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Soil microbiome characterization and its future directions with biosensing

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Abstract

Soil microbiome characterization is typically achieved with next-generation sequencing (NGS) techniques. Meta-barcoding is very common, and meta-omics is growing in popularity. These techniques have been instrumental in microbiology, but they have limitations. They require extensive time, funding, expertise, and computing power to be effective. Moreover, these techniques are restricted to controlled laboratory conditions; they are not applicable in field settings, nor can they rapidly generate data. This hinders using NGS as an environmental monitoring tool or an *in-situ* checking device. Biosensing technology can be applied to soil microbiome characterization to overcome these limitations and to complement NGS techniques. Biosensing has been used in biomedical applications for decades, and many successful commercial products are on the market. Given its previous success, biosensing has much to offer soil microbiome characterization. There is a great variety of biosensors and biosensing techniques, and a few in particular are better suited for soil field studies. Aptamers are more stable than enzymes or antibodies and are more ready for field-use biosensors. Given that any microbiome is complex, a multiplex sensor will be needed, and with large, complicated datasets, machine learning might benefit these analyses. If the signals from the biosensors are optical, a smartphone can be used as a portable optical reader and potential data-analyzing device. Biosensing is a rich field that couples engineering and biology, and applying its toolset to help advance soil microbiome characterization would be a boon to microbiology more broadly.

Keywords Soil health, Soil microbiome, Biosensor, Aptamer, Machine learning

Introduction

Soil is the foundation of all terrestrial environments; it can harbor billions of microbes per gram [1] and plays critical roles in nutrient availability, biogeochemical cycling, and bioremediation processes. Despite its importance, the soil is perhaps the least understood part of the environment [2]. Characterizing its physical and chemical properties has been essential for agriculture for thousands of years. However, soil's biological component has generally been overlooked until the past few centuries, and even then, scientists were usually limited to studying macrofauna like earthworms and small insects. The invention of optical microscopes powerful enough to resolve microorganisms enabled the visual study of microbes [3]. Only recently, with advances in sequencing and "meta-omics" technologies, have scientists been able to investigate soil microbes and their communities comprehensively. Today, there are many ways to determine various physical and chemical properties of soil and several metrics to evaluate soil quality as a whole. However, many soil quality metrics do not consider microbial community factors [4] despite their noted importance [4, 5]. The omission of community factors in these metrics is partly due to the difficulties of biological characterization. Determining what microorganisms are present is

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possible through next-generation sequencing (NGS), and though these methods have advantages over traditional culturing methods, NGS is not without its drawbacks. Moreover, knowing the microbes' functions is often essential, requiring different data with separate analysis methods. It can be challenging to interpret the large amounts of data generated by NGS, particularly when gaps remain in the databases used for analysis. Because of these challenges, many papers have called for developing novel techniques to complement NGS methods [6–11], and biosensing may offer an excellent suite of tools to do just that.

Biosensing has been defined as "a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles, or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" by the International Union of Pure and Applied Chemistry (IUPAC) [12]. In essence, biosensing detects a target compound or condition using some biological component. Such devices are often used in biomedical applications [13–18] and ecotoxicology [19–21]. Many biosensor studies use entire organisms to sense the target compound or condition [16, 19, 22–28], but other studies instead use only specific molecules without the surrounding cellular structure [11, 14, 15, 29, 30]. These molecules, or bioreceptors, are essential components in biosensing devices and are often the focal points of research.

Numerous research works and commercialization efforts have been made in the past couple of decades for biosensors, most notably toward reducing the device size, device cost, assay time, operation cost, and operation complexity while maintaining specificity and sensitivity comparable to more standard laboratory instruments. Such biosensing methods and biosensor devices may identify microbial species and their makeup in the soil, significantly reducing the cost and time associated with NGS methods. However, biosensors are inherently limited in specificity compared to NGS in identifying species. Substantial cross-binding can occur with antibody- or aptamer-based bacterial biosensing. In addition, biosensing may not be optimal for identifying a large number of species simultaneously. For example, detecting 100 microbial species could require 100 different bioreceptors pre-loaded on a biosensing platform, significantly augmenting the assay complexity and limiting its usefulness compared to current NGS technology.

This correspondence paper aims to summarize current methods and techniques for soil microbiome characterization and then to summarize and provide insights and recommendations for future opportunities with biosensing technologies. See Fig. 1 and Fig. 2 for visual overviews of these two technologies. These steps are described in detail in the following sections.

Soil microbiome characterization

In its entirety, the microbiome encompasses all the parameters that make up a community of microbes. This includes physical, chemical, and biological characteristics, but we will focus on the biological characteristics, like the microbial community dynamics and structure, for this correspondence paper.

Intrinsically, soil is a difficult medium to work with because of its physical properties. Soil is an opaque mixture, which significantly limits the use of optical microscopy techniques. Soil is also very spatially heterogeneous. Depth significantly affects parameters like moisture and the carbon–nitrogen ratio [31]. Also, roots create a unique environment rich in metabolites and signaling molecules; this environment is called the rhizosphere and is a critical region of plant–microbe interaction and symbiosis [23, 32–34]. There is spatial heterogeneity even at the microscale, such as an uneven distribution of nutrients, organisms, and microclimate conditions across millimeters or shorter distances [35]. This heterogeneity complicates soil analyses because average properties could be misleading for understanding microscale interactions [8]; however, measuring microscale properties is technically challenging. Lastly, soil properties can be time-dependent. Not only can soil parameters change across seasons, but the day-night fluctuations can be significant, along with the short-term disturbance or perturbation [36–38]. In addition, many of its components influence each other; no soil properties are genuinely independent [39]. There is also no "standard" or baseline for soil properties, complicating soil comparison studies [7]. Nonetheless, scientists can make loose comparisons within a soil type, which is characterized by physical parameters and geological history. Finally, most soil testing is destructive, so measuring any parameter over time for the same soil sample is generally impossible. This limits data to be snap-shots instead of continuous [40].

