Can. J. Plant Pathol. (2012), **34(3)**: 349–361

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Symposium contribution/Contribution à un symposium

Climate change and plant health: designing research spillover from plant genomics for understanding the role of microbial communities

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(Accepted 24 June 2012)

Abstract: Climate change presents new challenges for managing plant health. Simultaneously, the revolution in sequencing technologies offers an exciting new perspective on whole microbial communities – and on both microbial responses to climate and microbial effects on plant health. There is still the need for a comparable revolution in experimental approaches to understand the functional roles of microbial taxa within these communities. Two approaches leveraging advances in genomics tools and analyses may contribute. First, new soil mixing experiments may be developed, where analyses of quantitative trait taxa (QTT) may be analogous to analyses of quantitative trait loci (QTL). Second, new approaches for characterizing the extended phenotype or phenome of soil microbial communities may be developed, leveraging genomic tools for Arabidopsis and other model plant species through the construction of plant genotype panels in an 'Arabidopsitron'.

Keywords: climate change, metagenomics, next generation sequencing, pyrosequencing, quantitative trait loci, resilience, soil microbial communities

Résumé: Les changements climatiques posent de nouveaux défis quant à la gestion de la santé des plantes. Simultanément, la révolution dans les technologies de séquençage offre une nouvelle perspective passionnante quant à des communautés microbiennes entières, et ce, tant sur le plan des réactions des microorganismes aux changements climatiques que sur celui des effets de ces microorganismes sur la santé des plantes. Le besoin d'une révolution similaire quant aux approches expérimentales demeure essentiel pour comprendre les rôles fonctionnels des taxons au sein de ces communautés. Deux approches qui optimisent les avancées dans le domaine des outils génomiques et des analyses peuvent y contribuer. Premièrement, de nouvelles expériences sur le mélange des sols peuvent être conçues alors que les analyses des taxons à caractère quantitatif (QTT) peuvent être analogues aux analyses des locus à caractère quantitatif (QTL). Deuxièmement, de nouvelles approches permettant de caractériser le phénome (phénotype élargi) des communautés bactériennes terricoles peuvent être développées, optimisant les outils génomiques pour *Arabidopsis* et d'autres espèces modèles de plantes par la constitution de panels de génotypes de plantes dans un « Arabidopsitron ».

Mots clés: changements climatiques, communautés bactériennes terricoles, locus à caractère quantitatif, métagénomique, pyroséquençage, résilience, séquençage de la prochaine génération

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This paper was a contribution to the symposium entitled 'Emerging stresses on forests and agro-ecosystems resulting from climate change' held during the Canadian Phytopathological Society Annual Meeting in Vancouver, British Columbia, June 2010.

ISSN: 0706-0661 print/ISSN 1715-2992 online
http://dx.doi.org/10.1080/07060661.2012.706832

Introduction

The effects of weather on plant health and disease risk are well-known, so a change in climate will change the outlook for plant health in turn (Coakley *et al.*, 1999; Garrett *et al.*, 2006*a*). These relationships are of great interest because of the influence of plant disease on ecosystem services provided by agriculture and natural systems (Cheatham *et al.*, 2009). Plant diseases typically reduce agricultural production by over 15% globally (Oerke, 2006) and invasive pathogens can cause substantial damage to natural plant communities. Thus, climate change and plant diseases have been the focus of a number of recent reviews (Chakraborty & Newton, 2011; Garrett *et al.*, 2011; Juroszek & von Tiedemann, 2011; Luck *et al.*, 2011; Pritchard, 2011; Shaw & Osborne, 2011) as well as syntheses of reviews of plant disease responses (Pautasso *et al.*, 2010), of soil biota responses (Blankinship *et al.*, 2011), and of beneficial plant–microbe association responses (Compant *et al.*, 2010). Here, we discuss how new metagenomic data from next generation sequencing can help to address the new problems associated with climate change and its effects on plant health. New and much more complete views of the structure and function of microbial communities associated with plants are now becoming available. This information has the potential to be an important tool for understanding plant disease ecology and developing strategies to address climate change adaptation and mitigation.

In this paper, we address two main aspects of the potential for metagenomic tools to support climate change adaptation and mitigation related to plant diseases. First, the role of microbial communities in plant disease in general is an exciting frontier for plant pathology. Intriguing interactions among pathogens and microbial communities, such as those observed in disease-suppressive soils, provide inspiration for developing a fuller understanding of plant microbial community ecology. However, past limitations in techniques for analysis of microbial communities have made it difficult to study even the composition of microbial communities. Low frequencies of most community components complicate finding the organisms that contribute towards disease suppression or any other ecosystem services. New metagenomic tools, such as next-generation sequencing and microarray technologies, are helping to overcome this limitation. They support much more complete analyses of microbial communities, but are still hindered by the lagged development of reference databases and experiments that allow elucidation of organismal or gene function. The rapid generation of broad datasets allows for data mining at unprecedented global scales. This can support the development of baselines for microbial communities, and analyses of the expansion of new types of pathogens or beneficial microbes.

