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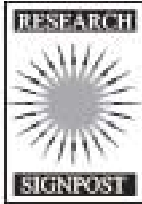

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Soil ecosystem health and its role in plant disease suppression

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Abstract

Quantitative and qualitative assessments of microbial biomass and microbial activity in agricultural soil ecosystems are essential to enhancing a soil's suppressive abilities and to identifying problems and monitoring changes in environmental quality related to agricultural practices. Soil microbial communities in which antagonists to plant pathogens occur in stable populations are the underlying components of suppressive soils. However, the development of methods that accurately quantify components of soil eco-systems and monitor how they change over time in relation to each other has proven particularly challenging. Major advances in molecular techniques

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combined with new microbiological and imaging technologies have potential to provide more complete information regarding soil ecosystems.

Introduction

Soil quality, soil health, and the role of microorganisms in maintaining healthy soil ecosystems and reducing incidence of plant diseases are important concepts in plant health management and sustainable agriculture. The terms soil quality and soil health are sometimes used synonymously, but a general consensus among researchers exists that soil health is the soil's biological component alone, while soil quality also includes chemical and physical components [1, 2].

Many excellent reviews have been written on various aspects of soil quality and health [3-7]. Also, a symposium on soil health was held at the joint national meeting of the American Phytopathological Society and the Entomological Society of America in 1998. The objective of the symposium was to increase awareness of the importance of soil organisms as indicators of soil quality and determinants of soil health [2, 8-12]. The development of methods to assess soil ecosystems accurately has proven particularly challenging. A key aspect of soil health is the capacity to control soilborne plant pathogens and improve the efficiency with which we produce food by maintaining or increasing production per unit area of land [13]. Doran and Zeiss [9] suggested key standards for indicators of soil quality or health. These included sensitivity to changes in management practices, correlation with beneficial functions, and usefulness for determining ecosystem processes. However, no one set of methods for assessing soil quality and health is universally accepted. Our objective is to present examples of how several agricultural practices affect soil quality, soil health, and plant health, and to discuss how the health of soil ecosystems is currently assessed.

Assessing soil health – A conceptual basis

Soil management practices should be based on an understanding of how ecosystems function and how soil management techniques affect their function. This considerable challenge is underscored by our lack of understanding of ecosystem function in the simplest natural or managed systems [10]. The function of soil ecosystems is particularly difficult to assess because the individual soil microbes live in communities in which an almost infinite number of specific interactions might be occurring. Terrestrial ecosystems are similarly characterized by diverse interactions among inhabitants, and one could argue that an increased understanding of soil microbial interactions could be gained by employing techniques used to study terrestrial ecosystems. However, due to the microscopic nature of their inhabitants and their ability to profoundly affect soil environmental conditions, soil microbial communities are inherently much more difficult to assess quantitatively and qualitatively than are terrestrial ecosystems.

As defined previously, the term soil health is most commonly used in reference to the soil's biological component. Microorganisms constitute a major portion of the biological component of soil in the absence of plants and define soil health in the presence of plants. Two similar yet distinct approaches for determining the vitality of soil microbes are soil microbial activity and soil microbial biomass. A review of literature, including basic textbooks on soil microbiology, indicates that microbial biomass is often used as a synonym for microbial activity, but these terms describe two different concepts. Microbial biomass refers to population densities or numbers of microorganisms. Microbial activity refers to the work or energy which results from a given microbial biomass. If the terms are used synonymously, the assumption is made that there is a direct relationship between populations of microorganisms in soil (soil microbial biomass) and the work or energy exerted by the microorganisms (microbial activity). The error in this assumption can be understood by the following analogy. Two rooms each contain a speaker addressing an audience of 10 people. In one room, the speaker is uninspiring and half the audience is asleep. In the other room, the speaker is engaging and the audience is taking notes, asking questions, and having small group discussions. So, although the two rooms have the same population (biomass), they have quite different levels of energy (activity).

