

COLLECTION REVIEW

A multi-omics approach to solving problems in plant disease ecology

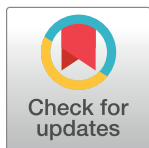
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Abstract

The swift rise of omics-approaches allows for investigating microbial diversity and plant-microbe interactions across diverse ecological communities and spatio-temporal scales. The environment, however, is rapidly changing. The introduction of invasive species and the effects of climate change have particular impact on emerging plant diseases and managing current epidemics. It is critical, therefore, to take a holistic approach to understand how and why pathogenesis occurs in order to effectively manage for diseases given the synergies of changing environmental conditions. A multi-omics approach allows for a detailed picture of plant-microbial interactions and can ultimately allow us to build predictive models for how microbes and plants will respond to stress under environmental change. This article is designed as a primer for those interested in integrating -omic approaches into their plant disease research. We review -omics technologies salient to pathology including metabolomics, genomics, metagenomics, volatilomics, and spectranomics, and present cases where multi-omics have been successfully used for plant disease ecology. We then discuss additional limitations and pitfalls to be wary of prior to conducting an integrated research project as well as provide information about promising future directions.



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Introduction

Plant disease ecology is inherently interdisciplinary and relies on the disparate fields of microbial ecology, epidemiology, plant physiology, and genetics to inform a critical observation: plants get sick. For more than a century, plant pathologists have observed that virulent microbes (e.g. bacteria, fungi, viruses, oomycetes) drive disease dynamics in susceptible plants given the right environmental conditions [1]. In natural systems, many plant pathogens have co-evolved antagonistic relationships with their hosts, and thus plant disease acts as an important force to regulate plant populations [2–4]. In managed systems, such as forest plantations or agro-ecosystems, plant health is important for maintaining yield and actively managing for plant diseases is a priority among land managers and farmers alike. Understanding and managing the ecology of these systems, however, has traditionally relied on a reductionist approach

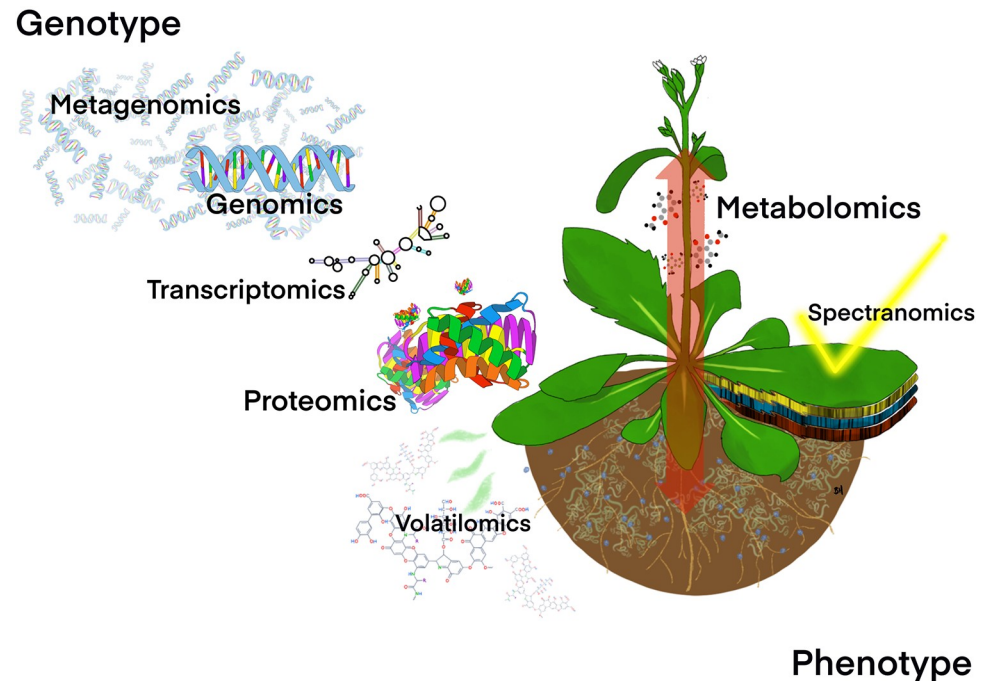


Fig 1. Multi-omics approaches inform the genotype to phenotype cascade. Omics approaches from the genotype (top left) to the phenotype (bottom right) inform plant disease ecology in a holistic manner and can shed light on microbial communities above and belowground. Red arrow on plant symbolizes dynamic intra-plant communication in the metabolome affecting the microbial community through released volatiles. Yellow check is light for spectranomics with multiple bands of sensing (below leaf).

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to research individual components (e.g., single or few microbe-plant interactions) related to specific disease pathologies rather than the complex ecological interactions between communities of microbes, hosts, and the environment.