Second, metagenomic information bears the promise to support plant health management under climate change, including spillover for new experimental and analytical methods. New sequencing tools have created a revolution in biologists' ability to study the taxonomic and, to a degree, the functional composition of microbial communities. A similar revolution is needed in experimental techniques to evaluate the effects of microbial community structure on outcomes such as plant productivity. It may be possible to design research spillover (Alston *et al.*, 2009), to leverage progress in experimental techniques in organismal genomics. We discuss the potential for two approaches to studying microbial community effects on plants. (1) In some respects, the identification of soil and other plant-associated microbes that contribute to plant productivity is analogous to the identification of genes that contribute to the productivity of particular plant genotypes. Methods for the analysis of quantitative trait loci may offer useful concepts for the analysis of the effects of microbial taxa on plants, though the analogy only goes so far, as we discuss. (2) The development of plant genomic resources such as mutant collections for Arabidopsis has the potential to be an important tool for the analysis of the 'extended metaphenome' of soil and other environmental microbial communities. For example, a panel of Arabidopsis genotypes with different mutations or other genetic variation relevant to plant–microbe interactions (an 'Arabidopsitron') could be used to characterize the extended metaphenome in terms of the productivity and other phenotypic traits of each genotype. We discuss how this type of information could be incorporated in analyses of microbial impacts on plant productivity.

Climate change and plant disease

Adaptation of plant disease management to new climate scenarios will depend on a number of types of information. Regarding the host, it will be important to understand how both native and agricultural plant species may move into new areas in response to changing climate, potentially creating new patterns of host sharing for pathogens. Likewise, it will be important to understand how different conditioning of hosts by new weather patterns results in different disease resistance phenotypes. For pathogens, it will also be important to understand the impact of geographic shifts and weather conditions. The new data available through metagenomic analyses include the composition of microbial communities associated with plants,

the profile of gene content within plant-associated microbial communities, and probably soon even the profile of functional variation within particular genes. How important these types of information will prove to be for formulating adaptation strategies remains to be seen. Many microbes interact with pathogens directly, or indirectly through their effects on plant phenotypic resistance (van Loon, 2007). Understanding how best to use and mine these types of information, and what level of complexity of information is most valuable, is an exciting frontier for plant pathology.

Experimental objectives determine the potential value of available data. A population of pathogens may be characterized in more detail in terms of its genetic composition, particularly in terms of its ability to overcome host resistance (e.g. Milgroom & Peever, 2003). The host population or community can be characterized in terms of induced and acquired resistance levels (e.g. Garrett *et al*., 2006*b*). The environment can be characterized with more detail about abiotic features, or with information about abundance of other organisms, such as the abundance of mycorrhizal fungi, though integration across such datasets can be challenging (Jumpponen *et al.* 2010). In some cases, greater detail about the spatial and temporal patterns of these features can also improve estimates of disease risk. The level of detail desired will depend on the goals of an analysis (Savary *et al.*, 2006). When the goal is short-term decision-making for management of a particular agricultural field, there will generally be strong financial incentives to minimize the amount of information needed for modelling. When the goal is to construct long-term strategies for agricultural or natural systems management, or to pursue curiosity-driven basic research, the potential range of predictors is much wider. Basic research will occasionally reveal predictors that are of great enough importance to include in the most practical modelling contexts, as well (Levins, 1966). The cost limitations for microbial community data are disappearing, so a new challenge emerges to incorporate relatively inexpensive and abundant information about microbial communities in disease ecology models. Epidemiological models often focus on a variable such as pathogen population size or disease severity, and on parameters that determine how that variable changes over time or space (Madden *et al.*, 2007). New summary measures are needed to make the incorporation of microbial community data feasible. Ideally, if microbial community information is available it could inform tactical decisions about what disease management methods are necessary and which crop species or varieties are practical. And it could also inform strategic decision-making about conservation policy and research investments. In some cases, the specific form of a virulence gene sequence may be a key driver in epidemic outcomes, yet would generally not be revealed unless primers specific for that gene were employed.

The role of plant disease in climate change mitigation has only recently been appreciated (Mahmuti *et al.*, 2009). The greenhouse gas budget for an agricultural system can be evaluated directly in terms of the greenhouse gases released per unit of food produced, for example. The many ways that plant-associated microbes influence crop productivity can bear on this relationship. If management results in an increased food production per greenhouse gas 'investment', this is a form of climate change mitigation. Beneficial microbes can increase food production through many mechanisms, such as increasing nutrient uptake, increasing drought tolerance, and reducing the effects of pathogens (Gentili & Jumpponen, 2006). In some cases there may be a monetary or greenhouse gas 'cost' associated with these benefits. For example, if a biocontrol microbe needs to be added to a field and it is applied using a tractor, the resulting greenhouse gas cost of the tractor use must be factored into the budget. If the benefit in increased productivity is greater than the cost of the tractor use, then the biocontrol microbe provides this type of climate change mitigation. Because the role of many microbes is not well understood, we are likely to underestimate the value of microbial mitigation. Understanding these processes can lead to valuation of microbial community contributions to carbon sequestration through disease regulation (an ecosystem service) in addition to nutrient cycling.