The obstacles listed above are mostly matrix effects, so the first option when characterizing microbes from the soil is to try to remove those effects as much as possible. Many studies have done this when working with soil or other complex matrices like feces [19, 24, 41–45]. Liquid extraction of soil is a common method to remove matrix effects. The sample is usually sieved through a 2-mm sieve to remove larger dirt particles and small rocks or leaves [46–49]. Then, the soil is incubated in a liquid medium, and the supernatant is extracted for further analysis. However, liquid extraction does not extract all of the microbes, and moreover, the precise method of transport to the lab and any pre-treatments can significantly

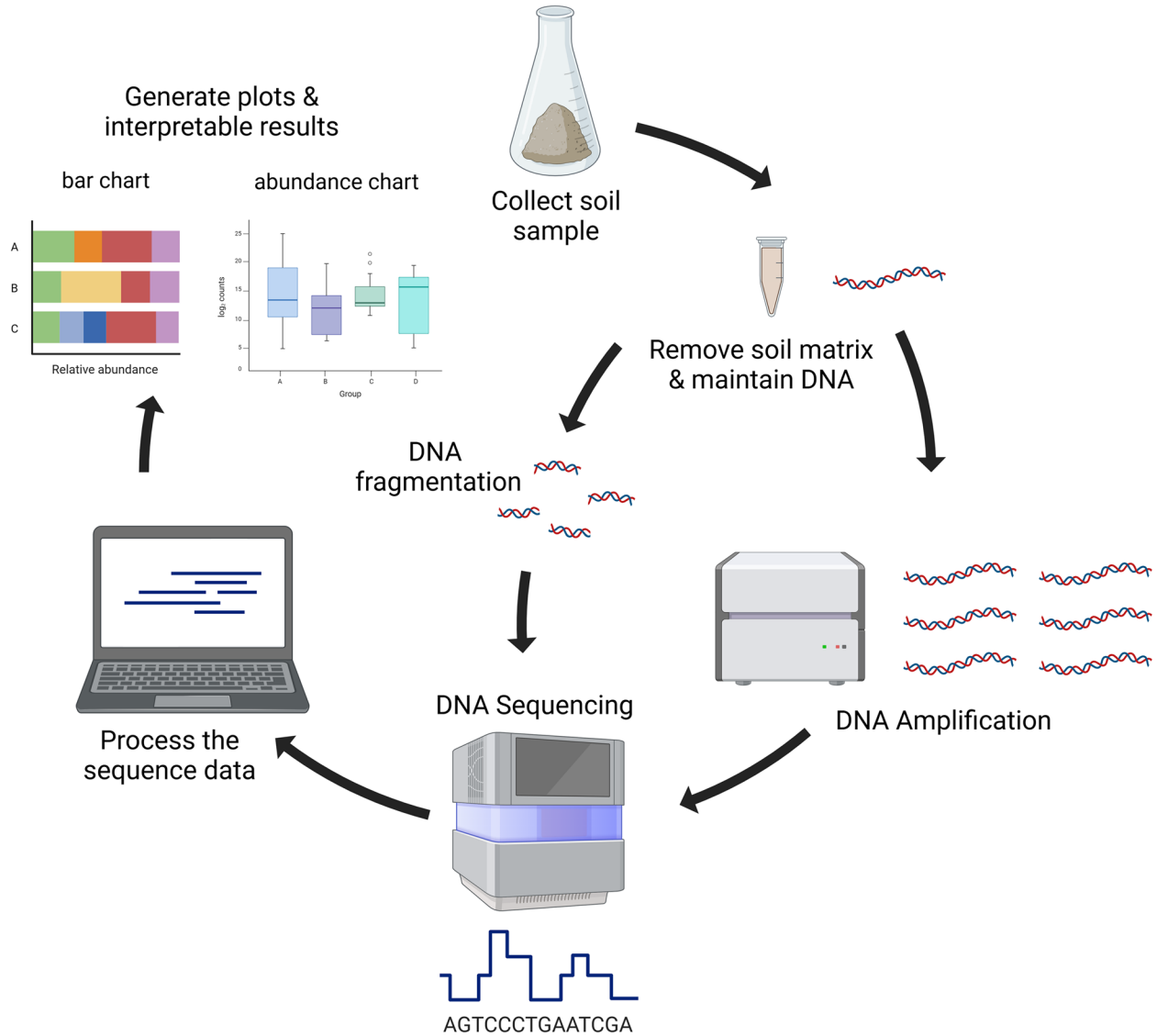

Fig. 1 Overview of next-generation sequencing (NGS) process. Amplicon-based methods include the amplification step, while shotgun methods instead include DNA fragmentation. Figure created with BioRender.com

affect the microbes detected by NGS [50, 51]. The sample could also be digested via chemical and mechanical means to create a homogeneous solution; there are many commercial kits available for this purpose that have been widely used in research for many years [4–6, 24, 25, 30, 33, 34, 36, 37, 44, 46, 47, 50, 52–71]. There are many different brands and products available, including FastDNA SPIN kits from MP Biomedicals, EZNA DNA kits from Omega Bio-tek, DNeasy Power kits from Qiagen, NucleoSpin kits from Macherey–Nagel, ZymoBIOMICS DNA kits from Zymbo Research, and INTEST.pro kits from BIOMES. Specific products are usually tailored to detect particular types of organisms from particular matrices or environments. Even with these kits, soil remains a difficult medium to work with. Until there are standardized procedures for soil analysis, various kits should be considered for different soil samples because each has its biases, pros, and cons [50, 72–74].

After dealing with matrix effects and generating a sample for further analysis, the microbes can begin to be characterized, and culturing in growth media is the traditional method for this. Different colony morphologies or absorbance characteristics can be detected for characterization. In addition, culturing allows for functional or biofilm assays in the selected presence or absence of various metabolites or conditions [4, 55, 64, 72, 75].

