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PERSPECTIVE

Predictive Ecology and Management of Phyllosphere Microbial Communities Through Cross-Scale Synthesis

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ABSTRACT

In this article, we summarize the main takeaways from a symposium and hybrid virtual and in-person participatory discussion focused on the challenges of scale in understanding the ecology and management of phyllosphere microbial communities. We provide an overview of the confounding effects of spatial scale on inference in microbial ecology, the spatial organization of microbial interactions in the phyllosphere, advances and remaining gaps in measuring phyllosphere colonization across scales, and the epidemiology in the phyllosphere. We hope to motivate further discussion and the development and adoption of creative approaches to solving the challenges of scale to enhance fundamental understanding and practical management of the phyllosphere microbiomes.

Keywords: hybrid participatory discussion, microbiome, phyllosphere microbial communities, scale dependence, spatial scale ecology and management

THE CHALLENGES OF SCALE IN THE PHYLLOSPHERE

The phyllosphere—the aboveground parts of plants and the boundary layer of air under its influence—is a microbial ecosystem characterized by change and variability. Within a few hours, the phyllosphere can shift from a xeric landscape bombarded by solar radiation to a cool, dark, mesic habitat, presenting microbes ample opportunities for growth. Likewise, the spatial arrangement of hospitable locales in the phyllosphere is conspicuously heterogeneous, with vast plains of barren epidermal tissue punctuated by the occasional fertile valley of a vein, outcropping of a trichome, or cavity of a stomate granting entrance into a leaf's interior (Fig. 1).

This variability in quality and quantity of habitat in the phyllosphere of even a single plant may select for uniquely adapted microbial communities of many different species which, in turn, exhibit unique interactions and dynamics and, therefore, must be studied at these fine spatial and temporal resolutions to be understood (Chaudhry et al. 2021; Kinkel 1997; Remus-Emsermann et al. 2012). On the other hand, some microbial processes in the phyllosphere (e.g., immigration and emigration) may operate at scales that are robust to underlying microheterogeneity, obviating a

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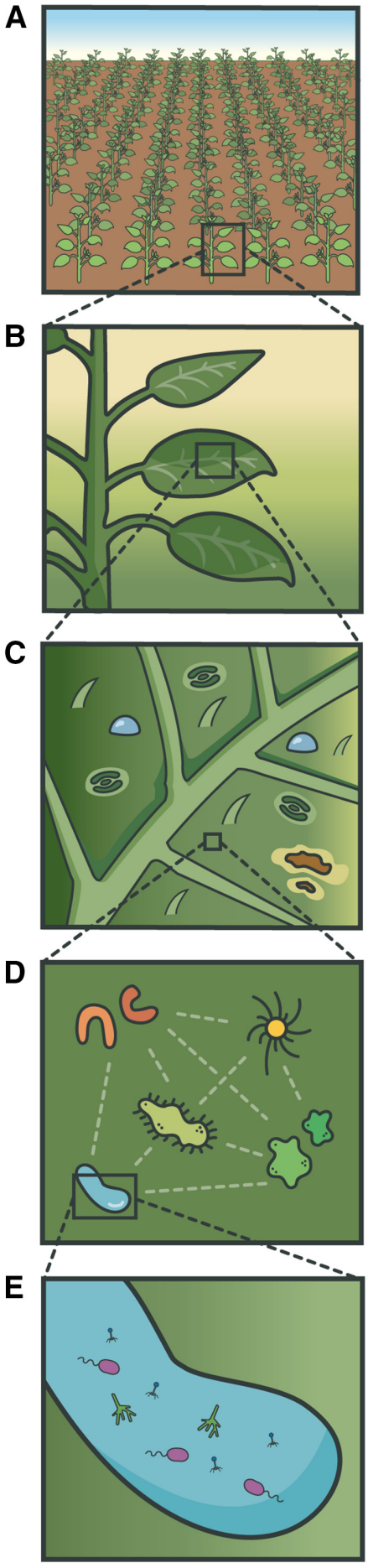

fine-grained approach and allowing for predictions based on course-grained observations. Furthermore, crop management that impacts phyllosphere dynamics and foliar diseases operates at scales ranging from tens to thousands of acres. In all cases, optimal sampling design and desired generalizability of the results for the question at hand require scale-dependent decisions that balance project costs or effort with the need to capture variance and sample deeply within or across plants at the relevant scales.