The role of microbial communities in plant disease

There are many potential roles for microbes in epidemics. They may alter plant uptake of nutrients, potentially affecting nutrient stress as well as susceptibility to infectious disease. They may alter plant susceptibility through processes such as induced systemic resistance and systemic acquired resistance (Vallad & Goodman, 2004; van Loon, 2007), with potentially complex network structures for information related to microbial associations (Garrett, 2012). The many ways in which microbial communities can influence these systems are an active area of study, but much remains to be known about what optimal levels of induction may be for plants, and when 'overstimulation' of these systems may have a net cost to plants. The full gamut of potential direct and indirect interactions among pathogens and other microbes occurs in soil and plants and on plant surfaces (Vellend, 2010). Soil microbes may directly interfere with plant pathogens by competing for

nutrients, producing antibiotics, or parasitizing the plant pathogens. New types of complex associations continue to be discovered: for example, a microbial association with roots supports heat tolerance in plants in a three-way symbiosis involving a fungus and a virus (Márquez *et al.*, 2007). Complete analyses of the ecosystem services provided by microbes will depend on much more extensive and reliable reference databases for relating taxa or genes to function, analogous to the Gene Ontology project (http://www.geneontology.org/), with attention to sources of inconsistency due to varying core and pan-genomes. In many cases, intraspecific functional variability for taxa of interest will need to be explored and better understood. Epidemiologists will need to assume an active role in contributing to the development of these databases with information about functional roles of specific microbes in epidemics.

Interactions between microbes that influence disease

Disease suppressive soils have been identified for several plant diseases (Garbeva *et al.*, 2004; Mazzola, 2004; Kinkel *et al.*, 2011). Although disease suppression is not equivalent to soil health (Hornby & Bateman, 1997), disease suppression is generally considered an important function of healthy soil (van Bruggen & Semenov, 2000). Soil heath is often considered an ecological characteristic (Karlen *et al.*, 1997) while soil quality generally refers to physical, chemical and biological characteristics (van Bruggen & Semenov, 2000). The concept of 'soil health', just as 'ecosystem health' and even 'plant health' (Döring *et al.*, 2012), is an intuitively appealing idea but difficult to define in practice. In many contexts, the quality of ecosystem services provided by a soil may be a practical measure of soil health, from the standpoint of benefits to humans. In a physical environment with the presence of a susceptible host and a virulent plant pathogen, disease suppression occurs when less disease is found in this conducive environment than would be expected (van Bruggen and Semenov, 2000). Disease suppression may be general or specific (Cook & Baker, 1983). For example, specific suppression occurs for take-all disease of cereals caused by *Gaeumannomyces graminis*, in which this plant pathogen has a specific interaction with an antagonist (Weller & Cook, 1983). The consequence of this specific disease suppression is disease decline, which has been attributed to increases in populations of specific antagonists such as a *Pseudomonas* that produces the compound phloroglucinol (Raaijmakers & Weller, 1998). Other examples of disease suppression include suppression of root diseases by endophytic fungi that asymptomatically colonize agricultural crops (Narisawa *et al.*, 2002; Narisawa *et al.*, 2004). The mechanisms behind this disease suppression are unknown, but may include direct antagonism, competition for colonization sites, or removal of signals that pathogens use to detect compatible host roots.

Bacteria use quorum sensing (QS), a cell-to-cell communication mechanism, to sense population density and coordinate regulation of gene expression (Dong & Zhang, 2005). One family of the well-characterized QS signals in many bacteria is *N*-acylhomoserine lactones (AHLs), which regulate a range of important biological functions, including virulence, and activate new gene expression during host invasion and biofilm formation (Mathesius *et al.*, 2003; Dong & Zhang, 2005). Eukaryotes, in turn, have evolved mechanisms to detect bacterial AHLs and respond to them. For example, the legume *Medicago truncatula* responded to AHLs from both symbiotic (*Sinorhizobium meliloti*) and pathogenic (*Pseudomonas aeruginosa*) bacteria (Mathesius *et al*., 2003). Similarly, microbial mutualists exchange signals with host plants to establish functional mutualisms (Held *et al.*, 2010).