Technological advances over the last few decades have enabled a new, more comprehensive approach to plant disease ecology allowing a holistic approach to identifying pathogenesis and its underlying mechanisms. Among these technologies are -omics tools that allow for the comprehensive examination of plant and microbial characteristics along the genotype-phenotype spectrum (Fig 1). Understanding these characteristics in a comprehensive fashion has facilitated discovery of mechanisms driving the ecology of plant defense and development of more effective management strategies ranging from improvements in plant breeding to field practices curtailing spread of plant pathogens.

These developments in omics-approaches to plant disease ecology have been particularly important in light of the current, rapidly changing environment. As increasing biotic and abiotic stressors impact plant health, adapting to and mitigating the effects of these stressors is critical for maintaining both healthy terrestrial ecosystems and productive agricultural systems. For example, globalization and the movement of people, plants, and microbes around the world can drastically change the composition and ecology of habitats. Invasive microbial pathogens and/or soilborne pathogens, especially those with broad host ranges, can cause the death of, in some cases, thousands of trees across a landscape [5, 6]. Both ecological invasions [7] and climate change are changing microbes and their environment [8]. For instance, shifting temperatures and the frequency and duration of weather conditions over time, result in phenomena such as the rapid evolution of microbial pathogens or environmental stress which can weaken plant hosts [8].

While some benefits from more holistic approaches have been realized, further opportunities exist through increasing integration of -omics technologies and platforms. Insights from genetics approaches can be tied to transcriptomics, metabolomics, and spectranomics to link multiple steps in the -omics cascade from genotype to phenotype. This integration, while still in its early stages, holds vast potential for unlocking fundamental and applied insights in microbial plant disease ecology. Understanding the genetic basis for plant pathogen susceptibility, how the plant microbial community influences expression, the cascading metabolomic and volatilomic pathways resulting from infection, and the spectranomic characteristics of foliage present numerous opportunities for intervention that can be tuned and optimized for plant disease management situations.

In this review, we discuss exactly these opportunities by beginning with an overview of -omics technologies including the unique challenges that each field faces. Next, we present recent case studies that highlight integration of multi-omics insights. We then offer additional opportunities and considerations for integrating these technologies and touch briefly on expected benefits resulting from this integration. These opportunities and considerations will be discussed in light of our principal goal: driving the development of healthy plants and their microbial communities in a constantly changing environment.

The omics

Genomics

The field of genomics, the study of the complete genetic makeup of organisms, has grown exponentially in the past 20 years since the milestone publication of the first draft of the human genome in 2001 [9, 10]. Although the word ‘genomics’ is somewhat recent [11], its origin dates back to the early 1900s when Johannsen introduced the concept of the gene and later when Hans Winkler coined the term genome in 1920. The first genomes sequenced were microbial, heralding an era of tool development and exponential generation of whole genome sequences: the bacterium *Haemophilus influenzae*, the first free-living organisms to have its whole genome sequenced via shotgun sequencing [12]; the fungus *Saccharomyces cerevisiae*, first eukaryote to have its whole genome sequenced [13]; and the nematode *Caenorhabditis elegans*, the first multicellular eukaryote [14]. Further development of Next Generation Sequencing (NGS) technologies has drastically reduced sequencing costs and accelerated the availability of whole genome sequences, *de novo* sequence assemblies and resequencing of multiple strains of a single species.

Microbial genomics is an interdisciplinary field that focuses on the structure, function, evolution, mapping, and editing of genomes for bacteria, fungi, archaea, viruses, and other microscopic organisms. Integration of genomics questions and tools can aid ecological questions, especially those that involve environmental change. For example, it is critical to understand the evolutionary history of the genome to understand if certain elements may change quickly under changing temperatures (rapid evolution). Sometimes, it may be necessary to draw comparisons across genomes to understand microbial functions in related species, or delve into transcriptomics and other -omics. Coupled with transcriptomics and proteomics, functional genomics uses genomic data to study gene and protein expression and function on a genome-wide or system-wide scale. The integration of genomics and transcriptomics has resulted in an increased understanding of various aspects related to plant pathogen biology, plant-pathogen interactions, and plant health [15, 16].

In investigations of plant disease ecology and plant health, it can be helpful to consider two main groups of microbial genomics. Structural genomics, focused on assigning and mapping genes and markers to individual chromosomes, results in a physical map of the whole genome.

Functional genomics integrates genome sequences with an organism's transcriptome (transcripts produced by a given organism) and proteome (encoded proteins) to describe gene functions and interactions [17, 18]. The combination of genome data and NGS-based RNA sequencing has significantly improved gene annotation and gene prediction [19]. However, there are many more “types” of genomics to be considered depending on the focus and context of research being done in contemporary plant disease ecology.