In ecological terms, two soils may have the same microbial biomass but different levels of microbial activity as a result of several factors. The physiological state of the microorganisms can be different, although the total microbial population density is the same. Bacteria such as species of the genus *Bacillus* may exist as dormant spores or as physiologically active vegetative cells. Similarly, many fungi can remain viable but inactive in certain resistant spore stages or may exist as physiologically active hyphae. Abiotic variables that can affect the physiological state and activity of soil microorganisms are changes in soil moisture, carbon/nitrogen ratios, minor nutrients, and temperature. Biotic factors, such as the diversity of microorganisms that constitute the soil microflora, also influence microbial activity. For example, a high population of one soil bacterium that produces a potent antibiotic will reduce physiological activity of soil microorganisms that are susceptible to the antibiotic. In addition, plant species and the management practices associated with crop production are known to affect both abiotic and biotic factors in soil.

Crop management strategies to promote beneficial soil ecosystems

Many currently employed agricultural practices do not contribute to beneficial soil conditions and are actually significant contributors to nonpoint source water pollution, destruction of the ozone layer, and increased human

health problems [14-16]. Practices such as use of synthetic fertilizers and intense soil tillage influence the composition of soil microbial communities which then affect water and atmospheric quality by changing the soil's capacity to produce or consume gases such as CO_2, nitrous oxide, and methane [14]. The continued use of urea or ammonia-based fertilizers eventually increases soil acidity which in turn leads to reduced diversity of soil fauna and flora, altered pathogen activity, reduced nutrient cycling, and reduced water infiltration and plant root development [17]. Mechanized agriculture that uses intensive tillage accelerates oxidation of organic matter and increases soil compaction, both of which lead to reduced root growth and enhanced susceptibility to soilborne plant pathogens [18].

Negative effects of pesticides on soil health are exemplified by a review of the use of soil fumigants. Use of broad spectrum soil fumigants such as methyl bromide during the last 40 years to control soilborne pathogens, nematodes, insects, and weeds has enabled intensive cultivation of more than 100 vegetable and nursery crops. The nearly complete biocidal activity of methyl bromide kills both deleterious and beneficial microorganisms, thereby creating a biological vacuum in fumigated soils. This vacuum can then be filled with an aggressive pathogen from outside the fumigated area. In such cases, disease can be much more severe in fumigated than in nonfumigated soils. In 1992 methyl bromide was determined to be an ozone-depleting substance. In 1997, the Montreal Protocol, an international treaty intended to protect the earth's ozone layer, designated a complete phase-out of production and sale of methyl bromide in developed countries by 2005. There has been a concerted international research effort to identify both chemical and nonchemical alternatives to methyl bromide. However, it is apparent that replacement of methyl bromide with other broad-spectrum fumigants will perpetuate dependence on pesticides and leave growers vulnerable to future regulatory policies. Fortunately, a segment of the research on alternatives has remained focused on developing long-term solutions to control of soilborne pathogens by increasing our understanding of soil ecosystems. Consequently, there are many examples of crops and/or crop management techniques that increase beneficial soil conditions and promote plant health through their effects on beneficial soil organisms.

Crop rotation has long been recognized as a beneficial production practice and has been extensively studied as a more sustainable pathogen control measure than use of chemical pesticides. Crop rotations were traditionally used in Europe by the Middle Ages; but when North America was colonized, monoculture became more commonly practiced in U.S. agriculture. Crop rotations often reduce plant disease by breaking the disease cycle of many pathogens. Rotations may prevent survival of pathogen propagules and thereby eliminate or greatly reduce inoculum density. Alternatively, rotations may

reduce disease by increasing the population or physiological activity of beneficial soil microflora. In such cases, the alternate crop or cover crop used in the rotation scheme is often selected for the development of a beneficial rhizosphere microbial community [5, 19-21]. Legumes such as clover, joint vetch, white vetch, and velvetbean have been shown to increase crop productivity through a variety of mechanisms including enhancement of nematode antagonistic rhizosphere bacteria [19].

In contrast, there are well described examples of how continuous cultivation of a single crop can induce soil suppressiveness, such as that of potato scab suppression by increased populations of nonpathogenic *Streptomyces* spp. in fields continuously cropped with potato [22, 23] and development of Fusarium-wilt suppression with successive plantings of watermelon [24]. However, it is more generally accepted that continuous cultivation leads to high levels of pathogens and other detrimental organisms in soil.