Identifying which microbial taxa are responsible for disease suppression is a challenge (Borneman & Becker, 2007), as is understanding the factors that determine whether microbial biocontrol agents persist in the environment. Unlike insect communities, where the full range of insect pest parasitoids and competitors may be well understood and sampled comparatively easily, the full components and interactions of microbial communities have remained mysterious even in well-studied systems. Typically, the only initial information available for drawing inference is the abundance of a set of taxa in suppressive soils in comparison to the set of taxa in soils which are not suppressive. The correlation between abundance of a particular taxon and the level of disease suppressiveness can be evaluated, but because there are generally so many taxa varying from one soil to another, it is difficult to draw conclusions about which taxa drive suppressiveness. The approaches described below are intended to address this problem. A second stage of analysis to support inference about the role of particular taxa is inoculation with the putative agent to determine whether disease suppression can be attributed.

Biological control agents added to systems to manage disease are another important component of microbial communities (Cunniffe & Gilligan, 2011). There have been some success stories with this approach, illustrated by the widespread use of *Trichoderma* spp. (Lorito *et al.*, 2010). These fungi often provide useful disease management and may also stimulate plant growth. It is exciting to consider the possibilities for identifying new unculturable taxa that are similarly important in modifying disease

risk. A challenge for adding biocontrol agents is the need to reduce the 'refuge size' (Johnson, 2010), or the proportion of the pathogen population that is not negatively influenced by the biocontrol agent. High-resolution spatial structuring can contribute to lack of interaction among pathogens and biocontrol agents, if the two do not come in close enough contact for influence. Weather, and thus climate, can similarly influence refuge size. Microbial communities associated with foliage and other plant parts also have the potential to be disease suppressive (Newton *et al.*, 2010*a*, 2010*b*).

Complex adaptive systems

The role of biodiversity in microbial communities and the importance of the diversity of microbial communities for providing ecosystem services remain unresolved. While some may argue that biodiversity enhances ecosystem function (Naeem & Li, 1997), our understanding of microbial diversity and species richness is rudimentary at best. Morally and in a value-based evaluation, we tend to argue that more biodiversity is better. However, empirical evidence to support this is contradictory and less clear. For example, Wardle *et al*. (1997) showed that increases in species richness did not lead to increases in function of decomposer communities. It is likely that the actual composition of the communities and the functional roles of the constituent species are more important as determinants of the function of a microbial community than the richness and diversity alone (Fukami *et al.*, 2010). In sum, in predicting the functional attributes of the microbial communities, we are severely limited by our lack of understanding of functional diversity and by our inability to connect taxon richness to function of the constituent species. Additionally, an even greater challenge than understanding the connections between microbial diversity and function are the connections to ecosystem functions and to the maintenance of these functions across ecosystem perturbations, i.e. sustainability and resilience during global change.

Resilience, the tendency of a system to return to its 'original' state after a perturbation, is a common goal for managed ecological systems (Neubert & Caswell, 1997; Gunderson, 2000; Walker *et al.*, 2004). Sustainability, the tendency of a system not to degrade from an original state, is another common goal. Transformability of a system refers to how readily it can be transformed to a new and potentially improved state if needed (Walker *et al.*, 2004). For example, an agricultural system may include disease-suppressive soils. In this case, a relevant state of the system is its quality of disease suppressiveness. If the system is resilient, the soils will maintain or return to disease suppressiveness after a perturbation, such as a change in patterns of temperature and precipitation, including a potential change in the frequency of extreme events (Rosenzweig *et al.*, 2001; Garrett *et al.*, 2012). This resilience is a desirable quality of the system, such that the original 'status quo' of the system will not be diminished when the environment changes. However, it may also be possible to transform the system to create a higher level of disease suppressiveness than was present in the original state. Or it may be necessary to transform the system to create a new type of disease suppressiveness because of an invasive pathogen species. As we understand microbial communities better, we may be able to efficiently modify agricultural environments to enhance populations of beneficial microbes, merging concepts from microbial ecology and intervention ecology (Hobbs *et al.*, 2011).

The concept of complex adaptive systems can often be applied to microbial communities (Crawford *et al.*, 2005; Coleman, 2011). Complex adaptive systems can be defined to have the following characteristics (Levin, 2005). First, complex adaptive systems exhibit 'sustained diversity and individuality of components'. Microbial communities meet this criterion by generally exhibiting very high diversity, as well as individuality in the sense that there are more-or-less distinct species present (though the species concept may be complicated for microbes). Second, it includes 'localized interactions among those components'. Microbes generally interact only with other microbes that are close enough in space to compete for resources and experience the same chemical environment. Third, it is 'an autonomous process that selects from among those components, based on the results of local interactions, a subset for replication or enhancement'. Those microbes that can reproduce most successfully in a small local environment (such as a leaf or root) will become more abundant there and may then successfully disperse to other environments. A complex adaptive system may be resilient if it tends to maintain its higher-level traits despite changes at lower levels. That is, a microbial community may be resilient if it maintains functions (such as disease regulation or nutrient cycling) despite changes in the structure of the microbial community (such as changes in abundance of particular taxa or strains). New work in individual-based modelling of microbial systems offers an approach to understanding potential interactions (Ferrer *et al.*, 2008), along with integration of mathematical models for different forms of microbial interactions (Klitgord & Segre, 2011).