Comparative genomics aims to identify structural and functional genomic elements conserved within species or across different species [20]. Using this approach, we now know that the genome architecture of many fungal plant pathogens is highly diverse and dynamic, comprising regions of rapid evolution that can range from transposon-rich chromosome compartments to entire accessory chromosomes [21]. For example, by comparing the genomes of four *Fusarium* species clear genomic compartmentalization has been identified at both structural and functional levels: a core component of the genome that encodes functions necessary for growth and survival and is shared among *Fusarium* species, and an accessory component [22, 23]. The accessory genome comprises a variable number of small (<2Mb), supernumerary or conditionally dispensable chromosomes that are enriched for transposable elements, and carry what seem to be horizontally-transferred genes, some of which are known to be involved in plant pathogenicity. Similarly, *Verticillium* genomes differ in four regions of approximately 300–350 kb each, which are present in *V. dahliae* Ls.17 and absent in *V. alfalfae* Ms.102. These four regions contain genes that seem to vary considerably between strains and have functions putatively involved in pathogenicity and virulence of the pathogens [24, 25]. As more genomes are available and compared, we realize that these chromosomal dynamics are prevalent in many other plant pathogenic fungi [26–28].

In addition to elucidating genomic architecture, phylogenomics and population genomics address similar questions, but at different biological scales: relationships among species, and individuals and populations within species, respectively. The types of questions addressed include genome-wide evolutionary and demographic processes affecting population structure, evolutionary processes affecting speciation and the divergence of closely related taxa, and locus-specific effects, acting on specific genes or chromosomes that affect adaptation or defined phenotypes [26, 29, 30]. Such data provide a foundation for understanding the evolutionary potential of plant pathogens. One of the challenges we face with microbial genomics is the difficulty to establish and define species boundaries and population communities given the complexity of genome dynamics and the amount of observed horizontal gene transfer [31–34]. How these dynamics are affected by a changing environment in which we may see more host jumps and extreme environments is unpredictable [22, 33, 35].

Metagenomics

Metagenomics builds on the tools and theories from molecular genomics to investigate DNA that is sourced directly from the environment. This field involves the extraction and amplification of microbial DNA targets from soil, water, air, plants and animals [36]. Although it is estimated that microbes are ubiquitous and found in every ecosystem on the planet, only approximately 1% of all microorganisms are culturable and identifiable using traditional first-generation, Sanger sequencing technologies [37]. The field of metagenomics grew out of the need for understanding the diversity and functional role of unculturable microbes. Microbiologists and plant pathologists first pioneered the techniques and tools in the late 1990s [38, 39].

Today the field has evolved to answer questions related to the ecological dynamics of microbial communities, multi-species regulation, mechanisms for microbe-microbe and host-microbe interactions, community coevolution [40]. The term metagenomics itself is

comprised of the Greek prefix meta- which means to ‘transcend’ and describes how this discipline goes beyond traditional genomic techniques to identify the genomic diversity and gene function of microbes of what some scientists have coined unculturable microbial ‘dark matter’ [37]. With recent advances in high throughput DNA sequencing techniques, we are able to sequence either partial or the entire genome of microorganisms to answer questions such as microbial species composition, structure, phylogenetic relatedness, and function on the scale of entire microbial communities [41]. Today, a typical metagenomic pipeline involves the initial collection and processing of environmental DNA, the bioinformatic pre-processing of DNA sequence reads, determining the taxonomic profile as well as any functional or genomic elements of interest, statistical analyses, data validation, and finally visualization and communication of the results [42].

A major challenge in metagenomics is defining the microbial species concept itself. The biological species concept works well with more derived taxa (such as ourselves, *Homo sapiens*) where reproductive boundaries and barriers are strongly delineated. Bacteria, on the other hand, are currently assigned to a common species if their reciprocal, pairwise DNA re-association values are $\geq 70\%$ during DNA hybridization experiments [43]. The common issue of microbial vertical transmission of DNA can complicate matters even further. Traditionally speaking, strains within a species must possess a certain degree of phenotypic consistency and species descriptions should be based on more than one type strain, such as a genetic variant or subtype. A microbial species name should technically only be assigned if its members can be distinguished from other species by at least one diagnostic phenotypic trait [43]. Of course it is difficult to assign microbial phenotypes to unculturable taxa. Another limitation for assigning taxonomic monikers is because of the lack of comprehensive public sequence databases available to map genetic reads to. Current databases such as GenBank and others such as GreenGenes for bacteria and UNITE are great start, but still lack genomic information about the vast diversity of microbes still undiscovered on the planet.

In the case of plant health, metagenomic studies have revolved around two major categories to date. The first is for promoting plant growth through understanding endophytic microbes either within the shoots or roots communities [44]. The vast majority of these studies are still conducted with model plant organisms, such as maize, and there is a need to expand to other crops and into more natural systems. Others have ventured beyond the root into the rhizosphere and rhizosphere and use metagenomics to explore the role of functional genes for plant and soil health [45]. The second type of plant health research involves understanding how soil microbes suppress plant pathogens [46]. Again, the few studies conducted here are in crop pathosystems and there is a need to study the phenomenon of suppressive soils in other ecosystems, for example, managed and unmanaged forests and grasslands.