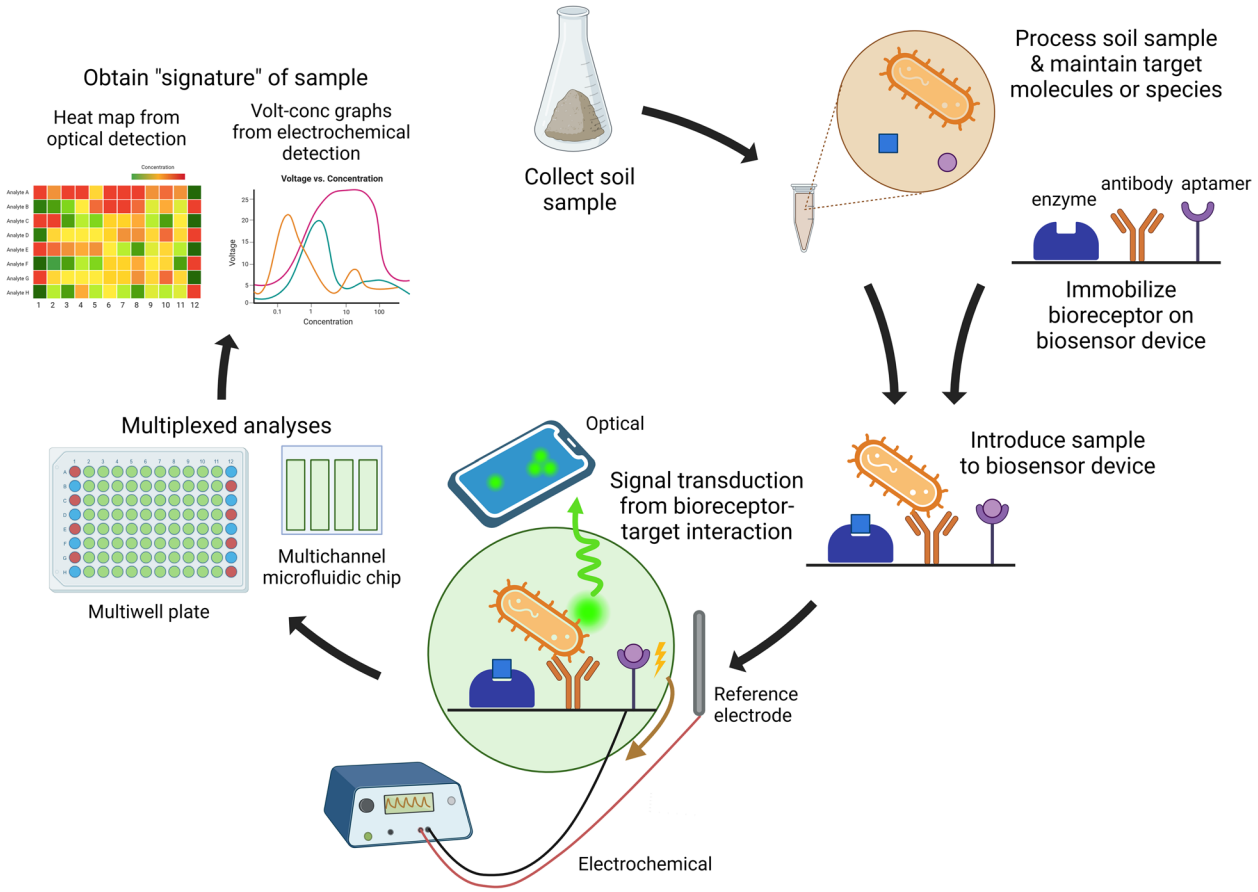

Fig. 2 Overview of biosensing process as potentially applied to soil microbiome characterization. Figure created with BioRender.com

However, many, if not most, microbes resist culturing in lab settings [76], even though many different growth media and methods have been created and used over the years. For example, the media could be missing a critical nutrient that the microbe cannot make on its own, or the growth of one species could depend on the presence of another [25, 77, 78]. Different microbes also have specific temperature, pH, moisture, oxygen, and salt concentration requirements that can be unknown before culture [76].

Some recent advances in 'culturomics' for soil microbes include modifying the particle size fractions and culturing in microwell plates. Such advances mimic natural spatial conditions in which organisms are separated and attached to various micro-surfaces like soil particles [76]. There are also novel in situ devices that allow natural media to permeate a membrane or filter where the microbes have been inoculated [79, 80]. These and other advances in culture techniques have enabled more species to be grown in lab settings, and indeed, these techniques will remain a central part of microbiology. Even so, sequencing technologies can overcome many of these challenges and are vital to microbiology.

Brief overview of sequencing technologies

Today, there are several different ways to sequence DNA. But, at the beginning of sequencing efforts, generating even one short DNA read was very laborious. Then, "next-generation sequencing" (NGS) arrived, which has become pivotal to microbiome research. It enabled the mass parallelization of sequencing reactions, even though read lengths were limited to several hundred base pairs. With these technologies, many strands of DNA could be read at once, which greatly shortened the time required to read lots of DNA. Then there were the "third generation sequencing" technologies which would enable longer read lengths (hundreds of thousands of base pairs) [81, 82]. Longer read lengths are especially useful for sequencing entire genomes; with longer reads there are fewer sequences to stitch together to create the whole genome. Together, these sequencing technologies exposed scientists to the wealth of previously unculturable microbes since a raw environmental sample could be

sequenced without culturing and without years of work to generate one sequence. While many NGS technologies are currently in use, a few representative technologies have been selected for a comparison of their key characteristics along with strengths and weaknesses in Table 1.

As sequencing technologies grew and matured, more organisms were sequenced, from bacteria and fungi to the less studied archaea and viruses. These genes and genomes were collected and deposited into massive databases, allowing molecular evolutionary testing and visualization. These databases also enabled sequence reads of unknown species to be matched and labeled; culturing was no longer a prerequisite to determine what species were present in a sample [83, 84]. In addition, with functional data from cultures, scientists could identify genes responsible for proteins that make up enzymes in metabolic pathways. Thus, just from the organisms' genes, microbiologists could make guesses about an organism's functional capabilities, its role in a community, and its responses to various types of stress [85]. The importance of these advances should not be understated; they make the field of microbiology what it is today.

"-Omics"

NGS is a cornerstone of microbiology, and there are several ways to use NGS. Perhaps the simplest application of NGS is to look at one gene from one organism. For example, there are several tests for oncogenes in humans [86, 87]. Though this is much more difficult for microorganisms, single-cell sequencing technologies do exist [88]. However, this approach only lets scientists learn about one part of one individual; it does not provide a full picture of one organism, nor is this approach suitable for community analysis.

To understand the individual more fully, scientists can look at all the genes in one individual, called genomics. Such an approach could be useful if one species is determined to be a keystone or indicator species for a microbiome. The genome can be very insightful for determining the potential function of an organism. However, the presence of genes alone does not guarantee that they function at all or as expected [89]. Analyzing all of the messenger RNA (mRNA) is a better way to determine gene activity, while characterizing the proteins or metabolites produced by an organism is a more concrete way to assess its functional ability [90]. These fields of study are called transcriptomics, proteomics, and metabolomics, respectively, and together with genomics, represent the core of the broader -omics field.

Ideally, each species study would include all of these -omics, but that is not feasible. There are general time and money limitations, but besides those, each -omics

Table 1 Selected current NGS technologies with their strengths and weaknesses. Adapted from [81]

Sequencing technology	Time of use, hours	Cost of machine, USD	Approximate machine size	Strengths	Weaknesses
Illumina: Sequence by synthesis[a] Fluorescent detection	4–56	19,900–335,000	Benchtop or standalone	High accuracy Good depth of coverage	Requires library preparation & assembly Long run times
ThermoFisher: Sequence by synthesis Ion Torrent detection	4.4–31	On request	Benchtop or standalone	High accuracy Good depth of coverage	Requires library preparation & assembly Long run times
Pacific Biosciences: Single molecule sequencing Fluorescent detection	< 30	525,000	Standalone	Long read length No amplification necessary Fast run times	Expensive Large footprint Higher error rate[b]
Oxford Nanopore: Single molecule sequencing Conductivity detection	< 72	1,400–530,000	Portable or benchtop	Long read length No amplification necessary Fast run times Portable instruments are available Real-time analysis is possible	Higher error rate[b] Higher signal-to-noise ratio Errors are systematic[c]
Sequencing technologies in general	*< 72*	*1,400–530,000*	*Portable, benchtop, or standalone*	*Accuracy* *Mature technology*	*Time* *Cost* *Portability* *Requires lab conditions & reagents*

[a] Sequence by synthesizing new DNA molecules

[b] These technologies have historically had higher error rates, but they continue to improve

[c] Systematic errors cannot be easily mitigated with increased coverage and thus are more challenging to resolve in practice

investigates different molecule types that work on different time scales and require different extraction and analysis methods [74]. In particular, proteomics and metabolomics commonly involve mass spectrometry [15, 37, 40, 77, 85, 91, 92], a highly technical and precise method requiring many non-standardized pre-processing steps. In addition, it is also difficult to compare mass spectrometry results across laboratories because their results depend on the previously tested libraries of molecules for that specific mass spectrometry machine and sample pre-processing method [93].