The development of three core areas is essential for successful cross-scale synthesis in phyllosphere microbial ecology. These areas are (i) fundamental understanding of microbiota at fine spatial scales, (ii) improving technology and throughput for characterizing microbial populations or communities at scales relevant to the processes or questions under consideration, and (iii) technical and modeling frameworks that can leverage high-dimensional community data to predict and manage microbial processes in the phyllosphere. To address these challenges and engage the community in identifying solutions, a hybrid symposium titled "Challenging Key Concepts in Spatial Scale Ecology and Management of Phyllosphere Microbial Communities" was held within the PHYLLOSPHERE 2022 11th International Symposium on Leaf Surface Microbiology in Davis, California in July 2022. We challenged four invited speakers and participants spanning a wide variety of disciplines and career stages, and representing more than 30 countries, to focus on two core questions: (i) How does scale challenge our abilities to understand and manage phyllosphere microbiomes? and (ii) What is the most important big question that we need to answer about scale in phyllosphere microbiology? Here, we summarize the main takeaways from our session.

THE CONFOUNDING EFFECTS OF SPATIAL SCALE ON INFERENCE IN MICROBIAL ECOLOGY

Phyllosphere microbial ecology has benefitted from high-throughput sequencing-based censuses of biodiversity. Such data are used to examine covariances between taxa and their local environments, which are used to infer interspecific interactions (e.g., competition versus facilitation) (Faust and Raes 2012) or community assembly mechanisms (e.g., stochastic versus deterministic) (Stegen et al. 2013). Although these approaches can yield valuable insights into the nature of a particular microbial community, it is rarely acknowledged that the signs and magnitudes of these effects are entirely scale dependent. The confounding effects of spatial scale have long been acknowledged in plant and animal ecology (Levin 1992) and, given recent dramatic increases in the use of pattern-to-process inference in microbial ecology, it is due time that microbial ecologists—particularly those working in heterogeneous landscapes such as the phyllosphere—begin interpreting their own results in the context of the scale at which they were collected. What

Fig. 1. Phyllosphere microbial communities have been studied at diverse spatial scales, each one of which can provide distinct insights into the biology and dynamics of phyllosphere populations. **A,** Field- or crop-scale studies may focus on mean population densities of pathogen populations in relation to functional outcomes (e.g., disease) or environmental conditions across the habitat, providing useful insights for disease prediction. **B,** Studies of individual leaves may be useful in understanding local-scale (e.g., in relation to position in canopy or field topography) variation in population or community characteristics. Zooming in on finer spatial scales can highlight the roles of **C,** leaf topography or **D,** microbial interactions on phyllosphere dynamics, while consideration of **E,** single bacterial or fungal colonies or populations on the leaf may highlight the underexplored roles of endosymbionts and biofilms in the ecology and functional capacities of phyllosphere populations.

follows are two simple examples concerning scale dependence in community assembly.

Consider a community of two microbial species competing for space over a heterogeneous landscape comprising discrete substrates of varying quality, as is the case for leaf surfaces (Fig. 2A) (Armitage and Jones 2019). In truth, these species strongly compete at the local scale of a single patch, such that they exhibit deterministically negative associations. However, it is rarely the case that censuses of such systems are made at the small scales over which interactions occur and, instead, a bulk sample of leaf tissue is collected for sequencing and enumeration (as is often done to satisfy minimum DNA concentration requirements). This sample will contain communities residing on substrates of varying quality to which the abundances of both species covary in the same direction. When such complex habitats are repeatedly sampled to infer species associational correlations, the resulting interspecific correlations between the two competing species will, on average, reflect neither the magnitude nor even the sign of the true interaction, unless samples are carefully selected to contain exactly the same proportions of different substrate types. In the illustrated example, we have mistakenly measured the interaction to be positive because we failed to account for underlying environmental heterogeneity in our samples.