Metabolomics

Metabolomics, the comprehensive profiling of all small molecules within an organism, is at the phenotypic end of the -omics spectrum and captures the results of the information cascade beginning with the genome and progressing through the transcriptome and proteome [47] (Fig 1). While metabolomics is firmly rooted in the chemical analysis of individual constituent compounds, the field has rapidly progressed in the past two decades to the ability to rapidly and exhaustively assess metabolites in multiple spatial and temporal dimensions [48–50]. While there are still limitations to attaining a complete understanding of the vast diversity of metabolites within a given organism or plant, targeted and untargeted approaches provide deep insights that, when coupled with complementary omics approaches, can yield an abundance of insights [51–53].

Metabolomics began in the 1970s with the expansion of medical analyses of compounds of interest using gas chromatography-mass spectrometry [54]. As technologies improved, medical profiling of human metabolites continued to expand for diagnostics and drug development. Applications of these profiling techniques in plants began in the 1990s with investigations into herbicide mode of action [55]. Linking functional genomics with metabolomics began in the late 1990s and has expanded in line with increasing technological capabilities in chromatography, mass spectrometry, and imaging technologies [54, 56]. Current metabolomics technology—typically ultra-high-pressure liquid chromatography coupled with high-resolution mass spectrometry or nuclear magnetic resonance spectroscopy—provides incredible resolution into the chemical phenotypes of study organisms able to capture and profile information on thousands of compounds [57–59].

These advances are not without limitations, however. A key challenge in metabolomics is the sheer diversity of potential metabolites present in any given sample. In contrast to microorganisms or animals producing on the order of 1,500 and 2,500 unique metabolites respectively, a single plant species can produce upwards of 5,000 unique compounds [52]. All told, an estimated 200,000 unique metabolites are present in the plant kingdom [52, 60].

Targeted metabolomics approaches address these challenges by constraining profiling to a known set of annotated compounds [61]. Precise quantitative analysis of these compounds becomes possible through comparison to known libraries of analytical standards [62, 63]. While necessarily less than comprehensive, targeted approaches nonetheless can yield detailed information on a wide array of compounds numbering in the hundreds depending on the techniques used [62, 63]. Targeted metabolomics approaches are particularly useful for examining known processes and responses and allow for methodological optimization and improved resolution into biological processes of known importance.

Untargeted metabolomics approaches, in contrast, provide insight into unknowns [64]. Approaching the ideal of truly global metabolomics, untargeted metabolomics seeks to profile vast numbers of compounds present in biological samples through comparison of relative intensities [64]. In some cases, these compounds may be annotated and putatively identified [65]. Eschewing constraints allows broad comparisons to be made within and between experimental units for discovery of pathways and compounds driving biological processes [53]. Particularly important for multi-omics approaches, untargeted metabolomics facilitates connection discovery linking genomic, metagenomic, volatilomic, and spectromic approaches [52, 53].

Volatilomics

Strictly speaking, volatilomics could be considered a subset of metabolomics [66]. However, the comprehensive profiling of high vapor pressure compounds released from organisms, volatilomics is much more than just a subset of metabolomics [67]. Where metabolomics seeks comprehensive profiling of predominantly intra-organisms compounds, volatilomics assesses those compounds released by an organism: the key components of chemically mediated inter-organismal communication [68]. Because volatilomics is concerned primarily with communication, it presents unique opportunities for understanding community dynamics in conjunction with a multi-omics approach.

As with metabolomics, plants use an astonishing array of compounds for communication ranging from plant hormones, terpenoids, green leaf volatiles, and volatiles produced and released upon attack from pests (herbivore-induced plant volatiles) [69]. These volatile compounds play numerous roles: influencing plant pollination, dispersal, herbivory and providing defenses against abiotic and biotic stress such as plant pathogens [68]. To date, it is estimated

that plants alone release on the order of 30,000 different volatiles into both the atmosphere and rhizosphere [69]. Indeed, volatile communication is potentially even more important below-ground where plant volatiles mediate everything from plant-pathogen interactions to plant-herbivore-natural enemy interactions in an environment where visual information processing is absent [70, 71].

The field of volatilomics grew out of the field of metabolomics as advances in gas chromatography coupled with mass spectrometry allowed for more in-depth profiling of gaseous volatile compounds [66]. Critical to the development of volatilomics as a field has been advances in collection methods of volatile organic compounds. To retain volatile compounds used in plant communication, headspace collection or sorptive materials are often used to capture volatiles in preparation for introduction to analysis by GC/MS [67]. These more modern approaches have begun to be used in high-throughput phenotyping where rapid assessment of plant volatilomic profiles are connected with gene by environment interactions in a non-destructive multi-omics approach [72]. Extending this approach to the plant-related microbiome holds the potential for discovery of new opportunities for identifying and controlling pests and pathogens [73, 74].