The incorporation of organic amendments into soil is another agronomic practice that can result in development or enhancement of beneficial microbial communities. Historically, organic amendments have been used to improve soil physical qualities and supply important nutrients for crop production. Additionally, there are many examples where the incorporation of crop residues or waste materials into soil results in disease suppression by either biochemical or microbial means [24-26]. Some amendments are converted to ammonia during decomposition, which is directly toxic to nematodes and can inhibit growth of many other pathogens [27, 28]. Similarly, release of tannins and phenolics from some amendments can directly inhibit fungal pathogens such as *Rhizoctonia solani* and *Sclerotium rolfsii* [29, 30]. In addition to causing biochemical inhibition of pathogens, organic amendments may result in less disease by alteration of the physical properties of soil. This alteration allows plant roots to grow better and through the growth increase tolerance of plants to pathogens. Specific organic amendments have been reported to increase soil aeration by formation of soil aggregates, buffer pH changes, and increase the water-holding capacity of soil [31-33]. Finally, organic amendments can lead to disease reductions by stimulating the physiological activity and population of soil microbes. This can have a cascading effect on other soil inhabitants including microbivorous nematodes, which can in turn reduce or prevent the increase in populations of plant parasitic nematodes and other soilborne plant pathogens [29, 34-37].

There are many examples of the use of microbial inoculants to promote healthy soil ecosystems. Inundative applications of biological control organisms to soils can enhance plant growth and yield or control plant pathogens. In most cases these organisms are originally isolated from plant roots or the rhizosphere of crops where noticeable reductions in disease have occurred. Among the microbes in the rhizosphere, bacteria that actively colonize roots are termed

rhizobacteria. The impact of root colonization by rhizobacteria on plants may be deleterious, neutral, or beneficial. Beneficial rhizobacteria that promote plant growth and suppress plant diseases have been termed plant growth-promoting rhizobacteria (PGPR) [38]. In some cropping systems, it has been shown that improvements in plant productivity are linked to suppression of pathogens and deleterious microorganisms by PGPR [39]. Rhizosphere bacteria affect deleterious microorganisms in several ways including as competition for limited resources, and suppression through production of antibiotics and lytic enzymes [40].

All agricultural management practices affect soil ecosystems. More accurate means of assessing changes in soil ecosystem composition would provide invaluable insight into how crop management practices may be employed to conserve beneficial functions. It is possible that some crop management practices considered to be less sustainable may be employed to a limited extent before becoming detrimental to soil productive functions.

Mechanisms responsible for disease suppression

As discussed above, practices of crop rotations, cover crops, organic amendments, and application of biological agents often lead to enhanced plant health via changes in the soil or rhizosphere microbial communities. Changes in soil microbial communities, particularly changes whereby there is an increase in the number of microorganisms that antagonize plant pathogens, are the underlying bases of soil suppressiveness. Soil suppressiveness is considered to be the "soil's resistance" to pathogen increase and is therefore an attribute of a healthy soil. General suppressiveness enhances total populations or physiological activity of the general soil microbial community and is not transferable [13]. This suppressiveness is often termed the biological buffering component of soil. Elimination of general suppressiveness by a drastic reduction in the population and physiological activity of soils, as in the previously mentioned case of fumigation with methyl bromide, results in severe disease upon soil colonization by an aggressive pathogen. Specific or induced soil suppressiveness is achieved when the pathogen suppression occurs through the activity of a specific species or group of soil microbes [13]. Specific suppression is transferable, and mechanisms for several classic cases including Fusarium wilts, potato scab, apple replant disease, and take-all of wheat have been determined as discussed by Weller et al. [5].

Uses of cover crops or organic amendments that result in increased soil microbial activity may lead to enhanced soil health through general suppressiveness. A greater ability to assess soil microbial communities accurately would enable a more targeted use of cover crops and amendments for causing induced or specific suppressiveness. For example, plants with known

antagonistic properties to plant-pathogenic nematodes were found to support a distinct rhizosphere bacterial community compared to nonantagonistic plants [19]. Vargas-Ayala et al. [41] reported that the use of velvetbean in crop rotations provided control of nematodes by selecting a taxonomically and physiologically distinct soil microflora compared to rotations without velvetbean. Other examples include the use of chitin as an organic amendment to reduce damage of roots by the root-knot nematode, *Meloidogyne incognita*, and increase populations of bacteria and fungi with antagonism to nematodes [42]. Amendment of soil with pine bark provided control of the soybean cyst nematode (*Heterodera glycines*) and was correlated with increases in soil fungal populations and trehalase activity of the soil [43]. In each of these examples, induced or specific suppressiveness via activity of a particular group of soil microbes seems to account for observed reductions in nematode damage.