Expanding this thought experiment, we might ask whether observed interspecific associations within our samples result from chance alone or, rather, from deterministic processes such as species sorting. To quantify this, we can compare the average dissimilarity of our sampled communities with that of communities assembled at random (Fig. 2B). Here, again, underlying spatial heterogeneity can mislead the practitioner by making a purely deterministic assembly process appear random, depending on the spatial scale at which the samples are collected. This strong scale dependence in community properties implies that the results of these studies are only general at the spatial grain over which they were collected, and comparisons between studies, even in the same environments, must use caution in drawing general conclusions. This is particularly important in metaanalyses looking to identify general trends in microbial community assembly. In the specific case of the phyllosphere,

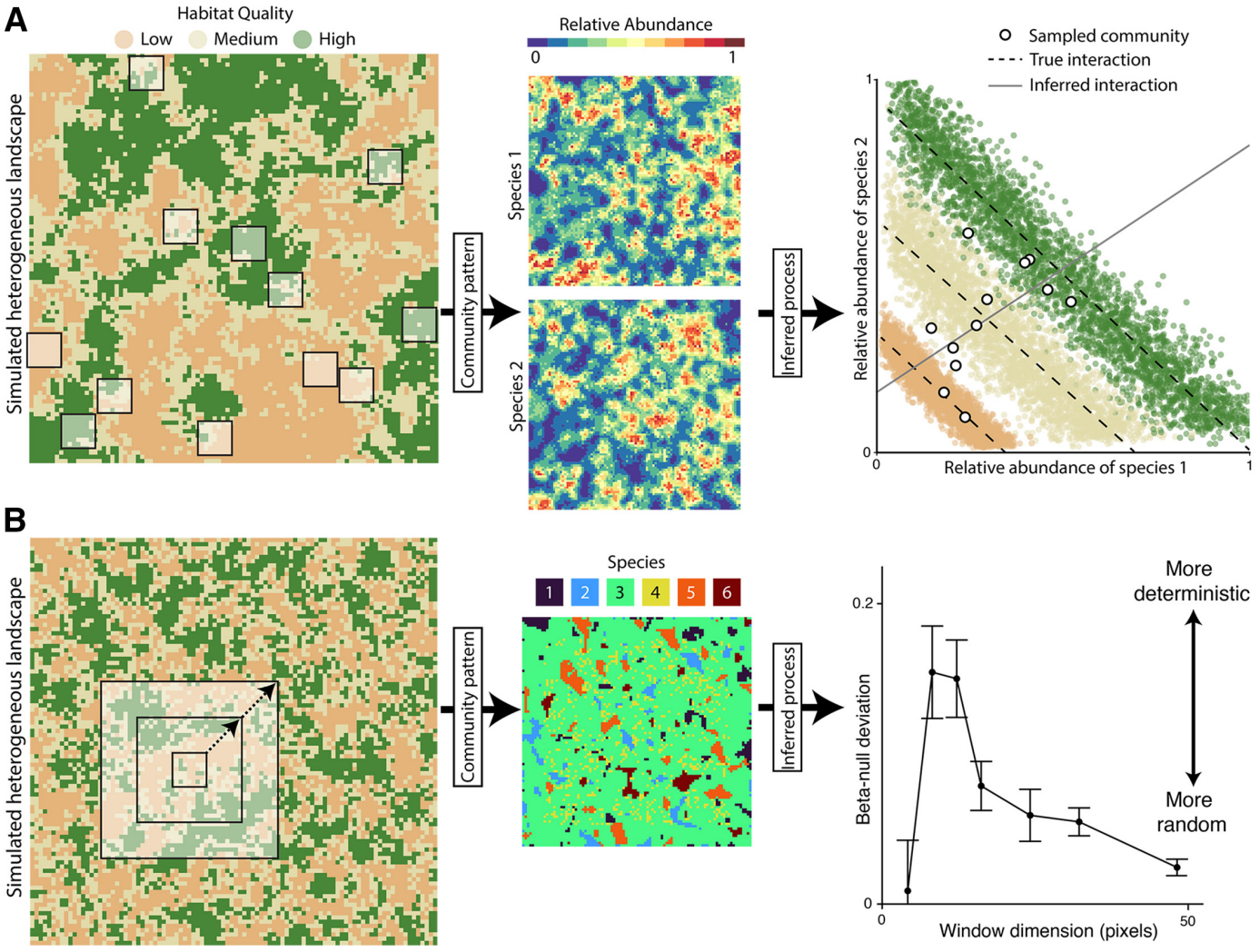

Fig. 2. **A,** Simulated example of how deterministic competition between two species over a spatially heterogeneous 96-by-96-pixel landscape can result in incorrect inference of species associations when random samples (white boxes) are collected from a subset of the habitat. **B,** Simulated example of how conclusions drawn about community assembly processes are dependent on the spatial scale of the random samples being collected (e.g., increasing size of white boxes). Here, the spatial arrangement of the six-species community is generated by purely deterministic competition at all spatial scales. This pattern, however, is obfuscated at certain high and low sampling grains, making them appear more random than they truly are.

collecting and comparing samples at multiple spatial scales—such as by censusing a size gradient of tissue punches from an individual leaf—will be important to identify the "deterministic scale" over which process-based models can most accurately describe and predict community dynamics (Esser et al. 2015; Pascual and Levin 1999).