Metabarcoding

Since -omics is focused on the individual, a more suitable application of NGS for microbiome research is metabarcoding, which looks at the breadth of the community and how its members are related phylogenetically. Metabarcoding uses specific sequences to identify what species are present and how they are related. This is typically done using polymerase chain reaction (PCR) to amplify an identifier gene like the 16S ribosomal RNA (16S rRNA) or internal transcribed spacer (ITS) gene throughout the sample (Fig. 1). Thus, the other genes are ignored, and only taxonomic information is extracted for analysis. However, care must be taken when selecting not only the DNA extraction method [44] but also the PCR primers, which can affect what taxa are detected and in what relative abundances [58, 74]. Since primers can internally differentiate some groups better than others, they inherently have more or less affinity for specific species or taxa. For example, for looking at fungi, amplifying part or all of the ITS gene is more useful than the 16S rRNA gene, while for bacteria, the 16S rRNA gene works well, though there are several variable regions to choose from [44].

After PCR, the amplified DNA is sequenced via one of several different sequencing technologies, each with its strengths and weaknesses (Table 1). Examining these methods is outside the scope of this review (the main emphasis is biosensing methods), but some excellent reviews have been completed [81, 94]. The sequence data must be processed via bioinformatics pathways. Again, there are many different ways to do this, and analyzing them all is outside the scope of this review. Finally, a collection of cleaned and annotated reads is returned to the user, and the scientist can see which taxa are present and in what relative abundances. However, these taxa are often not identified at the species level [95, 96].

After metabarcoding and omics data are generated, they must be analyzed. The chosen bioinformatics pathway will affect the analysis. In the past, these pathways were not standardized, though there are now substantial efforts to enhance standardization across labs and organizations [97]. Still, one drawback of metabarcoding and omics remains: they often rely on data collected from culture. Current data analysis tools often depend on previously studied organisms [7]; however, modern bioinformatics tools enable less reliance on culture data, and in some cases, genomes can be constructed de novo without prior knowledge of the organisms [98]. Still, culturing remains a critical technique, and advances must be made in that field to address the more intractable taxa.

Meta-Omics

For soil microbiome characterization, metabarcoding only gives scientists insight on what taxa or species are present; it does not give direct insight on what they are doing or how. To find out, scientists will study the -omics of many microbes, which introduces "meta-omics," in which community-wide data is taken and studied in aggregate. For example, metatranscriptomics looks at the mRNA of a community, metaproteomics looks at the proteins in a community, metabolomics looks at the metabolites in a community, and metagenomics looks at all the genomes in a community. Excellent reviews of these meta-omics have been published [99–103], and in this section, metagenomics will be used as an example of meta-omics use more broadly and its limitations.

Metagenomics is a zoomed-out view of genes and their potential functions in a microbiome. Microbiologists can look at the distribution of classes of genes throughout time or by taxa, or they can compare one metagenome from one community to another. However, meta-omics inherits many limitations of -omics, primarily related to feasibility. The sequence depth must be sufficiently deep to detect all of the genes of an entire community; a lot of DNA must be sequenced to get adequate coverage. Gathering this much DNA requires more precise preprocessing steps of the sample, and even so, detecting genes and genomes at low abundances is still challenging [104]. Meta-omics, like -omics, involves lots of data that require significant computing power to analyze, and this, rather than the ability to sequence, is now regarded as the limiting step for metagenomics studies [72], and several machine learning models have been developed for these analyses [60, 71, 85, 105–107]. In addition, meta-omics methods are not standardized, so comparing meta-omics data across labs is challenging because the pipelines are different, both for data generation and processing [72]. Lastly, as with -omics, even though culturing is not required to gather data, the analysis generally relies on previous culture data for labeling and interpretation; however, some de novo bioinformatics tools have been developed and tested to circumvent using any culture data [83, 84].

There are also more general obstacles to environmental sequence or molecule studies. Firstly, most of these

technologies require the samples to be taken to a lab and processed in a clean environment; these methods are neither fast nor suitable for point-of-care or in-situ monitoring programs. Also, meta-omics methods are typically too expensive for every lab to have access to them. Moreover, since DNA is relatively stable and can be bound up in inorganic complexes in the soil, DNA from dead organisms can be found and mistakenly interpreted as if it were from living organisms [108]. However, treatments like propidium monoazide can remove relic DNA from a sample [109, 110]. Finally, standard NGS only provides relative abundance data; to determine absolute abundance, techniques like quantitative PCR (qPCR), digital PCR, or spike-ins with known microbial concentration are required [111].

Biosensing technology

Researchers have long understood the limitations of NGS for microbiome studies, and there have been calls for developing novel techniques to help overcome these limitations [6–11]. We argue that biosensing is a vital avenue to consider in this endeavor. While biosensing has had many definitions over the years, a biosensor can be defined as a device or method that uses a biologically derived recognition element, or bioreceptor, to help detect or quantify a target molecule or condition [12]. Biosensors are inherently interdisciplinary devices incorporating chemistry, physics, engineering, biology, and sometimes nanotechnology. This field, like NGS, can be very useful for investigating the microbial world; however, few examples of biosensors applied to the soil microbiome have been published at the time of this manuscript's submission. Previously, biosensors have primarily been applied to the biomedical and ecotoxicology fields. However, biosensing has much to offer soil microbiology. Though many reviews are already available for detailed examinations of different biosensor examples, here we will discuss broad categories of biosensor types with potential uses in soil microbiology. Table 2 summarizes standard biosensors and novel technologies most suited for soil microbiome characterization. The following section will examine more direct applications to the soil microbiome.