In addition to direct antagonism, some rhizosphere bacteria are known to induce systemic resistance to disease [44, 45]. Induced resistance is the activation of latent resistance mechanisms that are expressed upon subsequent infection [46]. An example of naturally-occurring induction of resistance is the hypersensitive response upon infection by a pathogen. Triggers for induced resistance include avirulent or incompatible races of pathogens, nonpathogens such as PGPR, and some chemicals. Induced resistance can be systemically or locally expressed. Systemic resistance induced by chemicals and pathogens that cause limited necrosis is termed systemic acquired resistance (SAR). When systemic resistance is induced by nonpathogenic microorganisms such as PGPR, the effect is termed induced systemic resistance (ISR). SAR and ISR are further differentiated by the signal transduction pathways activated in plants. For a complete discussion of criteria for comparing characteristics of SAR and rhizobacteria-mediated ISR see Steiner and Schönbeck [47] and van Loon et al. [46].

Techniques for assessing microbial biomass and activity in soil ecosystems

Recent advances in microbiology and molecular biology have provided scientists with new tools to assess the effects of management practices on the microbial components of soil ecosystems. It is generally easier and more common to assess soil microbial biomass than microbial activity. Since biomass is the population density of microorganisms in soil, assessing biomass means measuring numbers of microorganisms. While this is simple in concept, quantifying microorganisms is complicated by the fact that they live in complex communities. There is a community behavior exhibited by microorganisms, and the ability to detect individual species or members of a microbial community will be partly influenced by the interactions among community members [48]. Ruinen [49] first described the multilayered composition of bacterial species in

what is now termed a biofilm. It has since been determined that plant surfaces can support dense patches of microorganisms that may facilitate horizontal gene transfer among microorganisms and regulate genes that code for phenotyptic traits such as antibiotic resistance and virulence [50, 51]. Examples of such density-dependent regulation of phenotypic traits include production and utilization of diffusible N-acyl-homoserine lactones (AHLs) by bacteria in the rhizosphere of wheat [52, 53]. These AHLs regulate production of phenazine antibiotics by *Pseudomonas aureofaciens* (now *P. chlororaphis*) strain 30-84, and phenazines contribute to rhizosphere competence and colonization of wheat roots [54]. Before these community interactions can be assessed, both biomass and activity need to be determined. Ultimately, this information could be used in the development of models that depict soil ecosystems.

As stated above, it is more common in the literature to find assessments of soil microbial biomass than soil microbial activity. In contrast to the large amount of research aimed at new techniques for measuring microbial biomass and species diversity of soil ecosystems, many of the methods available for assessing microbial activity are older. Given the conceptual differences between microbial activity and microbial biomass discussed previously, one could argue that assessing microbial activity is more important for determining soil health, as it relates to the physiological energy and work of soil microorganisms. Consequently, there is a critical need to develop more methods for quantifying microbial activity and determining when and how microbial activity relates to microbial biomass.

Soil respiration is the most common way of measuring general, or nonspecific, microbial activity. This method is based on the fact that heterotrophic microorganisms consume O_2 and give off CO_2 during respiration, thus creating energy to fuel metabolic processes. Therefore, measuring soil respiration gives a general indication of total microbial activity. Measuring respiration involves the following steps: a soil sample is moistened, a nutrient source such as glucose is sometimes added, the soil is incubated; and the evolution of CO_2 is determined by titration of CO_2 trapped in alkalai or by infrared gas analysis [55-57]. Instead of adding a specific carbon source, one can add an organic amendment such as ground plant material [58].

Another classical method of measuring general microbial activity in soil is by measuring dehydrogenase activity. Dehydrogenases are essential enzymes that catalyze removal of hydrogen atoms from energy-rich molecules such as NADPH, thereby providing fuel for cellular metabolism. The removed hydrogen atoms are transferred to various electron donors. Analysis of soil dehydrogenase activity is typically determined by repeated methanol extractions of soil, addition of one or more electron donors, addition of triphenyltetrazolium chloride as a colorimetric indicator of enzyme activity, and spectrophotometric quantification [59].