PROGRESS AND PITFALLS OF MEASURING PHYLLOSPHERE COLONIZATION AT THE MICRON SCALE

To investigate the impacts of the phyllosphere's numerous microenvironments on microbial colonization and spread, techniques that reach spatial resolutions in the micrometer range—the size of an individual bacterial cell—are required. This is generally limited to microscopical techniques, although high-resolution spatial mass spectrometry is also seeing increased use for these purposes. On leaves, bacteria are usually undetectable by light microscopy, requiring the use of either fluorescent markers or proteins. To be able to visualize individual cells of different bacterial taxa on leaves, fluorescence in situ hybridization (FISH) has been used, or genetically modified bacteria that, often constitutively, produce fluorescent proteins. This allows determination of spatial patterns of bacterial colonization in planta through visualization of bacterial bioreporters that reveal location or report on gene activities; responding, for example, to neighbor densities or resource availability. This has led to the observation that bacteria generally cluster with each other up to a range of approximately 10 μm (Esser et al. 2015; Remus-Emsermann et al. 2014). Surprisingly, based on current knowledge, this clustering seems to be independent of bacterial taxonomic identity. The biological reasons for this consistency are not yet clear but there is also the possibility that it is due to experimental limitations. For example, using FISH-based observations, the typically low phylogenetic resolution of standard probes prohibits the differentiation of cells beyond the rank of family. This potentially masks patterns of subfamily bacterial interactions or site preferences. By contrast, when working with defined bacterial communities expressing fluorescent proteins on leaf surfaces, only simple communities of two species have been employed thus far. Although promising as subjects of more complex and realistic experiments, such communities are inherently limited in scope because their members must be amenable to genetic transformation. Hence, their behavior and interaction may not be representative of the majority of leaf-colonizing bacteria.

To overcome these limitations, several avenues may be possible that parallel the holistic environmental FISH approach or follow the reductionist synthetic community approach using fluorescent proteins. With recent advances in mass spectrometry imaging techniques such as matrix-assisted laser desorption/ionization, it may be possible not only to scan leaves and query the metabolic landscape within microbial communities but also to identify the local inhabitants of a leaf (Ajith et al. 2022). Furthermore, other fields such as spatial transcriptomics may pave the way to using studies of microbial ecology on plant leaves with further improvements in spatial resolution (Saarenpää et al. 2022). Likewise, bringing novel phyllosphere microbes into lab cultivation and making them amenable to genetic manipulation, in combination with a wide range of different fluorescent proteins, will allow the observation of more complex synthetic communities and their behavior in planta, including the roles of genetic exchange and common goods production within these local communities. Both advances will facilitate further insights into how phyllosphere communities are shaped in planta, and identify opportunities for managing these communities to enhance plant health.

NESTEDNESS AND THE SPATIAL ORGANIZATION OF MICROBIAL INTERACTIONS IN THE PHYLLOSPHERE

Further complexity in mapping and understanding interspecific associations comes when we account for the great diversity of organisms beyond bacteria such as protists and viruses in the phyllosphere communities. Commonly considered impacts of interspecies and even interkingdom interactions on microbial behavior include predation, antagonistic compounds, or resource competition (Hassani et al. 2018). However, a particularly confounding aspect to these interactions is that they may be spatially nested, or vertical. That is, many eukaryotic microbes can harbor bacteria or viruses that significantly impact their relationship with a host plant. Whether two potential partner species are simply present or are closely associated can have drastically different resulting behavior. Endohyphal bacteria offer a useful lens for considering the diversity, prevalence, and impacts of the nested organization of microbes.