Table 2 Biosensing applied to the soil microbiome

Bioreceptor & signal type	Example & reference	Time of assay, hours	Approximate size	Strengths	Weaknesses
Enzyme Electrical	Glucose monitors [14]	< 1	Handheld	Very substrate specific Rapid Easy to use Mature technology	Limited stability Production is costly
Antibody Optical	LFIA, ELISA [117, 118]	< 2	Handheld	Very specific Can be rapid Mature technology Easy visual identification	Moderate stability Requires immune response to produce bioreceptor
Antibody Optical	Optical array [9]	< 1	Handheld	Very specific Multiplex array	Moderate stability Requires immune response to produce bioreceptors Complicated analysis of multiplexed visual data
Aptamer Optical	[131]	< 1	Handheld	Can be highly specific High stability	Need to generate aptamer libraries to increase specificity
Aptamer Electrical	Electrochemical array[a]	Not reported[a]	Handheld	Can be highly specific Multiplex array	Need to generate aptamer libraries to increase specificity Novel technology[a]
Genetic circuit Optical	[120, 123, 132]	< 2	Handheld	Can be highly specific Can incorporate logic into response Commercially available bioreceptors	Takes significant laboratory skill and time Could affect community under study
Biosensing in general		*< 2*	*Handheld*	*Rapid* *Low-cost* *Handheld and portable* *Already used for point-of-care tests*	*Newer technologies* *Often have trade-off between stability and specificity*

[a] Several papers have noted that aptamer-based electrochemical biosensors have great potential [6, 44, 68], but no papers were found that have already used this technology

Biosensing, like sequencing, is not a new technology. Though the classification of biosensors can be a bit nebulous, they can be categorized by the biological recognition element, or bioreceptor, and the signal they produce. The bioreceptor chosen significantly impacts the biosensor function. Enzymes are often used to detect metabolites (e.g., glucose monitor), and antibodies were used to detect target cells or viruses (e.g., rapid COVID-19 test). These two classes of molecules can be very sensitive and selective in their binding. However, purifying enzymes and generating antibodies are costly and time-intensive processes. Moreover, enzymes and antibodies are unstable and must be kept in controlled conditions. Otherwise, they denature and become inactive. A newer group of bioreceptor molecules are aptamers (Fig. 2). These nucleic acid polymers can specifically bind to targets, just like antibodies. These molecules are more stable than antibodies and more inexpensive to produce. Moreover, they do not require animals or an immune response in their production. However, it is difficult for a single aptamer to bind a unique microbial species consistently, so recent work has generated aptamer libraries that collectively bind a species with high specificity and sensitivity [6, 29, 44, 68]. There are other types of bioreceptors that are much bigger and more complex. Some studies use entire organisms, and these organisms are often "model" organisms, like *E. coli* cells or even mice [24, 112–114], that have been well characterized over many years of research. Some studies will genetically modify these reporter organisms to produce specific responses to the target molecules or conditions [114].

While the choice of bioreceptor is a critical design parameter, biosensing is also classified based on the type of signal they produce, which can be broadly divided into electrochemical or optical (Fig. 2). Electrochemical biosensors have been used extensively in the past couple of decades, and these sensors report biological activity via an electric signal. The classic biosensor that has been the field's flagship is the glucose monitor [14]. This electrochemical device measures the electron flow caused by enzymes interacting with glucose to determine how much glucose is present quantitatively. It is called an amperometric biosensor because it detects current. Another example is the potentiometric biosensor that measures voltage instead of current; it measures the difference in potential, or voltage, between two or more electrodes. One benefit of potentiometric sensing is that they generally do not consume the analytes; instead, these can be reused for multiple rounds of sensing. These are often used for the new class of wearable biosensors that can continuously monitor subsurface conditions [115, 116]. Voltammetric sensors report both current and voltage changes. Finally, electrical impedance spectroscopy (EIS) reports the impedance signal from an electrode, which is a complex number version of electrical resistance resulting from alternating current (AC). All of these have been applied to detect target analytes, and their success largely depends on the construction of the electrodes themselves. Generating the correct chemistry and understanding the electronics of the primary and secondary interactions is crucial to these biosensors.

The other large class of signals that biosensors produce is optical. Optical methods are varied, but one classic example is the lateral flow immunoassay (LFIA) [117]. For LFIA, a liquid sample is placed on a paper-based test strip, and the sample flows through the strip along with pre-deposited particles (gold nanoparticles or fluorescent polymeric particles) conjugated with bioreceptors (usually antibodies). The target molecule is sandwiched between the surface-bound bioreceptors and the bioreceptor particles at the test line, like in the enzyme-linked immunosorbent assay (ELISA). The presence of bioreceptor particles (and subsequently the target presence) can be confirmed visually via pink coloration (from gold nanoparticles) or fluorescence (from fluorescent polymeric particles). A commercial example of this is the urine-based pregnancy test. Though often designed to report a binary result, if the test and control bands are examined for fluorescent intensity, this method can also be used to generate quantitative data.

ELISA is a predecessor to LFIA, typically conducted in a lab setting, with better sensitivity and specificity. ELISA uses the sandwich method, with an antibody as a bioreceptor and an enzyme–substrate pair as a signaling molecule [118]. The latter can sometimes be replaced with a fluorescent dye conjugated with antibody-to-antibody. It is typically conducted on a microwell plate. While binding and washing are performed automatically via the capillary action through paper pores in LFIA, ELISA utilizes pipetting for adding and rinsing reagents. Automated pipettor systems are often used to automate and multiplex ELISA. Detection is conducted optically, typically utilizing a microplate reader.

Other optical biosensors include using whole, live cells that often contain engineered genetic circuits. These genetic circuits couple the genes of natural detection molecules with transgenic modules to produce a fluorescently active molecule like green fluorescence protein (GFP), luciferase, or violacein. Several studies detected quorum-sensing molecules or cell stress responses using these types of bioreceptors [23–25, 119–121]. These genetic circuits can even contain genetic logic such that the response is only generated when multiple conditions are met or a response is linearly dependent on a target concentration [122]. Genetic circuits have been made to detect and respond to various signals, and recent studies

have shown the ability to produce other classes of molecules at target detection [123, 124].

In recent years, nanomaterials have become an important part of biosensing research. Because of their high surface-to-volume ratios, they can accommodate more bioreceptors on a sensor surface or generate stronger electrochemical (or unique optical) signals [125]. Various nanomaterials also allow for customization and functionalization of their structure and surfaces [126]. They can also enter organisms and interact with their internal systems [126]. Quantum dots and carbon dots fluoresce and have been used for detecting targets [10, 127], and nanodiamonds have been used to prevent biofouling of submerged oxygen sensors [128].

Biosensing design considerations for the soil microbiome

Meta-omics technologies are excellent tools for microbiology, but they have limitations that biosensing can help address. Biosensors can be applied to soil microbiome characterization to add another tool to the microbiologist's toolbelt. Microbiome biosensing should strive for these characteristics: multiplexing [6, 18, 129, 130], stability of the biosensor itself in the field or non-ideal laboratory conditions, and ease of use and manufacture. These ideas will be discussed below, followed by more specific potential directions.

