism continuum - There is a considerable amount of variation in the relationship between root colonization and plant growth - Root colonization can change over weekly time scales
Bacterial and fungal pathogens	Presence and/or abundance of known pathogenic taxa	qPCR, high-throughput marker gene sequencing	Greater abundance of pathogens is detrimental to plant growth	- Other factors influence the severity of plant disease - Pathogen abundances may not predict disease prevalence
C and N mineralization rates	Production of CO_2 and net inorganic N per amount of soil or soil organic C per unit time.	Lab or field incubations	Greater C and N mineralization rates indicate more bioavailable C and N and a more active microbial community	- Lab incubations may not reflect C and N mineralization rates in the field - High C and N mineralization rates are not necessarily desirable
Nitrification rates	Production of net NO_3^- per amount of soil or soil organic C per unit time	Lab or field incubation	Greater nitrification rates indicate greater losses of soil N from nitrification and nitrate leaching	- Lab incubations may not reflect nitrification rates in the field - Does not account for uptake or leaching rates
Microbial community composition	Using DNA or RNA to infer the abundances of particular bacterial, archaeal, fungal and/or protist taxa in soil.	qPCR or high-throughput marker gene (typically 16S, 18S, ITS) sequencing	Microbial community changes correlated with other metrics of soil health. Diverse communities achieve higher nutrient cycling rates and defense against pathogens	- Contributions of many taxa to soil health often undetermined - Multivariate data difficult to analyze and interpret - Not all DNA comes from intact cells ('relic' DNA) - RNA is highly unstable and may not reflect activities of individual taxa
Microbial functional gene composition	Abundances of particular known genes (e.g. *amoA*, *nifK*) in soil. Analogous to			- Rare taxa overlooked

(*continued on next page*)

Table 1 (*continued*)

Microbial Metric	Description	Methods Used	Assumptions	Caveats
	microbial community composition, with genes instead of taxa	High-throughput shotgun metagenomic sequencing, qPCR of targeted genes	Greater abundance of a gene indicates greater rate of processes related to the gene	- Other factors may influence the actual rates of processes - Difficult to identify which genes are most relevant to measure
Nitrogen fixation	Rate of N fixed per volume of soil per unit time	Acetylene reduction assay	Greater N-fixation rates promote greater N availability	- Assay provides a snapshot of N-fixation which can exhibit a high degree of temporal variation
Plant growth-promoting rhizobacteria (PGPR)	Abundances of specific bacteria in the rhizosphere thought to be beneficial to plants	qPCR, high-throughput marker gene (16S) sequencing	Greater abundances of these taxa indicate greater benefits to plant growth Adding PGPR improves plant growth	- PGPR must establish in the community, which can be challenging in some soils - Not all PGPR are equivalent in their effects on plants and the magnitude or direction of those effects could be highly context dependent.

advancing soil health now demand empirical tests that are rapid, affordable, and highlight the biological, as well as physical and chemical, nature of soil health. Yet many existing methods were developed for research purposes, not explicitly to provide management guidance or plot-to-ecosystem scale assessments where causal links to outcomes are important. Therefore, these pre-existing biological indices may not necessarily be useful for making credible inferences about soil health status or to select and monitor strategies to improve soil health.

Our aim in this article is to review the microbial indices that could be used to assess and monitor soil health, and to provide guidance on their use. We focus here on microbial indices though we recognize that microbes are not the only group of organisms that could be considered in soil health assessments (e.g. Neher, 2001; Velasquez et al., 2007). We also acknowledge that this topic is not new. Soil biologists have long studied, and debated, how microbes and microbial activities can be used to quantify soil health (Lehman et al., 2015; Schloter et al., 2018). However, it is our opinion that these discussions are at a critical juncture because of the increasing demand for microbial measurements to provide management-relevant guidance.

2. What do we mean when we talk about soil 'health'?

Soil health can be defined both conceptually and operationally (Bünemann et al., 2018; Lehmann et al., 2020). As a conceptual definition, the U.S. Department of Agriculture Natural Resource Conservation Service (USDA-NRCS) offers: "Soil health, also referred to as soil quality, is defined as the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals, and humans." This definition attempts to define what a healthy soil is without defining how to measure it. However, the USDA-NRCS also offers a more operational definition by providing a key list of soil health indicators, that include physical, chemical, and biological properties (Stott, 2019) with an ongoing project to evaluate these indicators (Norris et al., 2020).