Diverse endohyphal bacteria can interact with their host fungi in relationships ranging from obligate to facultative and persistent to transient (Araldi-Brondolo et al. 2017). Plant-associated fungi represent most of the functional relationships identified, though the breadth of possible partnerships highlights the variety of outcomes, including developmental changes and increased stress tolerance (Araldi-Brondolo et al. 2017). For example, an obligate bacterial symbiont of an arbuscular mycorrhizal fungus alters its fungal host's primary metabolism to reduce oxidative stress in both the fungal and plant hosts (Vannini et al. 2016). We are increasingly discovering that plant pathogens harbor bacteria with diverse capabilities, such as the nitrogen-fixing *Bacillus* strains in the maize pathogen *Ustilago maydis* (Ruiz-Herrera et al. 2015). Notably, microbial symbionts that perform host-critical functions are not solely limited to endosymbiotic associations. One or more bacterial strains associating externally but persistently can modify fungal behavior as well, while bacteria can gain benefits, including movement along the fungal hyphae. One particularly striking example involves a *Fusarium oxysporum* strain that switches from plant pathogenic to plant growth promoting based on the presence of an ectobacterial consortium (Minerdi et al. 2011).

Although bacterial–fungal interactions in plants have been increasingly studied, knowledge of other microbe–microbe interactions, especially those involving protists, remain largely unexplored. Accounting for nestedness within the phyllosphere microbiome will require new tools and framing for how to capture and characterize a microbiome. We must move beyond single plant–microbe functional interactions and a simple inventory of what is present (genes, taxonomic units, and genomic bins). To fully understand microbiome function, techniques for capturing gene expression, regulation, epigenetic modification, and metabolism must be developed in ways that permit us to uncover the joint associations and contributions of nested symbionts to plant health through space and time.

EPIDEMIOLOGY IN THE PHYLLOSPHERE: MOVING FROM POPULATION TO COMMUNITY-LEVEL MODELS

Plant pathologists and epidemiologists often approach the challenge of modeling plant pathogen dynamics through a reductionist approach, using controlled-environment studies to focus on single plant–microbe interactions in isolation, from which they derive predictive models (De Wolf and Isard 2007). Controlled-environment studies can help resolve how phyllosphere microbial populations shift due to endogenous factors (e.g., growth rates and the forma-

tion of biofilms) as well as exogenous factors such as weather, climate, agricultural management practices, and host defense responses (Chaudhry et al. 2021). However, despite years of intensive studies for some well-studied pathosystems, we still struggle to accurately predict the population dynamics of many foliar microbes (De Wolf and Isard 2007). Ultimately, variation in our ability to predict microbial population dynamics may reflect variation in inter- and intraspecific interactions, necessitating a shift from single-population to community-level models (Vannini et al. 2016). The increasing availability of open-access data for the phyllosphere microbiome, coupled with relevant metadata on disease and crop yields, opens the potential to develop statistical models describing and predicting the dynamics of phyllosphere microbial communities on the leaf surface and their interactions with plant pathogens and plant health.

Microbial cooccurrence network models may build understanding of the community impact of particular microbial taxa, including pathogens, on phyllosphere composition and function (Agler et al. 2016; Poudel et al. 2016). Because these models are correlational, they cannot distinguish the mechanistic basis of coassociations (Armitage and Jones 2019). However, consistent shifts in phyllosphere populations over time in the context of other community members can generate testable hypotheses on the ecological roles of various microbes in the phyllosphere community and their impacts on plant health. Moving beyond the scale of individual species and focusing on networks and assemblages of microbes across populations of leaves provides critical information on the consistency of coassociations among specific microbial taxa in relation to crop health. Although coassociation-based predictive models of phyllosphere dynamics cannot replace experimental biology in developing a mechanistic basis for predictions, they offer a rich opportunity for the generation of novel hypotheses on the dynamics of the assembly of phyllosphere microbiomes and their collective impacts on plant health and productivity.

CHARTING A PATH FORWARD

Responses to our core questions—(i) How does scale challenge our abilities to understand and manage phyllosphere microbiomes? and (ii) What is the most important big question that we need to answer about scale in phyllosphere microbiology?—varied across 10 virtual and in-person discussion groups, and encompassed both fundamental and applied perspectives, from the conceptual to methodological. Overall, debate focused on dealing with fine-scale variability, upscaling knowledge from fine scales to understanding coarser scales, and adopting the appropriate scale of study depending on whether the objective is to obtain fundamental knowledge or to develop policy or advice for pragmatic applications.