To understand the community of a microbiome, knowing and understanding one aspect by itself is not very useful in understanding community dynamics; thus, multiplexed analyses are critical. In other words, multiple targets or conditions must be analyzed collectively for a given sample. Multiplexing is known to be essential and has been implemented in many studies and products over the years (Fig. 2). There are commercially available functional assays that are multiplexed. For example, multi-well microplates determine what kinds of substrates organisms in a sample can digest and process [55]. Often, the signal is a visual intensity originating from each well on these plates. Imaging the entire plate and analyzing the color intensities from each well has been the general practice, typically using a microplate reader. For simplicity or field use, this microplate reader can easily be replaced with a smartphone camera [118]. Functional assays require a culturing step for the organisms to degrade the provided substrates successfully, and as stated previously, culturing can introduce bias. Multiplex terminal restriction fragment length polymorphism is another common method that uses multiplexing to detect a "signature," "barcode," or "fingerprint" of the community, and again the raw data for this method is a visual image [46, 55, 133] (Fig. 2). This method involves many steps that must be completed in a lab setting. One study looked at generating an assay of antibodies to provide a signature of a disease state [9]; this study used a machine learning algorithm to process their image data and determine data trends. Multiplexing has already been successfully applied to microbiome analysis, but these methods are thus far limited to laboratory analyses. They have not been developed for field use, and most have not been designed for rapid analysis. Because the soil microbiome can change quickly over time, detecting characteristics in situ is essential. Biosensors attempting to characterize the soil microbiome should take these existing multiplexing technologies and apply them to field detection platforms that can quickly collect data.

Several factors need to be considered to develop a field-ready biosensor. Firstly, the effects of the soil matrix must be considered. As mentioned previously, common practice is to remove the matrix effects via a liquid extraction method. This step alone could require extensive research and experimentation to determine the optimal way to extract meaningful analytes from the soil while minimizing bias and process effects. However, because liquid extraction is currently standard, that is where any research into that method ought to start. After matrix effects have been overcome, the bioreceptor must tolerate fluctuating temperatures, pH, and humidity/moisture levels. This likely excludes enzymes and antibodies from these sensors, but aptamers, modified electrodes, and genetic circuits in robust microbes could still be useful (Table 2). The bioreceptor should also be specific and sensitive enough to detect the target in complex samples at environmentally relevant concentrations. This requires rigorous testing and careful selection of bioreceptors and overall biosensing methods for the desired target in its natural or minimally modified state. In addition, the signal must be comparably detectable in various field conditions; otherwise, the biosensor can only be used in limited areas or situations. For optical signals, the environmental lighting should not significantly affect the data. Either electrochemical data should be collected instead, or the optical signals should be corrected for ambient lighting variations. Another possibility is to have a small, lighting-controlled space around the sensor to take that image or other optical data. Electrochemical sensing can also have challenges in field conditions. Biofouling is a common problem, as is electrode degradation. Nanodiamonds and carbon nanotubes have been shown to limit biofouling [128, 134]. In addition, the electrodes can be coated with various layers to improve performance [135], and

electrode degradation can be minimized with material selection and manufacturing processes [136].

Potential biosensing applications to soil microbiome characterization

Multiplexing, field deployment, ease of use, and ease of manufacture are simple to discuss but can be tricky to implement. Still, researchers should consider those ideas when developing novel biosensing methods for characterizing soil microbiomes. Specific examples of biosensing applied to the soil microbiome are scarce, but several papers noted potential directions or future projects combining these fields. Examples are discussed below.

There are recognized challenges with optical methods, and electrochemical sensors have some advantages. Amperometric detection can be broadly applied; this method allows for low detection limits with reasonably simple devices, and current is easy to analyze relative to optical images. One issue with this approach is biofouling when the electrodes become impeded by organic matter build-up, though this can be overcome with nanomaterials, chemical layers, and material selection [128, 134–136]. Several studies have commented on the possibility of an aptamer-based electrochemical sensor [44, 68]. Perhaps an amperometric assay could be developed to sense a variety of metabolites all at once on a multi-well plate. These studies could follow the method [9] in which certain bioreceptors were bound to different areas of a larger plate. Thus, after applying the sample, each distinct plate region emits different signals to generate a sample signature. Detecting metabolites in this way would reduce the need for mass spectrometry, which is a technically challenging method to implement.

There are other ways to multiplex, like having different carbon dots with different colors for different targets [127]. Alternatively, studies have been conducted with microscopic beads coated with bioreceptors, and these have been used for multiplexed analysis when the beads have a variety of detection targets [91]. The LFIA method could be expanded to use various aptamers; perhaps there would be a way to create a multiplexed LFIA, e.g., multi-channel paper microfluidic chips [137]. If several channels radiated out from a central sample pad, it could be possible to seed different bioreceptor-coated beads at the beginning of each channel [138]. LFIA and paper microfluidic chips could be particularly insightful when the color intensity of the test band is quantified with an optical reader. Even if the data is purely qualitative, LFIA and paper microfluidic chips can provide results in minutes to enable field assessment of those targets.

A smartphone camera can be used to analyze optical signals rapidly and in the field from the LFIA and paper microfluidic chips; this is a viable method in previous works, particularly when used with a portable microscope (including a smartphone-based fluorescence microscope) [138–142]. The smartphone could be a vital component of a field-deployable biosensor because it is a mini-computer. It can take in high-quality visual data and run data analysis through a custom app. Or, if there is an internet connection, data could be remotely uploaded and processed on the cloud before researchers even get back to the lab. Figure 3 shows an example of analyzing bacterial species from environmental water samples, utilizing a set of peptides extracted from bacterial biofilms, a multi-channel paper microfluidic chip, a smartphone camera as an optical reader and data processing unit, and machine learning to analyze the data [115].

Regardless of the data being collected, machine learning will likely be needed to analyze these larger, multiplexed datasets. Several papers have successfully implemented machine learning, and many compare two or more algorithms to see which is better for their dataset [141, 143–146]. Machine learning has also been used with NGS datasets [83], and many algorithms have been used with both NGS and biosensor data. A comparison of algorithms used is shown in Fig. 4.

The standard practices of removing the matrix and then destroying the sample during analysis are inadequate for studying the soil microbiome in its natural matrix. The field of wearable biosensors could address the issue of destructive sampling. These devices are meant to continuously measure metabolites on or in the human body via electrochemical sensing [115, 116], and this technology could be applied to continuous monitoring of targets in the soil. One paper analyzed gases released from soil to investigate the soil microbiome [40], and there seems to be great promise in gas analysis for microbiome characterization [147] (Fig. 5). Gas molecules and their concentrations can reveal respiration rates and other metabolic processes [147] while maintaining the soil matrix's natural state. This minimizes processing effects while simplifying the biosensor and its method of use. Perhaps the genetic circuit technologies of fluorescent, whole-cell biosensors, which would have little direct application in opaque soil, could be reconfigured to emit alternate signals like gaseous metabolites that could pass through the soil matrix more easily than optical signals.