There is no 'optimal' soil nor a universal set of ideal soil characteristics (Bünemann et al., 2018; Lehmann et al., 2020). Although certain soil indicators may always be relevant when trying to assess soil health, including soil texture, bulk density, pH, and organic carbon concentrations (Stewart et al., 2018), their interpretation will always be highly context dependent. For instance, having low pH may be unsuitable for production of certain crops, but could be optimal for other desirable vegetation types or crop species (like blueberries). The functioning of soil will always depend on all six soil forming factors: climate, organisms, relief, parent material, time, and human activities (Jenny, 1994). Soil health, like beauty, is in the eye of the beholder. However, there are soil indicators that, when taken on their own, can be useful for quantifying specific soil processes and soil-related agronomic and environmental outcomes. What is needed is the ability to effectively monitor these indicators over time to guide management, recognizing that these metrics, and the desired values for these metrics, can vary depending on the soil in question.

Measurements of soil biology are similar to soil physical and chemical properties in that the interpretation of what is a "good" level will be context-specific. However, soil biological indicators differ in that what counts as a relevant indicator to measure also tends to be highly context-specific. In other words, the selection of which microbial metrics to measure to infer soil health will depend on the soil or site in question and the particular aspects of soil health that are of interest. Our recommendations rest on a change in mindset away from a universal set of soil health indicators, to a more nuanced recommendation of selecting indicators to meet specific management and/or policy objectives. Our goal here is to highlight which pieces of microbial information can be useful for monitoring soil health, from an agriculture and conservation perspective - whether the goal is to maximize soil carbon sequestration efforts or improve long-term crop production. We offer guidance for how to select microbial measures based on a set of common management and policy goals.

3. Limitations of existing methods

Soil microbes, including archaea, bacteria, fungi, and protists are associated with many aspects of soil quality and health (Bach et al., 2020; Fierer, 2017). Among the many facets of soil quality, microbes can regulate nutrient availability, aggregate stability, C sequestration, pollutant degradation, plant disease prevalence, and plant growth promotion. Soil microbes are neither 'good' nor 'bad' with regards to soil health - they just are. While some bacteria and fungi are well-established plant pathogens, others (even closely related taxa) may confer protection against pathogens (Schlatter et al., 2017). Likewise, microbes that are capable of pesticide degradation can effectively reduce soil pesticide concentrations, but the metabolites of active ingredients in pesticides can accumulate during degradation and end up being more toxic than the active ingredients themselves (Odukkathil and Vasudevan, 2013). As a final example, there is no ideal target for nitrifier abundances in soil. Increases in nitrifier abundances over time could be desirable in soils where the goal is to alleviate nitrogen limitation. Alternatively, such increases could be undesirable if the goal is to minimize excessive nitrogen losses. Context is key.

Given the importance of soil microbes to many aspects of soil functioning, it makes intuitive sense that there should be microbial metrics of soil health. This is a logical argument and it underlies much of the promise of using microbial data to quantify and monitor soil health. In fact, there are already companies offering microbial tests as potential metrics of soil health (e.g. Trace Genomics, Ward Laboratories, EarthFort, Prolific Earth Sciences, Woods End Laboratories). In Table 1, we describe some of the more commonly used microbial metrics of soil health, the assumptions underlying their use, and some of the caveats associated with these metrics. We note that these pre-existing metrics include those that involve measuring the abundances of particular taxa