Spectranomics

Foliar functional trait characterization has emerged in terrestrial ecology as a unifying concept to better understand both natural variability in vegetation function and variability in response to environmental change and stress. The idea of foliar functional traits has been enthusiastically embraced by the remote sensing community as many traits shown to strongly correlate with natural and stress-induced variation in plant function [75] can be detected and quantified from remotely sensed imagery [76–79]. Non-destructive, proximal and remote sensing of foliar functional traits with spectroscopy offers the capacity to fill gaps in space and time between labor intensive field measurements, reducing uncertainty in downstream analyses and decision making, as well as offering the ability to better evaluate hypotheses about plant function in response to abiotic and biotic stress. The use of spectroscopy combined with chemistry, taxonomy, and community ecology has been coined by leaders in the field as “spectranomics,” [80, 81]. The foundational components of the spectranomics approach is that 1) plants have chemical fingerprints that become increasingly unique when additional constituents are incorporated [77] and 2) spectroscopic signatures determine a portfolio of chemicals found in plants [82].

Only recently have plant pathologists begun to take advantage of the spectranomics trail blazed by terrestrial ecologists. Recent studies have helped establish in-situ (or foliar) and imaging spectroscopy (also known as “hyperspectral imaging”) as effective tools for early, non-destructive, and scalable biotic stress detection in natural and agroecosystems [83–86]. Both beneficial [87] and parasitic plant-microbe interactions [88] impact a variety of plant traits that can be non-destructively sensed. Microbes both directly and indirectly damage, impair, and/or alter foliar function, thus changing the chemical composition, such as through production of systemic effectors or secondary metabolites, or by physical presence of pathogen structures, such as hyphae and spores [89]. By taking a statistical approach to spectral data analysis, the sum total of the changes microbes impart to plant health can be quantified with proximal and remote spectroscopy [83, 84]. This approach has been successful in increasing efficiency and accuracy in agronomic crop breeding pipelines [90–92]. Thus, spectral quantification of foliar functional traits allows us to detect, map, and model the biochemical and physiological pathosystem processes that engender our capacity to use spectroscopy for plant-microbe interaction sensing [86, 93–99].

Broadband and multispectral methods relying primarily on visible (VIS) and near-infrared (NIR) reflectance indices, such as normalized difference vegetation index (NDVI), have been used to sense late stage plant disease since the 1980s [100–103]. Changes in continuous, short wave infrared wavelengths (SWIR) have proved valuable for plant-microbe interaction sensing due to SWIR sensitivity to a range of foliar traits [104], including nutrient content [79, 105–107], water [108], photosynthetic capacity [109], physiology [110], phenolics and secondary metabolites [94, 111, 112], that are all impacted by plant microbe interactions. The advent of VSWIR (400–2500nm) sensing has reinvigorated the discipline of plant-microbe interaction sensing with its newly established capacity for robust pre-visual disease detection [94–99, 111, 113–116].

Case studies in plant disease ecology under changing environments

Multi-omics in plant defense: The salicylic acid pathway

Plant defence pathways mediate plant microbial interactions in changing environments regulating responses to abiotic and biotic stressors above and belowground [117–119]. A comprehensive understanding of the mechanisms underlying this ability and the nature of plant-mediated effects on microbial communities necessitates an integrated multi-omics approach. Nowhere is this as apparent as with the role of the salicylic acid (SA) signaling pathway. While there are many important plant defence pathways involving other critical plant hormones such as abscisic acid and jasmonic acid, for example, the SA pathway plays a critical role in regulating plant disease ecology with regulation of plant systemic acquired resistance and mediation of the interactions between members of plant microbial communities [120].

Understanding SA signaling began with early molecular studies as realization dawned that this phenolic secondary metabolite was nearly ubiquitous in the plant kingdom and involved in everything from plant reproduction and photosynthesis to mediating responses to abiotic and biotic stresses [121, 122]. These approaches began with metabolomics; interest in this signaling molecule drove research into networks of plant metabolites related to salicylic acid and their role as chemical signals [123, 124]. Initially, biochemical pathways involved in the production of SA were established picking up where the shikimic acid pathway left off and further developing through parallel isochorismate synthase and phenylalanine ammonia lyase pathways to production of the actual SA molecule which can be further modified downstream through addition of molecular tags such as methylation, glucosylation, and conjugation with amino acids [123–125].

Following pathway elucidation, modern targeted and untargeted metabolomics approaches have characterized SA signaling cascades from plant stressors [126–128]. These metabolomics approaches have shed light not only on the manner in which plant responses are induced and effected, but also on SA induction cascades within the plant to affect other metabolic processes including cross-talk and regulation of other plant hormones [129, 130]. Particularly relevant for understanding microbial changes in the rhizosphere, metabolomics approaches are beginning to resolve how plant-microbe communication can shape microbial communities in changing environments [71]. Specifically, in the context of defense against plant pathogens, SA plays a role mediating pattern and effector triggered immune responses, producing of specific defense compounds ranging from anti-microbial peptides to terpenoids, programming death of infected and affected cells, and inducing systemic acquired resistance (SAR) [131–136].