Concerns about fine-scale temporal heterogeneity arise from the fact that daily measurements of microbial population size might not capture diel variation in microbial or plant–microbe interactions that determine population dynamics as captured in the metatranscriptome, for example. Potential remedies to this problem could involve collecting preliminary samples at representative times during the day (morning, afternoon, and night) to identify whether the response data are time variant over a diel cycle. Fine-scale spatial heterogeneity also represented a significant concern; for example, in accounting for the phyllosphere's numerous microclimates. Although it is currently possible to monitor phyllosphere organisms using advanced microscopy, it is not clear how to integrate diverse features such as the boundary layer atmosphere, fine-scale leaf topography, and availability of local resources to patterns of microbial abundance or behaviors. The leaf surface is, indeed, a landscape. However, we lack the diversity of tools to address questions of phyllosphere landscape ecology that are available for macroscopic landscapes.

A fundamental challenge for phyllosphere researchers lies in the lack of understanding of the relationship between small-scale (e.g., localized plant–microbe interactions or microbial interactions) and large-scale (plant population or landscape) phenomena (e.g., foliar disease and source- or sink-population dynamics). In some cases, outcomes can be a strong linear function of predictors, as in the case of disease severity and pathogen abundance, thereby leading to straightforward upscaling. However, nonlinear responses such as changes in microbial density or composition across an elevation or temperature gradient (Cordier et al. 2012) are more challenging but can, with the correct data, still be scaled up to the landscape (Chesson 2012). On the other hand, context-dependent responses such as variation in fungal infection of a host in response to the presence of specific fungal endophytes must be studied and measured at the spatial and temporal scales at which the microbial interactions occur, making prediction or management at practical scales substantially more difficult. Without insight into the scale dependence of phenomena or relationships of interest, effective management recommendations will remain out of reach.

Cross-scale sampling is probably the best approach to identifying the scale dependence of a response variable. More knowledge about the life histories of the taxa under study will also help contextualize scale dependence. Another key limitation is the availability of appropriate funding opportunities. Lack of funding for cross-scale replication was highlighted as a primary roadblock to assessing scale dependency. Participants underlined the need for modest funding streams for preliminary data collection and independent replication of results across experimental systems and at multiple spatial and temporal scales as particularly important for advancing phyllosphere biology.

The question about the most important challenge or knowledge gap concerning scale in the phyllosphere elicited a plurality of responses. These ranged from problems in fundamental ecology (What is the relevant scale of study or understanding for a particular system? How do we scale simple, high-resolution results to coarser scales or more complex systems?) to those of a practical or philosophical nature (How do we balance tractability and realism? How do we advise policy in light of scale dependence?). Although this lack of consensus may seem frustrating, participants agreed that all of these questions can begin to be addressed by community-wide incorporation of scale-explicit approaches in our research. To conduct scale-explicit studies, phyllosphere scientists will need to expand their collaboration networks and incorporate the expertise of people knowledgeable about approaches appropriate at multiple scales. A minimal effort to this end involves identifying other researchers working on a similar system or question but at a different spatial, temporal, or ecological scale. Focused meetings such as the PHYLLOSPHERE conference series and others present ideal opportunities for developing precisely this type of collaborative study that captures the range of habitats and approaches considered among phyllosphere researchers. It is our hope that, through the diversity of participants in this symposium, its inclusive hybrid format, and our emphasis on documenting outcomes of formal and informal discussions, the topic of scale will permeate the phyllosphere microbiology community and encourage a more nuanced consideration of scale in our research endeavors.

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LITERATURE CITED

Agler, M. T., Ruhe, J., Kroll, S., Morhenn, C., Kim, S.-T., Weigel, D., and Kemen, E. M. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. PLoS Biol. 14:e1002352.

Ajith, A., Milnes, P. J., Johnson, G. N., and Lockyer, N. P. 2022. Mass spectrometry imaging for spatial chemical profiling of vegetative parts of plants. Plants 11:1234.

Araldi-Brondolo, S. J., Spraker, J., Shaffer, J. P., Woytenko, E. H., Baltrus, D. A., Gallery, R. E., and Arnold, A. E. 2017. Bacterial endosymbionts: Master modulators of fungal phenotypes. Pages 981-1004 in: The Fungal Kingdom. J. Heitman, B. J. Howlett, P. W. Crous, E. H. Stukenbrock, T. Y. James, and N. A. R. Gow, eds. American Society for Microbiology, Washington, DC, U.S.A.