or genes of interest, quantifying rates of microbial activities, or estimating pools of microbial biomass or their enzymatic capacities (Table 1). These pre-existing metrics may be useful in some situations; however, the science underlying the use and application of these pre-existing metrics is often insufficient to guide clear interpretation for management and policy purposes (Table 1). For example, fungal:bacterial ratios have been used as indicators of effective nutrient cycling in ecosystems (de Vries and Bardgett, 2012; Six et al., 2006; Wardle et al., 2004), but the utility of these metrics has been strongly questioned due to a lack of evidence for clear distinctions between fungal and bacterial-dominated pathways (Rousk and Frey, 2015; Strickland and Rousk, 2010). Likewise, it has been proposed that bacterial diversity could be a useful indicator of soil health (Maron et al., 2018; van Bruggen and Semenov, 2000; Van Der Heijden et al., 2008), but higher soil bacterial diversity is not always 'ideal' (just as maximizing plant species diversity is not always desirable) and bacterial diversity is often well-correlated with soil pH (Fierer and Jackson, 2006; Griffiths et al., 2011) and changing soil pH levels is not always feasible nor desirable. As yet another example, a soil with higher abundances of bacterial and fungal pathogens could be considered less healthy than a soil with lower pathogen loads. However, the mere presence of soil pathogens does not necessarily correlate with elevated likelihood of plant disease (Lievens et al., 2006). Even the same dataset could be interpreted in opposite ways. This is the case with the potential activities of extracellular enzymes that are commonly measured to infer N and P availability where higher activities could be interpreted as evidence of nutrient limitation (Sinsabaugh et al., 2008) or as evidence of greater nutrient availability (Nannipieri et al., 2012). As a final example, the measurement of soil microbial biomass, or the ratio of microbial biomass to soil organic carbon (Anderson and Domsch, 1989), may not necessarily provide a useful, or readily interpretable, assessment of soil health as there are many biotic and abiotic factors that could contribute, directly to indirectly, to changes in soil microbial biomass (Dalal, 1998). While pre-existing microbial metrics can be useful under some situations, many of these metrics are not well-supported by the available scientific evidence, or the evidence supporting their utility is simply unavailable (Table 1). Relying on these metrics could be a waste of money or, at worse, lead to incorrect inferences about soil health status. Caveat emptor.

3.1. The potential utility of microbial community analyses

It is easy to highlight the flaws in pre-existing microbial metrics of soil health, or at least to point out when these metrics are, or are not, useful. However, a key question remains: How do we move forward in trying to leverage microbial information to provide more relevant, and actionable, assessments of soil health?

We argue that DNA-based analyses of microbial communities represent an under-utilized metric of soil health that has the potential to transform how we measure and understand soil health. Such analyses most commonly involve either quantitative PCR to quantify the abundances of particular microbial genes or taxa, amplicon sequencing of marker genes for broad community analyses (e.g. ITS or 16S rRNA gene sequencing for fungal or bacterial analyses, respectively), or shotgun metagenomic sequencing for an untargeted survey of both the microbial taxa and functional genes found in a given sample. These are by no means the only molecular methods that could be used to characterize soil microbial communities (Prosser, 2015; Schloter et al., 2018), but they meet a number of criteria for widespread adoption:

- **Microbial analyses can be reasonably cheap, fast, and high-throughput.** Hundreds of samples can be analyzed per week in a reasonably small laboratory at a cost that is on par, or perhaps even cheaper, than many of the pre-existing methods. For example, high-throughput DNA sequencing to assess bacterial and fungal community composition can now be conducted for a per-sample cost that is lower than the cost of the commonly used Haney test (~$50 USD per sample) and improvements in DNA sequencing technologies will continue to drive these analytical costs down even further. Furthermore, while processing and analyzing these data still requires expertise and training, the bioinformatics pipelines are becoming more user-friendly and accessible.
- **Microbial communities are temporally variable, but not too variable.** Soil characteristics that change very slowly are not useful for monitoring how soils respond to changes in management practices. For example, the soil organic carbon pool is large and annual changes in the size of that pool can often be very difficult to detect (Bradford et al., 2016). Likewise, soil characteristics that change very quickly are often of limited utility as collecting samples a few weeks apart could yield very different results. This is a problem when measuring extractable NH_4^+ or NO_3^- concentrations which can change appreciably over days to weeks. Microbial communities could be considered to fall into the 'Goldilocks' sweetspot - variable, but not too variable. Communities can change over seasons to years, but the DNA pool does not typically exhibit a lot of variability over days to weeks (Carini et al., 2020; Lauber et al., 2013). This could be important for efforts to predict emissions of N_2O and CH_4, both important greenhouse gases, from agricultural lands. Direct measurements of gas fluxes from individual plots are often stymied by the high temporal variability in emission rates (Groffman et al., 2009; Hendriks et al., 2010), but microbial analyses could provide a temporally-integrated metric of the variation in the emissions of these trace gases (Conrad, 2007; Deslippe et al., 2014; Nazaries et al., 2013).
- **Microbial communities are highly diverse and this diversity can be used to characterize many aspects of the soil environment.** The high diversity of soil microbial communities is often considered a daunting analytical problem as a single soil can harbor thousands of different microbial taxa and functional genes, many which remain poorly characterized (Delgado-Baquerizo et al., 2018; Howe et al., 2014; Tedersoo et al., 2014). However, this diversity also presents opportunities for exploring many different aspects of the soil environment in much the same way that satellite hyperspectral images (where hundreds of different spectra can be measured for any given location on Earth) are more informative than simple black and white photos of the Earth's surface. Just as leaf nutrient concentrations can be inferred from hyperspectral imagery (Martin et al., 2018), even if the specific mechanisms underlying the relationships between particular spectra and nutrient ratios are undetermined, we may be able to infer specific soil properties by quantifying the relative abundances of specific microbial taxa or genes, even if we do not necessarily know why such relationships exist (as discussed in more detail below). The potential of this approach was demonstrated in a recent cross-site study that used information on soil bacterial community composition to predict key physico-chemical variables associated with soil quality (Hermans et al., 2020).