While understanding of the SA pathway may have begun with metabolomics, it quickly progressed to inquiries into the genetic basis for observed phenoma, particularly related to systemic acquired resistance (SAR)—the ability of plants to acquire long-term resistance to

pathogenic microbes [136, 137]. Genomic studies identified critical nonexpressor of pathogenesis (NPR) genes involved in SAR and how these genes can be systemically and transcriptionally programmed in situations where resistance is induced [138–140]. In addition to these NPR genes, attack by microbial pathogens and induction of the SA pathway can influence a wide range of gene expression regulating transcription networks that control plant defense responses involved in systemic acquired resistance [141, 142]. These genomics studies hold particular importance for plant breeding where desired genes encoding for SAR against pathogens could be bred into plants to ensure advantageous manipulation of microbial communities [143–145].

The SA signaling pathway and systemic acquired resistance not only affects the plant pathogen microbial community, but also a whole host of other organisms [120]. To do so, mobile volatile signals are used such as the methylated form of SA, methyl salicylate (MeSA) [146]. Volatilomics approaches to SA signaling have revealed that volatile profiles of plants change upon induction of the SA defense pathway [120]. These changes in volatile profiles can result in the induction of SA responses in neighboring plants and recruitment of natural enemies aboveground and belowground in the rhizosphere [147–150]. Exogenous induction of SA responses in perennial and annual plants can recruit entomopathogenic nematode natural enemies over long distances altering community structure and relationships with other nematodes and nematode microbial predators [151].

Multi-omics for plant stress detection

The story of how these aspects of SA-regulated plant disease ecology were discovered is inextricably intertwined with the early history of multi-omics approaches. As integrated multi-omics has emerged, our resolution of the processes underlying SA plant defense signaling has improved and, with it, our ability to use this knowledge for positive intervention in managed natural systems. Early vegetative spectroscopic studies addressing the retrieval of foliar biochemicals primarily targeted pigments, nitrogen, protein, structural elements (cellulose and lignin) and mineral compounds [152, 153]. Soon after, this capacity was expanded to include phenolics and other classes of secondary metabolites associated with defense and the salicylic acid pathway [94, 111, 112]. In the past five years the boundaries of specificity in the characterizable trait range has expanded significantly to include specific parameters related to photosynthesis such as maximum carboxylation and photosynthetic rates, sucrose, starch, fructose and free amino acids in leaves [91, 110, 154].

Spectroscopic methods such as fluorescence spectroscopy, Fourier-transform infrared spectroscopy, and chemometrics are used to better assess and understand plant-microbe interactions, but these methods require sampling and processing [155, 156]. The ability to quantify and predict plant metabolites from passive monitoring with vegetative spectroscopy, especially under field conditions, would enable *in vivo* metabolomics and thus opening the door to a new generation of plant, and plant-microbe, phenotyping approaches. *In vivo* metabolomics, with understandably less specificity than traditional metabolomics, with hyperspectral sensing and its value to precision agriculture has been hypothesized by multiple sub-groups in the plant sciences, and most recently by Martins et. al [157]. Pioneering work by Vergara-Diaz et al. [158] showed for the first time, the ability of hyperspectral field-sensors to non-destructively estimate foliar metabolite profiles. This work found that about one-quarter of metabolites detected in wheat leaves and ear bracts by GC-MS profiling could be satisfactorily predicted with hyperspectral data with validation accuracy over 50%. Many of the metabolites they could predict included sugars, amino acids and organic acids that play a central role in primary and secondary metabolism. Vergara-Diaz found the blue region proved to be the

most relevant waveband for metabolite prediction regardless of the metabolite examined in both plant organs studied (leaf and ear). Likewise, these authors found consistency in their canopy models: the 1300–1400 and 2200–2400 nm regions were always the best determinants for metabolite prediction, and coincident with absorption bands known to be associated with sugars and nitrogen compounds.

Combining spectranomics and metabolomics would not be without its challenges: the assessment of plant biochemical traits at the canopy scale still faces considerable limitations from complexities related to canopy structure/density and the subsequent impact on light reflectance. Additionally, historical knowledge of the VIS-NIR-SWIR spectral features (spectral signals in plant reflectance) associated with plant metabolites is limited because typically other approaches have been used such as UV, MIR, X-ray, Raman or FTIR spectroscopy [155]. These more sensitive approaches can far more precisely quantify isolated metabolite concentrations, but do not account for how plant macro- and micro-physical properties impact how light interacts with these bonds. Prior knowledge of VIS-NIR-SWIR spectral features, such as those outlined in Curran [104] and Carter & Knapp [159], greatly aids in transferability and scaling, allowing us to make better sense of spectral data and the information it provides about underlying plant processes. Spectroscopic approaches sense genuine yet subtle changes to plant biochemistry, physiology, and morphology, all of which are impacted by plant-microbe interactions, and engender the origins our ability to use spectroscopic methods for plant sensing.