Microbiological methods

In the context of determining the effects of soil organisms on plant health, the composition and function of rhizosphere communities are of primary interest. It has been estimated that hundreds, and possibly thousands, of genotypically distinct fungal and bacterial species inhabit the rhizosphere of an individual plant [50]. Activity of bacteria in the rhizosphere has been estimated to be over 60 times more than that in bulk soil, while fungal activity in the rhizosphere has been estimated to be 12 times more than in bulk soil, primarily due to the high level of carbon released from plant roots [60, 61]. Many factors influence the composition of microbial communities in the rhizosphere including the quantity and quality of root exudates, plant species, environmental conditions, and other rhizosphere inhabitants like nematodes [62, 63]. These relationships are complex and interactions between nematodes and micro-organisms can be competitive, additive, or synergistic [63].

Quantifying populations of rhizosphere microorganisms has historically been done through culturing on various fungal or bacterial growth media, a technique referred to as culture-dependent. There have been many examples where culture-dependent techniques have successfully been applied to determine the types of microorganisms associated with various soil conditions and disease suppression [5, 64-66]. However, researchers comparing direct microscopic counts to the number of microorganisms cultured on artificial media estimated that only 0.1% of microorganisms in typical agricultural soils grow on artificial media [67, 68]. The inherent limitations of culture-dependent techniques should be considered when interpreting data on microbial community composition.

A significant improvement in using traditional physiological characteristics to identify culturable organisms is the development of an automated system by BioLog, Inc. (Hayward, CA) that produces profiles based on carbon source utilization patterns. This system is based on the reduction of tetrazolium or similar chromogens by actively metabolizing organisms, resulting in a metabolic fingerprint for 95 carbon sources in a microtiter plate. Databases are available for identification of over 2500 species of microorganisms including Gram-negative and Gram-positive bacteria, anaerobic bacteria, filamentous fungi, and yeasts. The scientific community has generated considerable data using the BioLog system to develop metabolic fingerprints for assessing functional diversity of microbial communities. Functional diversity of a soil microbial community refers to the capacity of the community to catabolize different organic compounds [69]. In this method, suspensions of soil samples are placed in BioLog microtiter plates containing the 95 simple carbon substrates [70]. The assumption is that the greater number of carbon substances catabolized, the greater the microbial activity of the soil [71].

It should be noted, however, that this functional diversity approach measures a series of individual metabolic processes rather than general microbial activity, which is measured by respiration and dehydrogenase activity, and is dependent on the capacity of microorganisms to grow on an artificial medium. Multivariate statistical analysis is commonly used to determine if substrate utilization profiles are functionally similar or different from each other [72-74]. As with all techniques for assessing microbial communities, certain considerations need to be addressed. These include condition of cells in the inoculum and the efficiency with which organisms utilize substrates. Although this method for evaluating functional diversity of soil microbial communities remains constrained by issues facing all culture-dependent methods, due to the ability to measure utilization of large numbers of carbon sources over time it has great potential to provide insight into functional differences among agricultural soil communities and should be considered as an important component of a multifaceted approach [75].

Analysis of fatty acid methyl esters (FAMES) is another commonly accepted culture-dependent practice for bacterial identification. This technique is based on fatty acid profiles compared using the MIDI Sherlock microbial identification system (MIDI, Inc., Newark, DE). The Sherlock system identifies aerobic and anaerobic bacteria and yeasts based on gas chromatograph (GC) analysis of cellular fatty acid content. Like the BioLog this system was developed for microbial identification, and methods were modified to assess complex soil ecosystems. A derivation of this technique for soil ecology is culture-independent and analyzes phospholipid fatty acid (PLFA) profiles in soil communities. Phospholipid fatty acids are found in membranes of primarily viable cells and are broken down quickly after cell death. Thus, these molecules can be used to estimate both culturable and nonculturable viable biomass, and profiles can be used to determine changes in microbial communities with soil treatments [76, 77]. There are unique fatty acids that can also be used to identify groups of organisms. Ibekwe and Kennedy [78] used fatty acid methyl ester (FAME) profiles to characterize soil microbial communities and found that shifts in bacterial community structure differed among plant species studied. Gram-positive and Gram-negative bacteria were identified by characteristic peaks that included short chain hydroxyl acids and cyclopropane acids for Gram-negative bacteria.