Climate change will tend to change the importance of particular crop diseases at any given location. To some degree, natural biocontrol agents, such as microbes in disease-suppressive soils, can help to buffer this effect.

Use of more diverse mixtures of biocontrol microbes can sometimes produce better results, as different components of the mix colonize different plant niches. However, selection of compatible components of such biocontrol mixtures is likely to prove difficult and can vary across environmental conditions. New environmental conditions might exceed the tolerance of biocontrol agents.

There is global interest in shifting agricultural systems to emphasize 'conservation agriculture', such that tillage is reduced and plant material (crop residue or cover crops) is maintained in fields for a greater percentage of the time (Hobbs *et al.*, 2008). Primary ecosystem services motivating conservation agriculture are reduced soil erosion and increased carbon sequestration. But increased risk from soilborne pathogens is sometimes an outcome when crop residue is present, because crop residue may maintain viable propagules of pathogens that cause diseases such as wheat tan spot. This trend will exert increased pressure for effective integrated management of soilborne pathogens, including use of biocontrol microbes.

New tools for understanding microbial communities

Metagenomic techniques provide a huge advance in the study of microbial communities by supporting the study of unculturable microbes and the study of a previously unapproachable number of taxa simultaneously through high-throughput sequencing and microarray technologies (Handelsman, 2004; Acosta-Martinez *et al.*, 2008, 2010; Das *et al.*, 2008; Yin *et al.*, 2010; Mendes *et al.*, 2011; Studholme *et al.*, 2011; Rosenzweig *et al.*, 2012; Xu *et al.*, 2012). Previously, microbial community assessments relied on cursory evaluation of shallowly sampled cloned nucleotide fragments (Amann, 1995), that rarely came as close as newer approaches to saturation of the organismal diversity (Jumpponen & Johnson, 2005). The vast richness of microbial communities in environmental samples became apparent in the very early studies, which used DNA–DNA hybridization and estimated that perhaps as little as 1% of the organisms were detected by traditional means of analyses (Torsvik, 1990). In contrast, nearly every other taxonomic subgroup of ecology has at least been able to work with lists of species present in a community, though perhaps with some taxonomic questions unresolved. Microbial ecology is now expanding this opportunity and has the potential of producing extensive, if not perhaps comprehensive, lists of resident microbial entities (species or operational taxonomic units – OTUs). These techniques were first applied for extensive characterization of the metagenome, or set of genes from all sampled organisms, in applications such as characterizing a single sample of sea water or soil (Handelsman, 2004; Woyke *et al.*, 2006). The volumes of data from the metagenomic next-generation sequencing studies enable microbial ecologists to characterize microbial communities in greater detail than before. At the same time, while these data are powerful, these tools must be developed hand in hand with reference databases to permit molecular identification.

The study of microbial metagenomes has been evolving in multiple ways. First, more sophisticated experimental designs are being employed that support better statistical hypothesis testing and construction of confidence intervals. Some of the first studies using high-throughput techniques tended to focus on comparisons of samples with little or no replication (Sogin *et al.*, 2006; Roesch *et al.*, 2007). The use of DNA tags for sample identification has permitted wider multiplexing and thus helped to make it more practical to use replicated designs. Unique DNA tags can be added to each sample prior to pooling of samples, so that each sequence obtained can be assigned to its original sample based on that DNA tag sequence. Researchers can determine a balance between sequencing depth per sample and total number of samples to sequence: increase in sequencing depth inevitably leads to lower replication and vice versa. Higher levels of replication are often necessary for understanding ecological processes, where many factors may influence system variation, so DNA tagging makes it practical to incorporate metagenomic information in studies of disease ecology. Simultaneously, greater sequencing depths may be desirable for a more accurate approximation of the richness and diversity maintained within a system, if that is an important goal.

The development of high-throughput sequencing has been an important advance for disease ecology, by making analysis of microbial communities in replicated experiments practical for typical project budgets. For example, an epidemiological experiment that includes, say, 16 experimental units could now include generation of a list of the relative frequency of the most abundant microbial taxa associated with each experimental unit at a couple of time points for approximately US $2000. New sequencing advances will likely make community characterization even less expensive, and open up new possibilities. Illumina and Ion Torrent platforms, which generate large numbers of short sequence reads, will support sequencing of metatranscriptomes (expressed portions of the genome), where repetitive regions are less common, as well as metagenomes at much lower costs.