Multi-omics in suppressive soils for plant disease management

The observation that soils have the capacity to suppress plant disease has been known for decades, some would argue for almost a century or longer in agricultural settings [160]. Yet understanding the specific soil biota involved, in what combination and under which environmental conditions elicit a suppressive response has eluded microbial ecologists [161]. Recently, with the advent of -omics approaches, we are beginning to better understand the ecology behind the suppressive effect [162]. Disease suppressive soils hold a subset of microbes, typically bacteria, that prevent the infection of a root by a plant pathogen or the development of the disease. Suppressive soils can act in either a general or specific manner. Broad soil suppression occurs when the suppressive capabilities of broad microbial communities are harnessed, versus specific biocontrol which can occur using a single microbial taxon or a few taxa [163]. Understanding which taxa are involved in soil suppression using a metagenomics approach could reveal their various functions and shed light on the biological mechanism of suppression.

In order to apply basic knowledge of the soil microbial community identities and functions to address problems in the applied plant sciences such as conservation, restoration, forestry, and agriculture, it is essential to consider a multi-pronged approach. For instance, plant nutrient deficiencies have long been known to affect both crop yield, but also overall defense against pathogens. Using *Arabidopsis* as a model, researchers were able to tease apart the relationship between plant nutrient levels and host defense [164]. The authors found that the phosphate starvation response (PSR) plays an important role in shaping the root microbiome. The tools used to come to this result were a mix of amplicon-based sequencing to identify the host associated microbiome bacteria (16S) as well as genomic modeling and finally various metagenomic tests to reveal the functions of specific genes that modulated the response for the defense process [164]. The authors were able to conclude that certain beneficial microbial communities that help with plant defense against pathogens are shaped by the PSR.

Another application of a multi-omics approach is identifying more specific microbes that could be involved as biological control agents (BCA) against soilborne plant diseases and the creation of synthetic communities. Biocontrol research has typically taken a targeted approach that focuses on using a single or a few microbes to suppress pathogenicity. With the development of high throughput sequencing techniques, such as amplicon-based sequencing, entire microbiomes were and are continuing to be identified. There is now need to shift away from community description toward functional understanding of these microbial communities in their capacity to not only suppress disease, but potentially have one microbe or a suite of microbes elicit antagonistic interactions to hinder plant disease [165, 166].

What is still needed for understanding the microbial ecology of soil suppression and the potential for biological applications to suppress plant disease are studies that seek predictive patterns and results. Specifically the spatial-temporal distribution of soil suppression and whether certain pathogens are consistently suppressed by specific taxa or functional groups of soil microbes. For instance certain sustainable land management practices such as the use of cover crops [167], organic cultivation [168, 169], or the use of diversified crop systems rather monocultures [170] have all been found to suppress plant diseases at varying levels of success depending on the crop pathogen, season, and climate. Decoupling microbial identity, function, pathogen identity, function, and environment are the next challenges for microbial ecologists—and doing so outside the lab and greenhouse to account for environmental factors is sorely needed.

Frontiers in multi-omics for plant disease ecology

A promising area of -omics research is understanding the role of endophytes in plant disease. Endophytes are microbial organisms that live within plant tissue without causing visible symptoms and are essential to plant health and well being [171]. There is evidence for the critical role endophytes play in reducing herbivory both above and below ground [172], modulating plant immune response pathways and in some cases, systemic plant disease resistance [173], protecting from oxidative bursts and reduced gene expressions and remediating of abiotic and biotic plant through the specific downregulation of abscisic acid (ABA). Most research to date focuses on the function of one or a handful of endophytes rather than entire communities. One excellent study focused on the singular potato endophyte *Burkholderia phytofirmans* PsJN which should how various extra-cytoplasmic functional group elements (sigma factors, group IV) were critical in facilitating other bacteria to sense changes in their surrounding environment such as temperature or moisture and shift their metabolic activity to survive the change. This paper used a dual -omic approach, by using high throughput sequencing to identify both the key player, *Burkholderia*, other bacteria, and basic metabolomics [174]. More recently, studies have been conducted to understand the diversity and structure of foliar endophytic fungi in forest ecosystems [175], however, there is still much to be understood about endophyte ecology in other systems and at the biome scale [176].

An additional promising frontier is the integration of spectranomics with genomics to construct multi-dimensional phylogenies that capture the evolutionary dynamics of leaf chemistry, structure, and more. Recent work by Meireles et al. showed that spectroscopy is capable of robustly capturing phylogenetic signals and that broad plant groups, orders, and families can be identified from reflectance spectra [177]. Using evolutionary models they found that different spectral regions evolved at different rates and under different constraint levels, mirroring the evolution of the underlying traits they correspond to. This breakthrough finding establishes that spectroscopy and spectranomics can provide novel insights into leaf evolution and plant phylogenetic diversity at scale. This approach could be applied to plant disease ecology to

better understand the evolutionary dynamics of and history of beneficial plant microbe relationships as well as antagonistic.