Armitage, D. W., and Jones, S. E. 2019. How sample heterogeneity can obscure the signal of microbial interactions. ISME J. 13:2639-2646.

Chaudhry, V., Runge, P., Sengupta, P., Doehlemann, G., Parker, J. E., and Kemen, E. 2021. Shaping the leaf microbiota: Plant–microbe–microbe interactions. J. Exp. Bot. 72:36-56.

Chesson, P. 2012. Scale transition theory: Its aims, motivations, and predictions. Ecol. Complexity 10:52-68.

Cordier, T., Robin, C., Capdevielle, X., Fabreguettes, O., Desprez-Loustau, M.-L., and Vacher, C. 2012. The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*) varies significantly along an elevation gradient. New Phytol. 196:510-519.

De Wolf, E. D., and Isard, S. A. 2007. Disease cycle approach to plant disease prediction. Annu. Rev. Phytopathol. 45:203-220.

Esser, D. S., Leveau, J. H., Meyer, K. M., and Wiegand, K. 2015. Spatial scales of interactions among bacteria and between bacteria and the leaf surface. FEMS Microbiol. Ecol. 91:fiu034.

Faust, K., and Raes, J. 2012. Microbial interactions: From networks to models. Nat. Rev. Microbiol. 10:538-550.

Hassani, M., Durán, P., and Hacquard, S. 2018. Microbial interactions within the plant holobiont. Microbiome 6:58.

Kinkel, L. L. 1997. Microbial population dynamics on leaves. Annu. Rev. Phytopathol. 35:327-347.

Levin, S. A. 1992. The problem of pattern and scale in ecology: The Robert H. MacArthur award lecture. Ecology 73:1943-1967.

Minerdi, D., Bossi, S., Maffei, M. E., Gullino, M. L., and Garibaldi, A. 2011. *Fusarium oxysporum* and its bacterial consortium promote lettuce growth and expansin A5 gene expression through microbial volatile organic compound (MVOC) emission. FEMS Microbiol. Ecol. 76: 342-351.

Pascual, M., and Levin, S. A. 1999. From individuals to population densities: Searching for the intermediate scale of nontrivial determinism. Ecology 80:2225-2236.

Poudel, R., Jumpponen, A., Schlatter, D. C., Paulitz, T., Gardener, B. M., Kinkel, L. L., and Garrett, K. 2016. Microbiome networks: A systems framework for identifying candidate microbial assemblages for disease management. Phytopathology 106:1083-1096.

Remus-Emsermann, M. N., Lücker, S., Müller, D. B., Potthoff, E., Daims, H., and Vorholt, J. A. 2014. Spatial distribution analyses of natural phyllosphere-colonizing bacteria on *Arabidopsis thaliana* revealed by fluorescence in situ hybridization. Environ. Microbiol. 16:2329-2340.

Remus-Emsermann, M. N., Tecon, R., Kowalchuk, G. A., and Leveau, J. H. 2012. Variation in local carrying capacity and the individual fate of bacterial colonizers in the phyllosphere. ISME J. 6:756-765.

Ruiz-Herrera, J., León-Ramírez, C., Vera-Nuñez, A., Sánchez-Arreguín, A., Ruiz-Medrano, R., Salgado-Lugo, H., Sánchez-Segura, L., and Peña-Cabriales, J. J. 2015. A novel intracellular nitrogen-fixing symbiosis made by *Ustilago maydis* and *Bacillus* spp. New Phytol. 207:769-777.

Saarenpää, S., Shalev, O., Ashkenazy, H., de Oliveira-Carlos, V., Lundberg, D. S., Weigel, D., and Giacomello, S. 2022. Spatially resolved host-bacteria-fungi interactomes via spatial metatranscriptomics. bioRxiv 496977.

Stegen, J. C., Lin, X., Fredrickson, J. K., Chen, X., Kennedy, D. W., Murray, C. J., Rockhold, M. L., and Konopka, A. 2013. Quantifying community assembly processes and identifying features that impose them. ISME J. 7: 2069-2079.

Vannini, C., Carpentieri, A., Salvioli, A., Novero, M., Marsoni, M., Testa, L., de Pinto, M. C., Amoresano, A., Ortolani, F., and Bracale, M. 2016. An interdomain network: The endobacterium of a mycorrhizal fungus promotes antioxidative responses in both fungal and plant hosts. New Phytol. 211: 265-275.