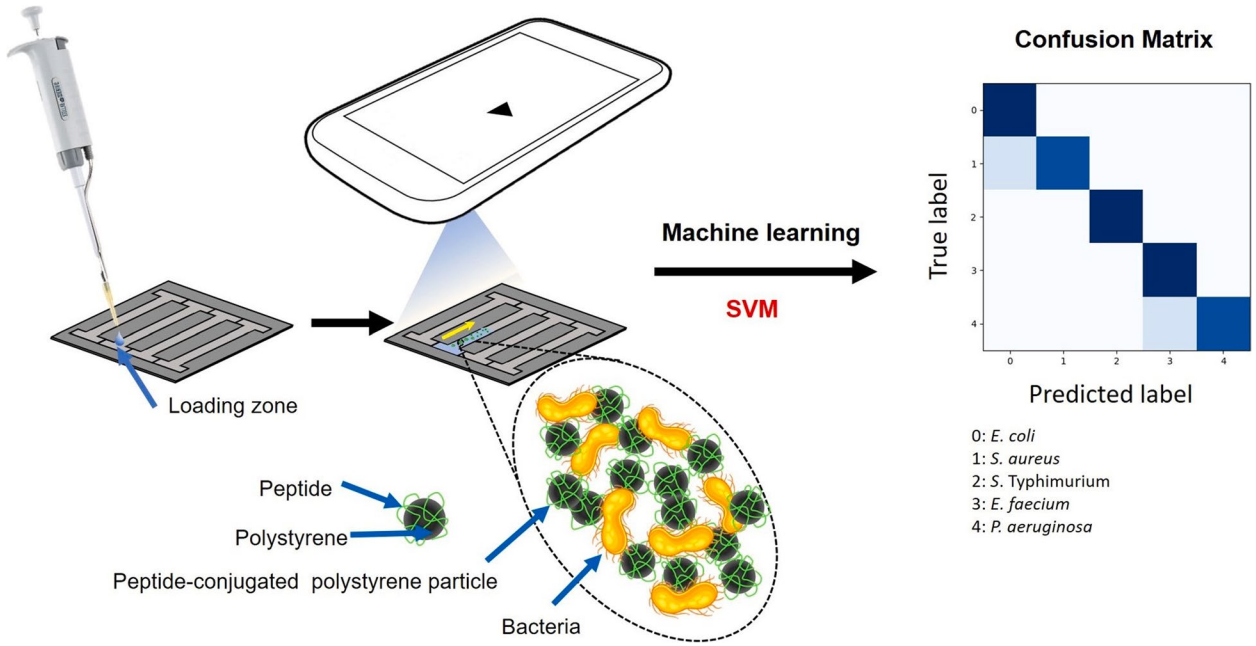

Fig. 3 An example of analyzing bacterial species from environmental water samples. It utilizes a set of peptides extracted from bacterial biofilms, a multi-channel paper microfluidic chip, a smartphone camera as an optical reader and data processing unit, and machine learning to analyze the data. Reprinted with permission from [141].

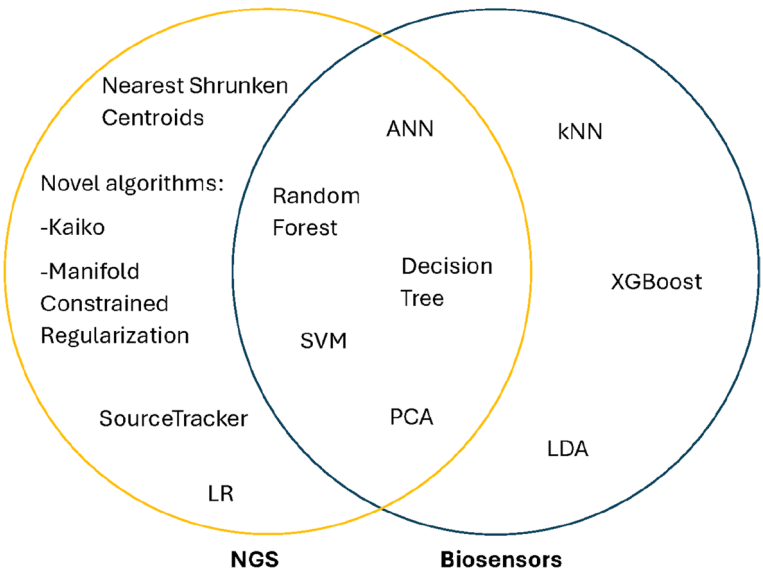

Fig. 4 Venn diagram showing a sample of machine learning algorithms applied to NGS and biosensor data. Most NGS algorithms found in the literature were supervised learning methods except for PCA. There were also several papers that detailed novel algorithms for NGS data. A great variety of algorithms are used with biosensor data, and many of them are also used with NGS data; there is significant overlap. ANN = artificial neural networks; kNN = k-nearest neighbors; SVM = support vector machine; XGBoost = extreme gradient boosting; LR = logistic regression; PCA = principal component analysis; LDA = linear discriminant analysis