3.2. Microbes as bio-indicators

Microbes not only drive many important soil processes, they also respond to biotic and abiotic soil conditions. For example, consistent changes in soil microbial communities have been associated with changes in P availability (Hermans et al., 2017), soil pH (Delgado-Baquerizo et al., 2018), labile organic carbon pools (Ramírez et al., 2020), and soil moisture levels (Isobe et al., 2020). Likewise, we can often identify particular microbial taxa or functional genes associated with specific soil processes, including nitrification, methane production, denitrification, and cellulose degradation. Instead of focusing on particular microbial attributes (including taxa or genes) that we think should be indicative of soil health, we can instead use taxa or genes to determine changes in soil characteristics and processes that we already know to be important components of soil health, leveraging the

potential advantages of DNA-based microbial analyses. Of course, the complexity of microbial communities and their functional attributes makes microbial community data difficult to analyze and interpret. Giving a gardener a list of microbial genes or taxa found in their soil may be scientifically interesting, but it is not practically useful. Instead, if such data are used to identify particular bio-indicators of particular soil conditions or processes, the microbial information is far more useful - making it easier to generate the relevant microbial information and the results will be easier to interpret. We note that the idea of using microbes as soil bio-sensors or bio-indicators is not new (Visser and Parkinson, 1992; Waksman, 1927), but the approach has not yet been widely adopted.

Microbial-based indices of soil conditions are most useful if they are broadly applicable across a wide range of soil and ecosystem types. Just because particular taxa increase or decrease in response to P availability at a single site does not mean those same taxa are broadly indicative of P availability in different soils. Not all taxa or genes will be found in all soils, but identifying microbial bio-indicators requires validating that those taxa, or genes, are consistently associated with particular aspects of soil health for the soil or ecosystem types in question. Identifying these microbial bio-indicators thus requires comprehensive, cross-site analyses of soils that are well-characterized (e.g. Hermans et al., 2017). This is analogous to the use of spectral libraries to calibrate infra-red measurements of soil health properties (Sanderman et al., 2020). These measurements use infrared reflectance of soils to correlate with known, measured soil properties, which requires large libraries of spectral signatures across many soil types, with the results validated against independent sample sets to assure that the signatures are robust.

Identifying microbial bio-indicators greatly simplifies the integration of microbial data into decision-making processes as there is often already preexisting information on the parameters of interest. For example, assessing the degree of soil contamination with heavy metals is critical to monitoring and managing soil pollution, but heavy metal concentrations can be expensive to measure. Thus, microbial indicators of heavy metal contamination can provide information directly relevant to determining the suitability of sites for agriculture, designing remediation strategies, and monitoring the success of the remediation efforts (Tang et al., 2019). We are not suggesting that chemical analyses of heavy metal concentrations should be replaced with microbial indicators of metal contamination, but rather that microbial data could help prioritize when or where more detailed chemical analyses of metal concentrations could be useful. The same idea applies to the measurement of P bioavailability in soil, a notoriously difficult problem given the limitations of chemical testing approaches (Das et al., 2019). Instead, we can likely use microbes as bio-indicators of P availability (Hermans et al., 2017), providing information that can be directly integrated into decades of accumulated knowledge on management strategies to alleviate P limitation in agricultural systems.