Promise, limitations & future directions

Integrating multi-omic approaches can help us understand who are the microbial players in the system, how they disperse and are distributed across time and space, and what are their individual and combined functions. As detailed in the case studies for this review, -omics can provide a richer picture of the mechanisms behind fundamental topics in plant disease ecology such as plant defence, stress response, and the potential for disease suppression. This is especially the case when compared to simply utilizing one -omic alone. These approaches, when integrated correctly at the right scale and for the right research question, promise to reveal a multi-dimensional view of plant disease. However, there are still many pitfalls and limitations toward integrating and using these approaches that researchers must be aware of before designing a research proposal and project. Below, we outline some of the promises as well as the pitfalls of -omics driven studies, as well as future directions for using these approaches for plant disease research.

When designing projects that could benefit from multi-omic approaches, there is a need to move away from strictly observational research questions to those that are hypothesis driven. Although the number of published research articles has grown exponentially in the past decade to reflect breakthroughs in high throughput sequencing, few have been applied to questions within the fields of environmental and conservation genomics that ask experimental questions [178]. A shift from hypotheses that measure correlation to those that decipher an underlying causation for an ecological phenomena would benefit the field of plant disease ecology. For example, there is a dire need to design hypothesis-based studies that test ecological theory in order to understand how microbial diversity and dynamics shift under a changing environment. There is merit for observational studies that describe the microbial players in a system; these studies allow a researcher to hone their ideas and questions once they explore the taxa present and certain environmental covariates. These initial studies often use predominately amplicon-based sequencing tools. While using amplicon-based sequencing to identify taxa is a valiant first-pass into defining the taxonomic and correlative boundaries of a system, descriptive studies can not reveal underlying mechanisms or drivers for the patterns observed. For instance, conducting ordinal statistical analyses to find patterns in microbial similarity in plant roots that are drought stressed versus those that are not can allow us to gain a better picture about microbial taxonomic presence or absence under environmental stress. Understanding why we see these patterns can not be gauged from strict observation. It is critical to understand the processes that drive the patterns we see with amplicon data, namely through designing and developing field, greenhouse, and laboratory experimental designs that elucidate community functional roles and dynamics and at different spatio-temporal scales [179, 180].

Another major limitation to conducting multi-omics are taxonomic or chemical compound databases that are currently in their nascent phases as more microbes are sequenced and discovered worldwide. Still, a handful of databases do exist that are well curated and are the standard for conducting -omics research. For both amplicon-based studies and metagenomic studies, open-source databases include RDP [181], Silva [182], Greengenes [183], and UNITE [184]. A Python-based tool that can be used to parse fungal operational taxonomic units (OTUs) by ecological guild independent of sequencing platform or analysis is FUNGuild [185]. FunFun is a novel database for fungal functional traits which is able to interface with other databases to explore and predict how fungal functional diversity varies by taxa, guild, and other evolutionary or ecological groups [186]. Common databases used in metabolomics

and volatilomics studies include MassBank [187], Metabolomics Workbench [188], among other more specific databases [189]. Limitations in the scope and ability to use them as true reference databases for compound matching persist, however, with many researchers maintaining private instrument-specific libraries. Spectroscopy (spectranomics) databases are ecosml.org (Ecological Spectral Model Library) and ecosis.org (Ecological Spectral Information System) which are useful for finding spectral models and data on leaf nutrients, cellulose, and other physiological parameters.

Additional limitations to integrating multi-omics approaches in plant disease ecology include cost-per-sample (especially when considering multiple evaluation techniques) and difficulties in integrating disparate data-sets. -Omics information inherently comes in different formats with different means of preprocessing, analyzing, and interpreting the final results. Integrating those results across the genotype-phenotype spectrum can be challenging.

Addressing these limitations and realizing the potential of multi-omics research necessitates collaboration. Indeed, the motivation for writing this review was to spark such collaborations. No one person can be an expert across multi-omics domains; effectively conducting multi-omics research necessitates an integration of expertise just as it necessitates integration of data-sets. This aspect of collaboration is perhaps the most promising feature of multi-omics research. Integrating a diversity of disciplines engenders an integration of perspectives. The challenges apparent in one discipline will likely benefit from fresh perspectives from another. Integrating these approaches will allow research collaborations to answer questions and address intractable problems that were previously not possible.

A multi-omics approach to solving problems in plant disease ecology has already led to breakthroughs in understanding plant defense, detecting plant stress, and managing disease with suppressive soils. This approach also seems poised to create breakthroughs in our understanding of endophytes. The promise of multi-omics in plant disease ecology extends beyond these areas, however, and looks to create breakthroughs in our understanding of how microbial communities respond in a changing environment for years to come [190].

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