Imaging techniques

Direct microscopic identification can be used with some soil organisms such as nematodes, microarthropods, flagellates, and protozoa. These secondary consumers can also be used as indicators of shifts in populations of fungi and bacteria in lower trophic levels, where direct microscopic identification of species in soil is not always possible. However, recent

advances in light and electron microscope imaging technology, confocal laser scanning microscopy, video microscopy, digital image processing, and use of fluorescent probes have increased our capacity to visualize microorganisms [79]. New techniques in electron microscopy that have improved observational power for microbe-microbe and plant-microbe interactions include the use of high-pressure freezing for fixation of tissue for transmission electron microscopy and the use of low-temperature chambers in scanning electron microscopy [79].

Light microscopic techniques have been combined with molecular techniques for the detection of gene expression by *in situ* hybridization. Recently, development of fluorescent probes has enabled detection of molecules and activities within living cells. These include visualization of nucleic acids and various enzyme activities [79]. Fluorescent *in situ* hybridization (FISH) uses 16S or 23S rRNAs from whole fixed cells that are hybridized with fluorescently-labeled, taxon-specific oligonucleotide probes. The labeled cells are then viewed by scanning confocal laser microscopy. Microorganisms across phylogenetic levels can be clearly detected by FISH. Also, FISH probes can be generated without prior isolation of the microorganism [80]. Detection of single cells in complex settings, such as the rhizosphere, is also possible with FISH [81, 82]. This technique has potential to gain a more complete representation of complex microbial communities by locating microorganisms, determining spatial relationships among organisms, and providing confirmation of PCR-based quantitative estimates [83]. The short-comings of this technique are that in nutrient-poor environments, such as soil, penetration of cells by probes can be a problem [80]. Detection of microorganisms is dependent upon the accurate generation of probes. Further, cells must be metabolically active in order for cell walls to be permeable enough for the probe to penetrate [81, 84]. Use of FISH combined with microautoradiography involves the incubation of cells in the presence of radiolabelled substrates and enables microscopic visualization of active cells like the previously described FISH but avoids the need for production of antibodies from pure cultures of microorganisms [83]. Stable isotope probing (SIP) can be used to determine the components of a community capable of incorporating certain heavy-isotope labeled substrates, enabling the identification of actively metabolizing organisms *in situ*.

The use of green fluorescent protein (GFP) has greatly advanced the visualization of gene expression and cellular processes using light microscopy [79]. GFP is a protein isolated from jellyfish (*Aequorea victoria*) that retains its fluorescence in chimeric fusions. Because only molecular oxygen is required, GFP will fluoresce in almost any aerobic organism. For more information on new trends and techniques in microscopy, consult Heath [79] or Gilroy [85].

Molecular methods

Molecular methods based on nucleic acid composition have the potential to answer certain questions that culture-dependent techniques cannot. Molecular techniques can be used to determine the relative abundance of a taxonomic range [86] and, to a certain extent, the functional diversity of microbial populations [83]. Some of the more useful techniques for assessing soil microbial communities are those in which nucleic acids are extracted from soil and the sequence of rRNA genes are determined after PCR amplification. Most molecular analyses have used genes encoding the small sub-unit (SSU) of rRNA, with 16S rRNA genes used to assess bacterial populations and 18S rRNA and internal transcribed spacer (ITS) regions used to assess fungal populations. These genes can then be compared to those from known organisms and used to develop probes for tracking and visualizing microorganisms [11]. Once the DNA is extracted from the soil and amplified using PCR, it is necessary to separate individual amplicons through cloning procedures using a variety of bacterial vectors [11]. Denaturing- or temperature-gradient gel electrophoresis (DGGE/TGGE) can also be used to separate amplicons and is based on the influence of primary sequences on electrophoretic mobility under partially denaturing conditions. This technique allows mixtures of PCR products of the same length to be separated by differences in sequence composition and is based on how the temperature or denaturant concentration affects the secondary structure of individual sequences, thereby affecting mobility in the gel [11]. Complexity of banding pattern resulting from this analysis reflects the microbial diversity in the sample. Single-strand conformation polymorphism (SSCP) can detect differences in primary sequence composition under partially denaturing conditions such as those described for DGGE/TGGE [87]. Public-domain databases can then be searched for similarity once the individual amplicons are cloned or separated and sequenced. Although useful, this technique can only be considered semi-quantitative as sequence abundance may not directly correlate to cell numbers due to differences in rRNA copy number among organisms [83].