4. How do we move forward?

While microbial data have the potential to supplement pre-existing soil health metrics, we are not yet at the point where these microbial community metrics should be widely adopted. There are some important caveats and limitations that need to be carefully considered before such DNA-based microbial analyses can be, or should be, widely adopted.

- **Methodological issues can limit the utility of DNA-based microbial metrics.** DNA-based microbial analyses are no panacea and some of the issues that limit the interpretability of pre-existing microbial health metrics (Table 1) will also plague alternate approaches unless the metrics are carefully validated. There are also some particular aspects of DNA-based microbial community methods that need to be considered. For example, even subtle differences in approaches (including differences in DNA extraction efficiencies, PCR primers, or bioinformatic pipelines) could make it difficult to compare results in a consistent manner. Likewise, not all DNA in soil is exclusively derived from 'active' microorganisms and the presence of 'relic' DNA (Carini et al., 2020) could obscure relationships between the microbial data and soil conditions. Finally, the complexity of soil microbial communities and the multivariate nature of many microbial datasets can make downstream analyses challenging and results difficult to interpret.
- **Choose microbial tests that match soil health parameters of interest.** Rather than focusing only on broad profiling of the microbial community, we believe it will be most productive to develop specific microbial indices for specific soil health outcomes. If the goal is to limit nitrate losses from soil, measuring the abundances of taxa (or genes) known to be key to nitrification and denitrification will be more useful than trying to characterize the overall diversity of the bacterial community. Simply conducting more tests in hopes of finding a hit will not provide more actionable information and will add cost. In the medical field, for example, tests are conducted based on expert assessment of a likely problem; we recommend this same approach when using microbial measurements of soil health.
- **Show how microbial measures can yield actionable information.** Many soil microbial measurements lack clear, actionable interpretation. For instance, the USDA's soil health measurement recommendations (Stott, 2019) acknowledge that microbial community data have no normative interpretation and this is needed for these data to be useful as a soil health metric. The USDA guidelines also acknowledge that their recommended non-genomic measures of microbial activity, including short-term C mineralization rates and enzymatic activity, have uncertainty about interpretation. By contrast, data on soil pH can yield very specific guidance as to whether liming a field is required and how much should lime be applied to achieve optimal crop growth. Likewise, if there are robust microbial indicators of soil phosphorus bioavailability or soil oxygen levels, such microbial information could be directly used to guide decision making processes. For microbial soil health measures to be broadly useful, they need to have clear interpretability.
- **Provide guidance for how to interpret microbial data in specific contexts.** Because of strong geographic differences in soil properties and microbial communities, it is critical to interpret results of microbial analyses in a context-specific manner. It is of limited utility to compare microbial biomass in one field against the biomass levels reported in other fields across a broad area because microbial biomass can be influenced by many factors that may not be directly relevant to soil health. Analogously, the weight of a specific person compared to the U.S. average should not, by itself, be used to determine if that individual is healthy or not. However, changes in the weight of a given adult could indicate health problems, or at least suggest that more detailed assessments of health status may be required. Microbial-based metrics should not be used to indicate whether soil health is 'good' or 'bad' using arbitrarily defined cut-off values. However, comparing how samples from particular locations *change* is likely to provide more insight as it provides the necessary site-specific context required for informed interpretation.
- **There is no 'ideal' soil microbial community.** Just as healthy humans can have highly variable gut communities (Falony et al., 2016) and even highly productive, undisturbed ecosystems can have very distinct plant communities (that are not always high in diversity), we should not expect healthy soils to have a single 'optimal' community type - or that more microbial diversity is always better. Comparing a given soil to an idealized 'optimal' soil microbial community will never be useful as such an 'optimal' soil microbial community simply does not exist. Rather, we can use microbial taxa, or their functional attributes, as metrics of particular soil characteristics to track how soil conditions change across time, space, or in response to shifts in management practices.
- **Use microbial measurements when pre-existing methods are insufficient.** There are microbial taxa that are consistent bio-