Sequencing technologies are evolving at an extremely rapid pace. The recent development of the Pacific Biosciences (http://www.pacificbiosciences.com/) platform supports single molecule sequencing by fluorescent

nucleotides and provides impressive lengths with a potential for analyses of intraspecific variability in genes or their expression (McCarthy, 2010). These and other emerging fourth-generation sequencing technologies such as the Ion Torrent (www.iontorrent.com) or Oxford Nanopore (www.nanoporetech.com) are likely to even further expedite these advances. It is very likely that these platforms will allow even greater expansion in data volumes and parallelism. These developments will further reduce the cost of sequencing and make the tools and hardware more accessible to the research community at large. Microarrays can also be used to characterize communities by evaluating sequences known a priori to be of interest, even if the sequences are very rare. In contrast, sequencing, unless very extensive, tends to yield relatively more common taxa, including those that may not be known a priori.

The taxonomic identity of microbes has the potential to be used to infer functional roles, and our ability to draw such inference should increase rapidly as more microbial community data sets are generated and analyzed. Inference about role may be straightforward for some taxa, such as those that are always plant pathogens. For other taxa, such as *Fusarium oxysporum*, inference about function may be complicated by the wide variation in ecological roles observed within the taxon. To fully utilize these inferences, well and correctly accessioned databasing of vouchered organisms is essential. Voucher organisms need to be maintained in culture collections such as the American Type Culture Collection (ATCC) and Central Bureau für Schimmelcultur (CBS) not only for rDNA reference, but for future genome sequences and proteome characterizations, physiological characterization, and potentially for future experimental manipulations.

Leveraging new genomic tools to understand plant health under climate change

Baselines

While maps of invasive plant species observations have been developed for many areas of the world, a comparable approach for invasive microbial species associated with plants has been inconceivable for any but the most economically important plant pathogens. An important application for new sequencing tools will be characterization of microbial communities as baselines for understanding the effects of global change, including the effects of climate change and the redistribution of invasive species. While it is likely that major changes have already occurred through human influence over recent centuries (as, for example, with earthworm invasions), it will be important to catch up in our understanding of these structures. As environmental microbe datasets become more commonly available, tools can be developed to synthesize long-term trends and to identify important new movements of pathogens and invasives.

There may be feedback loops for some types of environmental effects. For example, disease management strategies that work under lower pathogen pressure may have reduced efficacy when abiotic environments are more conducive to disease and so become 'saturated' with pathogens (Garrett *et al.*, 2009). Biocontrol may be subject to this type of constraint. Likewise, forms of disease management such as sanitation (removing infected plant materials within and near a field) may have reduced efficacy if abundant regional inoculum sources make within-field inoculum sources irrelevant. Because of these types of feedbacks, small changes in climatic favourability to disease may be magnified.

Indicator taxa and indicator genes/functions

One relatively straightforward benefit that could be derived from understanding microbial communities would be the identification of indicator taxa, or indicator genes. An ideal outcome in some scenarios would be development of a simple indicator analysis (a colour change test strip!) for the presence or absence of indicator taxa or genes, including beneficial community components. The test could indicate (a) the need for addition of particular microbes to improve the environment for plant health, or (b) the need for abiotic additions such as organic matter to support shifts in the microbial environment.

A second 'revolution' in microbial ecology, corresponding to our new ability to characterize whole communities, would occur if tools were designed to effectively manipulate microbial communities in natural environments such as soil without introducing substantial artifacts. This could ultimately help to avoid accumulation of genomic information that is not 'translated' into better management (Evans *et al.*, 2011).

Associating microbial community composition with plant phenotypes: analogies from QTL and association mapping experimental designs

The development of high-throughput sequencing technologies has led to changes in the way in which connections are made between plant phenotypic variation and the underlying causative genetic variation, increasing the speed and resolution at which quantitative trait loci (QTLs) can be mapped to the nucleotide level. These

changes have been a boost to plant breeding strategies for increased productivity, and it may be possible to adapt some of the tools and concepts developed in mapping plant QTLs to address the problem of dissecting the functional role of individual members of soil microbial communities.

Although several methods exist to detail connections between phenotypic variation and genetic variants, the most popular and perhaps simplest conceptually is QTL mapping (Mauricio, 2001). In this method, two parental strains must differ at genetic loci that affect variation in the trait of interest. A large mapping population is created by crossing the parental strains and thus shuffling the parental alleles in different combinations. The phenotype of each member of the mapping population is measured, as are the genotypes of genetic marker loci that differed between the parents, and a statistical association between trait and genotype at individual loci implies linkage of that marker to an underlying causative locus. No longer is the development of molecular markers the rate-limiting step in QTL analyses. The low cost of sequencing and genotyping has meant that the determination of quantitative trait nucleotides (QTN) is now impeded by the resolution provided by the number of genetic recombination events in the mapping population. Many crossing schemes have been devised to maximize the shuffling of linked parental alleles through recombination to provide maximum resolution of the location of causative loci.