Other molecular fingerprinting techniques that involve restriction analysis of PCR products include RFLP analysis, which when applied to ribosomal gene sequences is termed amplified ribosomal RNA restriction analysis (ARDRA) and is typically used for comparing microbial isolates. Applying a terminal restriction fragment fluorescent labeling method (T-RFLP) to the ARDRA technique provides a "community fingerprint" giving information on species richness [88, 89] and can be used for comparison of other genes as well [90]. While FT-ARDRA is more useful for differentiating taxonomic groups at the level of genus, the DGGE/TGGE and SSCP methods can determine diversity in species within a genus.

Analysis of genes other than 16s rRNA genes in environmental samples can be used to determine gene function and which organisms are metabolically relevant to ecosystem processes [83]. Genes controlling many microbial processes have been sequenced making it possible to construct primers for their amplification and analysis and allowing for characterization of their abundance within environments. For example, techniques including colony hybridization, slot-blot hybridization, and PCR have been used to evaluate specific microbes responsible for suppression of take all decline using a gene probe specific for producers of the antifungal metabolite 2,4-diacetylphloroglucinol (2,4-DAPG) [91-93]. This technique can be used for phylogenetic grouping if the genes are on the chromosome. However, if the genes are plasmid-borne, potential horizontal transfer would prevent their usefulness for phylogenetic grouping. Hence, it is important to choose functional genes carefully [83].

It is likely that genomics and associated high-throughput sequencing and bioinformatics will be used extensively in the near future for analysis of soil communities. DNA arrays (high density arrays or microarrays) may eventually provide rapid, quantitative analysis of soil microbial communities [94]. These arrays consist of thousands of oligonucleotides of known sequence fixed to a membrane or glass slide which is then hybridized with a labeled nucleic acid sample. The arrays can be customized with respect to the oligonucleotides used depending on the specific application. For example, for analysis of community structure, oligonucleotides directed towards various phylogenetic groups of organisms would be used while analysis of individual strains would be performed using oligonucleotides directed toward very specific genomic sequence differences among members of a phylogenetic group. Alternatively, arrays could be used to measure the metabolic activity of a community by focusing on the quantification of genetic transcripts for, and thus, the level of activity of, defined biochemical pathways within a soil community.

Many new and emerging molecular techniques have great potential to provide insight into the complex and dynamic nature of soil ecosystems. Molecular techniques address inherent problems with culture-dependent methods but also suffer from inherent bias. It is advisable that PCR-based results be confirmed by complementary techniques when possible due to the limited capacity for quantification and the intrinsic uncertainties regarding amplification products from mixed templates [5]. A combination of methods will likely be required in order to make significant advancements in under-standing relationships among organisms in soil and how they affect plant health.

Current challenges and future directions

Decisions regarding agricultural soil management practices are based primarily on incomplete data when measurements of the soil microbial

ecosystem are not considered. Countless opportunities exist to advance the science of how soil ecosystems relate to plant health. Studies that more clearly characterize relationships among organisms, physiological activity of soil microorganisms, and ecosystem properties and functions are required [10]. Studies need to be designed to answer the question of which organisms most consistently reflect the long-term potential of a soil to resist or recover from disturbance [10]. Further, a great deal of confusion surrounds the topic of ecosystem stability and how the complexity of a system relates to its stability [95].

Integration of new imaging, microbiological, and molecular techniques is likely to provide a wealth of information on the dynamics of soil ecosystems and how they function. Use of molecular approaches can overcome limitations of culture-dependent techniques by identifying which organisms are important and preventing the selection of microorganisms presumed to be important in ecosystems based on their ability to grow under artificial conditions. Molecular approaches may also help determine conditions under which currently nonculturable organisms may be cultured [83].

Developing a better understanding of the dynamics of agricultural soil ecosystems and how these dynamics influence plant health is essential to reducing dependence on energy-intensive agricultural practices such as use of synthetic pesticides and fertilizers. This understanding will enhance the performance and consistency of cultural control measures and of biologically-based pest management strategies by enabling users to make informed decisions regarding the conditions under which these practices are used. Ultimately, a greater understanding of the effect of soil ecology on host/pathogen relationships and crop productivity is necessary in order to design and implement sustainable crop production systems. Research challenges should be addressed through multidisciplinary, collaborative efforts that consider many perspectives and employ all the techniques available to develop, evaluate, and apply soil quality management systems. Strategies that limit crop loss and reduce reliance on environmentally damaging inputs would conserve beneficial soil organisms and enhance the sustainability of agricultural production systems.

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