indicators of soil temperature (Oliverio et al., 2017) and soil pH (Delgado-Baquerizo et al., 2018). However, both pH and temperature are reasonably cheap and fast to measure so using microbial data to infer these soil characteristics would be slower, more expensive, and provide less interpretable information. Using microbial analyses to assess pH or temperature would be akin to using microbial analyses of an individual's feces to infer antibiotic usage, instead of just asking someone if they had recently taken antibiotics. Efforts should focus instead on identifying microbial bio-indicators of soil characteristics that are important, but can often be difficult (or expensive) to measure directly. These may include: O_2 levels, N/P availability, amounts of labile C, gross N/P mineralization rates, potential 'hot spots' of biogeochemical processes (e.g. denitrification, nitrification, methanogenesis), and concentrations of certain pollutants. While these characteristics could be measured using other means - these measurements are often logistically difficult or cost-prohibitive.

- **Soils are spatially and temporally heterogeneous.** We know that many soil conditions can vary dramatically across time and space. For example, N mineralization, nitrification, and denitrification rates can vary by several orders of magnitude across a single 0.5 ha field (Robertson et al., 1988). Likewise, soil emissions of N_2O measured at a single location can vary week to week by multiple orders of magnitude (Kaiser et al., 1998). This variation can be daunting, but it can be handled with an appropriate sampling design. Thus, microbial metrics are likely more useful for identifying potential 'hotspots' (Kuzyakov and Blagodatskaya, 2015) of particular microbial processes, rather than for quantifying specific rates of a given process at a given point in time. To phrase this another way, microbes are unlikely to tell us when methane emissions are highest, but these data could be used to infer whether one field likely has higher potential for methane production or where in a given field conditions are likely to be conducive to methane production.

The more spatial or temporal variation in a given microbial parameter, the more samples will need to be analyzed to adequately capture that variation and make robust decisions based on the analytical results. We note that this problem of spatiotemporal variation is not unique to microbial metrics of soil health. Soil attributes are rarely homogeneous and even subtle differences in soil conditions can have direct consequences for soil management efforts.

- **Start by doing, but don't overpromise.** Despite the limitations and potential utility of microbial data, we recommend that more efforts, especially those outside of the 'basic' research domain, be taken to measure microbial parameters and build the knowledge base needed to make them more practical. Such efforts could include the collection of comprehensive datasets spanning a wide range of soil and ecosystem types to directly compare microbial metrics against more traditional metrics, targeted assessments of how particular microbial bio-indicators respond (or do not respond) to changes in management practices, the implementation of longer-term studies to quantify the relationships between microbial metrics and plant health or plant productivity, and the cross-validation of methods to assess whether microbial analyses conducted in different labs yield consistent results. We believe that such activities and other "learning-by-doing" models are necessary to take these measurements outside of research labs and into real-world settings. However, it is essential that these efforts not over-promise on the usefulness of these data until it is well established how and when the measures can effectively be interpreted for action.

5. Summary

The development and validation of new microbial indices of soil health is clearly needed. We argue that some microbial metrics of soil health are currently poorly validated and lack interpretability, but the increasing affordability and availability of microbial data offers great potential to improve this interpretability and make these measures more useful. We also propose that there is under-recognized utility in using microbes as bio-indicators for soil attributes that we already know are important components of soil health, but are difficult to measure directly. To fully leverage this approach, we need cross-site studies of well-characterized soils so we can begin to determine what community attributes consistently provide relevant indices of soil health across time and space (and to determine when and where those indices may be less appropriate). We note that such validation efforts are already underway (Norris et al., 2020), but additional efforts to screen other potential microbial metrics across a range of sites and conditions are clearly needed. We believe that there is a strong opportunity for companies, governments, non-profits, farmers, and universities to collaborate to produce these data. Such efforts will help guide the application and interpretation of new and emerging metrics, while helping to reduce the time and money potential users may spend on microbial-based assessments that are of limited utility.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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