An alternative to QTL mapping employing classical linkage analysis that has gained traction in plant genetics is genome wide association (GWA) mapping (Nordborg & Weigel, 2008). GWA makes use of the variation segregating in a large sample from a natural population without explicit information about pedigree relations rather than variation segregating between two parents. GWA assumes causative loci found in different individuals share a common origin, and the standard cosegregation of linked alleles has been shuffled by the large number of recombination events in the ancestry of the sample. GWA mapping can provide higher resolution than classical linkage analysis and can detect more causative loci from the broader population, but GWA is also more susceptible to false positives due to the structure of the population sample. GWA mapping also requires a much higher density of genetic markers than classical linkage analyses, but this is no longer a problem in an era where genome resequencing and high-throughput genotyping are becoming more common.

Finally, the combination of classical linkage mapping with association mapping is being achieved in specially designed mapping populations in plants (Kover *et al.*, 2009; McMullen *et al.*, 2009) and other model organisms (Aylor *et al.*, 2011). These populations provide fine-scale mapping resolution without the confounding common to standard GWA studies. Both QTL and GWA gain power to the extent that they can minimize sources of variation.

Correlative studies of microbial taxa associated with increased plant productivity, powered by high-throughput DNA metagenomic sequencing, are similar to modern GWA studies. Different microbial communities sampled at different locations do not have a clear shared history with each other, but confounding may arise due to the association of microbial communities to common physiochemical properties of their abiotic environment. Certain microbial taxa may also be associated with each other across samples, and pinpointing the causative role of individual taxa requires breaking up these associations by a process analogous to genetic recombination.

Soil mixing experiments can be devised as analogues of plant crosses used in plant QTL analyses, in what could be considered a *quantitative trait taxon* (QTT) analysis (Table 1). Soil mixing experiments can be thought of as a type of artificial ecosystem selection experiment (Day *et al.*, 2011). If two (or more) soils known to have different microbial communities are mixed together, this will tend to result in new microbial communities, new combinations of microbial taxa, which will vary from one mixed replicate to another. This variability in taxa from one mixture to another is key to the potential success of a QTT experiment; if all mixtures have exactly the same microbial profile there is no variation to study, yet variation within a mixture must be low enough that an individual mixture can be characterized well through sampling. Variability from one mixture replicate to another is likely to be present because of the high diversity of microbial communities even across small spatial scales (Fulthorpe *et al.*, 2008). If the same proportions of each soil are added to each mixture, the physical and chemical properties of the mixtures will tend to be similar (though this may need to be confirmed). When a replicate study plant type is grown in each soil mixture, the differences in productivity from one replicate to another can be related to the differences in soil microbial communities.

The potential value of soil mixing or quantitative trait taxon experiments would come from being able to generate and analyze a range of microbial communities and their effects on plant productivity. Mixing of two soil types may produce fewer artifacts than other methods for manipulating microbial communities, such as soil sterilization, the application of selective biocides, or the application of particular nutrients. The effect of this range of microbial communities could be studied in different environments representative of current and future climate scenarios.

Table 1. Comparison of soil mixing experiments (quantitative trait taxa (QTT) experiments) and QTL experiments.

Aspect	Soil mixing experiment (QTT experiment)	QTL experiment
Goal	Identify microbial taxa associated with changes in plant productivity	(In this context) Identify genetic loci associated with changes in plant productivity
Methods	Physically mix soils with different microbial communities, grow plants in the mixtures, and study which taxa in the resulting mixtures are associated with changes in plant productivity	Cross parents with different genetic profiles and study which genetic markers in their offspring are associated with changes in plant productivity
Why this method can work	Generates soil mixtures with a range of different combinations of microbial taxa	Generates offspring with a range of different combinations of genetic markers
Changes over time	Microbial taxa most adapted to a soil mixture have the potential to reproduce rapidly	No substantial change in an individual's genetic make-up over time
Limitations of association with particular taxon or locus	Microbial taxon associated with higher productivity may simply be spatially associated with another taxon that has the causal impact	Gene associated with higher productivity may simply be spatially associated with another gene that has the causal impact
Limitations for inference about causality	It is possible that greater plant productivity causes higher abundance of some microbial taxa	No substantial limitation; plant productivity does not change genotype (though it can change gene expression)
Interactions/Epistasis	Certain taxa may only have effects if other taxa are present	Certain genes may only have effects if other genes are present
Importance of abiotic environment	New soil physical and chemical characteristics may modify microbial taxon's effect on productivity	The choice of abiotic environment may modify genetic effects on productivity
Spatial structure	Spatial structure of association of microbial taxa in soil matrices is challenging to study and has the potential to change	Spatial structure of association of genes on chromosomes can be mapped and is unlikely to change quickly