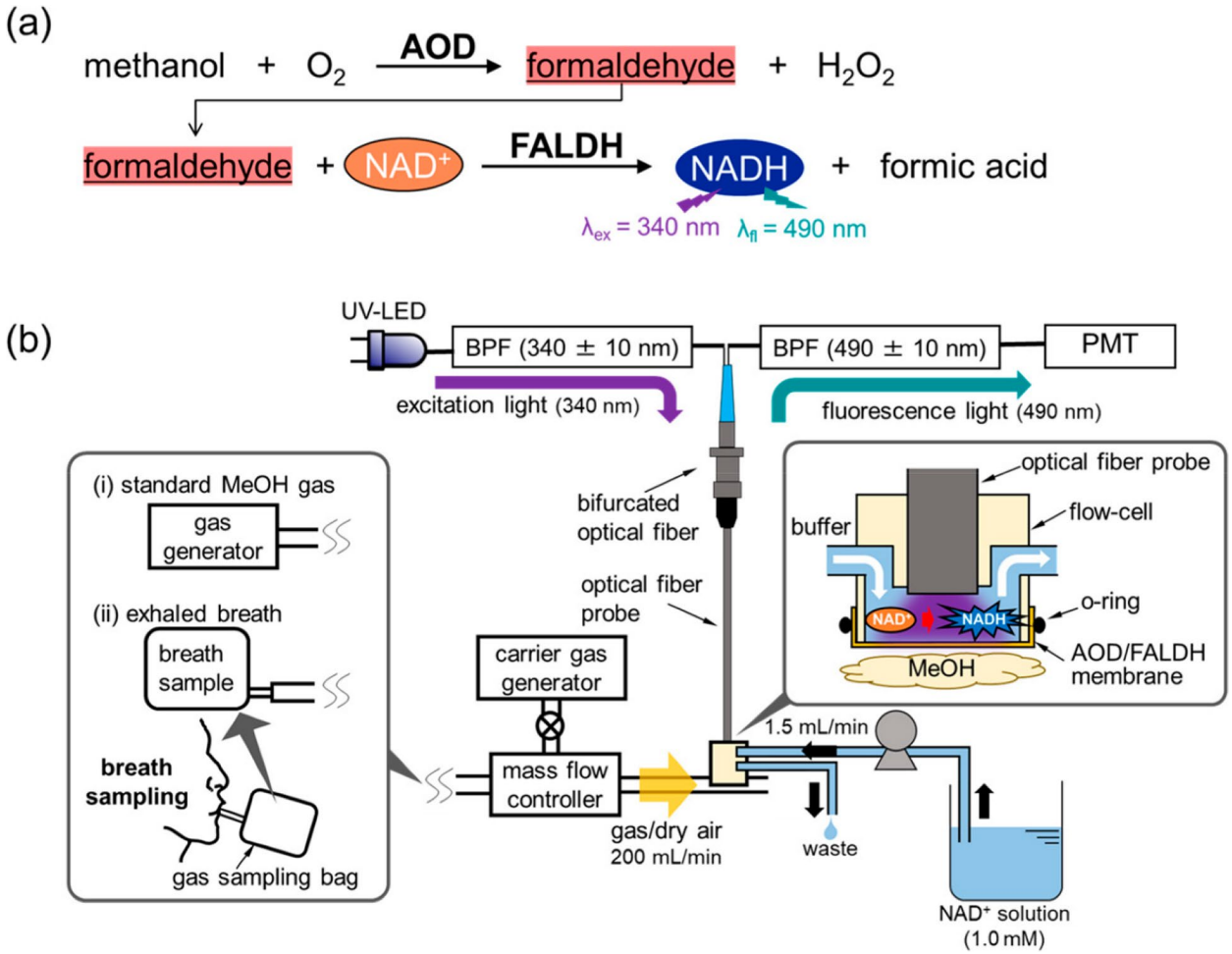

Fig. 5 An example of a gas sensor for assessing the microbiome. While this work was designed to assess the intestinal flora, e.g., gut microbiome, it can be adapted to the soil microbiome. Reprinted from [147] under Creative Commons Attribution License

Our group recently applied biosensors to the soil microbiome. This study used induced natural fluorescence, a smartphone to capture optical intensity, and machine learning to categorize bacteria derived from the soil matrix. See Fig. 6 for a depiction of the process used. This process could be a model for future works applying biosensors to soil microbiome characterization.

Conclusion

In conclusion, the field of biosensing has a lot to offer soil microbiome characterization. NGS and meta-omics methods are central to microbiology, and they can generate data for in-depth analyses of communities and community structures via DNA and RNA analyses. However, these technologies typically require extensive time, money, expertise, and computing power to be effective. Moreover, these techniques are restricted mainly to controlled laboratory conditions; they are not applicable in field settings, nor can they rapidly generate data. Biosensing technology can be applied to soil microbiome characterization to overcome these limitations and to complement NGS and meta-omics techniques more broadly. Biosensing as a field has not been applied to the soil microbiome until recently, and some methods are better suited for this new application. Aptamers are more stable than enzymes or antibodies and are more appropriate for field-use biosensors. Given that any microbiome is a highly complex system, a multiplex sensor will be needed, and with large, complicated datasets, machine learning might benefit the analysis. If the signals from the biosensors are optical, a smartphone can be used

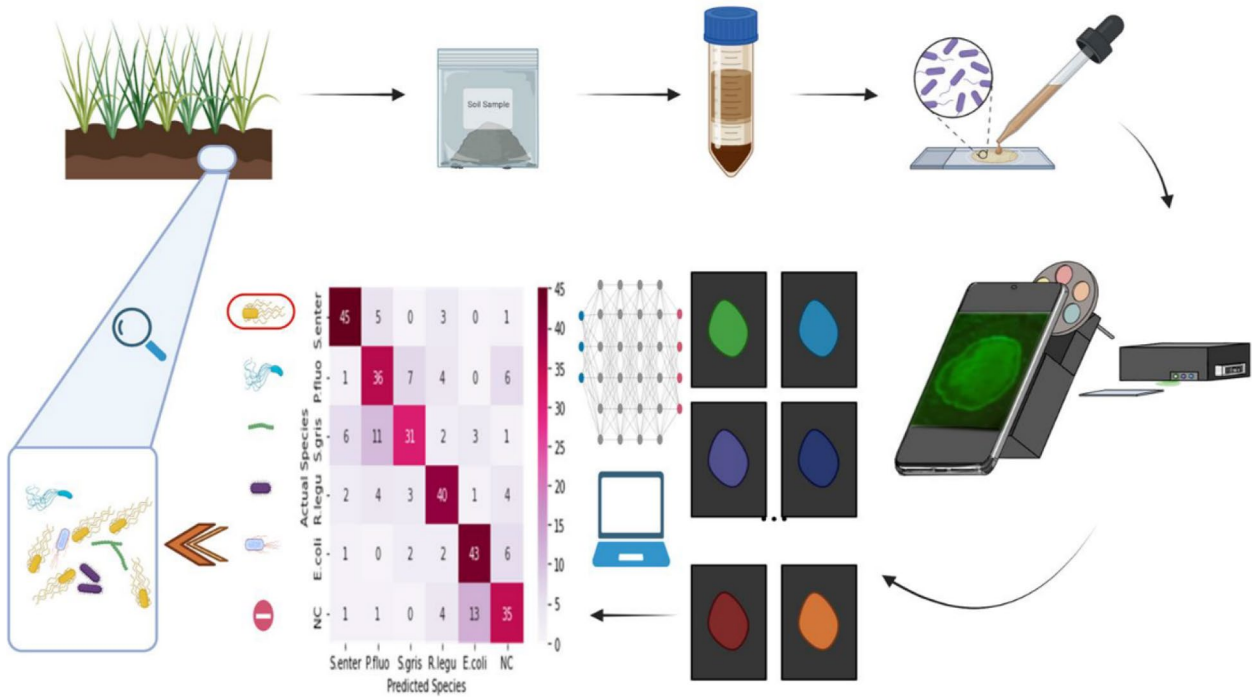

Fig. 6 An example of optical biosensor to soil microbiome characterization. A smartphone is modified with an acrylic film wheel (12 filters) and three LEDs to capture multiple autofluorescence images of various bacterial mixtures and soil samples. Learning databases are collected from various bacterial mixtures, and the field soil samples are used as a test set to predict the dominant bacterial species

as a portable optical reader and potential data-analyzing device; however, optical signals can be affected by environmental lighting conditions. In contrast, electrical signals might suffer less distortion in the field. Biosensing is a rich field that couples engineering and biology, and applying its toolset to help advance soil microbiome characterization would be a boon to microbiology more broadly.

Authors' contributions

Lexi DeFord: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Visualization. Jeong-Yeol Yoon: Conceptualization, Methodology, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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