A major complicating factor in microbial community analysis is accounting for the interactions among individual taxa. One-dimensional genome scans of phenotype-marker associations that ignore possible interactions between loci (epistasis) are extremely common, although many experimental studies indicate epistatic gene interactions play a prominent role in the genetic control of many traits. As long as the genes underlying phenotypic variability have relatively large additive effects, they will be detected in QTL mapping, though in some cases gene interaction will prevent the detection of loci unless these interactions are incorporated into the model (Carlborg *et al.*, 2006). The complex interactions of taxa in microbial communities make this type of epistatic interaction more likely in soil mixing experiments. Potential problems for such soil mixing experiments include the following issues for which there are not comparable problems in QTL analyses. (1) Perhaps most importantly, the microbial taxa present can change over time as populations expand or die out. This is a problem if communities in all mixtures change to become more and more similar, and studies would be needed to address the timescale at which microbial communities reach a stable composition. (2) It is possible that any difference in microbial communities from one mixture to another will be associated with a particular change in the physical and chemical properties, such that the effects of taxa cannot be teased apart from the effects of soil properties. (3) It is possible that higher plant productivity has a causal effect on some microbial taxa, rather than vice versa – in other words, our attempt to measure the extended phenotype of the soil microbial community changes its composition. (4) Limitations to current understanding of the spatial structure of soil matrices, and the spatial structure of microbial taxa within these matrices, may be greater than limitations to current understanding of plant chromosomes.

Of course, most of these problems are not unique to soil mixing experiments, but are equally a problem in any observational studies of soil microbes linking microbial community structure to plant disease or plant productivity. The key is to have a lower level of variability within an experimental unit and a higher level of variability among experimental units.

The Arabidopsitron: leveraging plant genomic tools to define the extended phenotype of the soil metagenome

The extended phenotype of a genotype can include a range of responses (Dawkins, 1982; Whitham *et al.*, 2003). The structure of a soil, itself, can be considered an extended phenotype for the organisms that contribute to

its structure (Phillips, 2009). From the standpoint of plant health, and the effects of climate and climate change on it, the extended phenotype of an environmental microbial community might be measured in terms of the productivity and other characteristics of plants growing in that environment.

Identifying components of microbial communities that increase plant productivity by comparative studies of natural or mixed soil samples requires a highly efficient phenotypic screening tool. Because the molecules or conditions present in the soil samples that promote productivity are generally unknown, measuring plant productivity as an extended phenotype of the soil metagenome would be the most direct assay. As technology to characterize genomes has improved rapidly, methods for high-throughput, highly parallel phenotyping have lagged somewhat. This is an important problem to plant breeders, who are manipulating plant genomes for clues to increase their productivity, and they have developed imaging technology to increase the efficiency of measuring phenotypes (Furbank & Tester, 2011). This 'phenomics' approach could also be used to identify the key functional genes in microbial communities that are important for plant productivity.

There is precedence for using plants as an extended phenotype of soil microbial communities (Swenson *et al.*, 2000). The use of small model plants such as Arabidopsis allows for more plant genotypes at higher replication to be grown on common soil samples. Smaller soil requirements per experimental unit also make it easier to reduce the variability in microbial communities within that unit, making it easier for many plant genotypes to experience a similar microbial community and making it easier for a representative sample of microbes to be collected. The choice of Arabidopsis is attractive, as it is arguably the easiest model to work with and has the largest number of genetic resources, including natural accessions and mutations in nearly every gene available (Alonso *et al.*, 2003). Different components of the soil metagenome may have an effect on some plant genotypes and not others, so an 'Arabidopsitron' could be composed of a panel of many genotypes – either a selection of mutants most likely to reflect interactions with microbes, or a panel of natural ecotypes that reflects the diversity of interactions with microbes across its native range. In the second case, the genetic variation that explains differential interactions with microbes could easily be mapped using GWA if the panel ecotypes are chosen from the large set with their genome already resequenced (Weigel & Mott, 2009). Panels could be calibrated by reference to selected crop plants, and might be expanded to include other small and efficient model plant species such as *Brachypodium* that may more readily represent effects for important monocots, or *Medicago truncatula* for evaluation of legumes and symbiotic processes with rhizobia.

Policy

At the same time that our understanding of microbial communities is in the early stages of development, there is the need for policies to conserve microbial resources. The UN Food and Agriculture Organization has begun the formulation of concepts for the conservation of microbial genetic resources (Beed *et al.*, 2011). The legal and economic context for sharing of microbial resources is also being developed (Dijkshoorn *et al.*, 2010; Kothamasi *et al.*, 2011). The potentially complex interactions among microbes may need to be considered explicitly in formulating and evaluating policy (Stirling, 2010). Understanding of the contributions of microbial communities to plant health and food security will be important for setting policy priorities.

Acknowledgements

Thanks to T. Forge, Z. Punja, and other symposium organizers and discussants at the meeting of the Canadian Phytopathological Society. We appreciate support from The Ceres Trust, US DOE BER Grant ER65000, US NSF Grant EF-0525712 as part of the joint NSF-NIH Ecology of Infectious Disease program, US NSF Grant DEB-0516046, USDA APHIS Grant 11-8453-1483-CA, USDA NC RIPM Grant 2010-34103-20964, and the Kansas Agricultural Experiment Station (Contribution No. 12-446-J).

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