ANNUAL REVIEWS

Annual Review of Phytopathology

Facilitating Reforestation Through the Plant Microbiome: Perspectives from the Phyllosphere

Posy E. Busby,[1] George Newcombe,[2] Abigail S. Neat,[1] and Colin Averill[3]

[1]Department of Botany and Plant Pathology, Oregon State University, Corvallis, Oregon, USA; email: posy.busby@oregonstate.edu

[2]Department of Forest, Rangeland and Fire Sciences, University of Idaho, Moscow, Idaho, USA

[3]Department of Environmental Systems Science, Institute of Integrative Biology, ETH Zürich, Zürich, Switzerland

Annu. Rev. Phytopathol. 2022. 60:337–56

First published as a Review in Advance on May 18, 2022

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

https://doi.org/10.1146/annurev-phyto-021320-010717

Keywords

plant pathogens, endophytes, biodiversity, plant phyllosphere feedback, climate change, tree planting

Abstract

Tree planting and natural regeneration contribute to the ongoing effort to restore Earth's forests. Our review addresses how the plant microbiome can enhance the survival of planted and naturally regenerating seedlings and serve in long-term forest carbon capture and the conservation of biodiversity. We focus on fungal leaf endophytes, ubiquitous defensive symbionts that protect against pathogens. We first show that fungal and oomycetous pathogen richness varies greatly for tree species native to the United States (n = 0–876 known pathogens per US tree species), with nearly half of tree species either without pathogens in these major groups or with unknown pathogens. Endophytes are insurance against the poorly known and changing threat of tree pathogens. Next, we review studies of plant phyllosphere feedback, but knowledge gaps prevent us from evaluating whether adding conspecific leaf litter to planted seedlings promotes defensive symbiosis, analogous to adding soil to promote positive feedback. Finally, we discuss research priorities for integrating the plant microbiome into efforts to expand Earth's forests.

To keep every cog and wheel is the first precaution of intelligent tinkering.

—Aldo Leopold (75)

INTRODUCTION

Forests, particularly those found in the tropics, have long been known to be hotspots of biodiversity, inhabited by a wide variety of plants, insects, birds, amphibians, and mammals. More recently, we have come to appreciate the multitude of tree-associated microbes (fungi, bacteria, and archaea inhabiting various tree habitats), their complex interactions, and the mechanism by which they can promote forest biodiversity (8). The best understood and longest studied part of the tree microbiome is its most ecologically important symbiosis: mycorrhizal fungi (63). The ectomycorrhizal (EM) symbiosis is limited to approximately 2% of all plant species (although 60% of Earth's 3.1 trillion trees), whereas the more ancient, arbuscular mycorrhizal (AM) symbiosis is much more common across Earth's 60,065 tree species (122). Mycorrhizal symbioses are recognized as being fundamental to plant nutrition and the structure and function of forest soils (7, 63). In contrast, the organismal groups that make up the majority of the tree microbiome, mostly bacteria and fungi, are often characterized either as pathogens or as nonpathogens of uncertain role. Moreover, their identities are frequently represented simply by sequence-based approximations at the genus level or higher. Plant disease protection, conferred by many of these members of the plant microbiome, is increasingly documented, with communities of endophytic microbes in soil, roots, stems, leaves, flowers, and seeds combating or competing with pathogens and priming host immunity (34, 100, 130, 135). Together, this body of work suggests that the functional influence of the microbiome on its tree host is beneficial and protective (82). In this review, we address strategies for integrating diverse tree microbiomes into a massive effort to expand the global forest.

Ongoing tree planting and natural forest regeneration efforts are components of a larger strategy to address interrelated crises aggravated by deforestation. Over the course of human history, we have reduced the number of trees on Earth by half, from six trillion to three trillion (36). In many regions of the world, losses are continuing (121). The loss of forest habitat caused by land-use conversions is a major contributor to Earth's sixth mass extinction (69), most acutely seen in the tropics (57, 58). These large-scale losses are one crisis. A second crisis exacerbated by deforestation is climate change (96). Deforestation returns carbon stored in tree stems and soil to the atmosphere, contributing to warming (87). Half of all terrestrial carbon stored in vegetation is stored in tropical forests alone (23). Earth's largest tropical forests are in Amazonia, where a 17% loss in forest is associated with profound, system-wide effects, including regional shifts from carbon sinks to sources (53).

Reforestation is one way to address these interrelated crises. This includes conserving existing forests, promoting natural forest regeneration, and actively planting trees. These efforts are of special urgency for people living near forests, totaling 1.6 billion, or 21% of the current human population (93). Most of these people are in the tropics where the shade of trees offers critical protection against heat waves (91, 119). Many groups are contributing to the massive effort to restore Earth's forests; examples include the United Nation's Trillion Tree Campaign, Plant-for-the-Planet, One Tree Planted, Trees for the Future, the Arbor Day Foundation, Trees for Cities, International Tree Foundation, The Woodland Trust, Tree Canada, Casey Trees, Trees for Life, and Trees, Water & People. New groups emerge frequently. In the Netherlands, for example, one group collects volunteer tree seedlings where they are not wanted (e.g., a flower bed or vegetable patch) and gives them away to those who will plant them. To coordinate the many tree-planting efforts, the World Economic Forum established 1t.org (**https://www.1t.org/**) to "serve the global reforestation community by mobilizing private sector ambition and engagement, accelerating restoration in

priority regions, and empowering a new generation of ecopreneurs to take action on climate change."

In the most basic sense, success in global reforestation hinges on the survival of planted and naturally regenerating tree seedlings. The seedling stage is the most vulnerable in a tree's life cycle (12, 46, 59), and plants depend heavily on their symbionts to tolerate both abiotic and biotic threats to their existence (139, 147). Plant disease has always been a concerning threat to the establishment of tree plantings (111); this threat is now exacerbated by climate change and the movement of pathogens by human activities (21, 123). Yet with few exceptions, restoration efforts are planting trees without knowledge of, or consideration for, their microbial symbionts, including pathogens. This is problematic given that historically, large-scale tree-planting projects often failed for reasons that involved microbes.

Tree-planting practices are typically borrowed from plantation forestry, which is rooted in tree genetics, silviculture, pathology, and entomology, a worldview in which productivity is prioritized, and microbes are narrowly viewed as pathogens to be avoided. These views persist today, where the microbiome is not considered relevant to forest management (129). For plantation methods that have been developed for a few of the world's 60,065 tree species (14), success has been achieved by planting trees outside their native range, where their native pathogens do not occur. For example, disease has been avoided by introducing pines of the Northern Hemisphere into plantations of the Southern Hemisphere, Amazonian rubber into southeast Asia, or African oil palm into the neotropics. Alternatively, when trees have been planted within native ranges, plantations can fail due to native pathogens and unnaturally close spacing of the trees; Henry Ford's failed rubber plantation in the Amazon (i.e., native range of *Hevea brasiliensis*) in the 1920s is one infamous example. Plantations under attack by pathogens are often managed with specific remedies (e.g., genes for resistance) (141) that apply only to a particular tree species, pathogen, and environment. Silvicultural methods of disease control are effective in some cases (20), but here we focus on a more general approach involving microbial symbionts to combat the wide array of pathogens (e.g., parasitic plants, fungi, bacteria, nematodes, phytoplasmas, and viruses) that affect trees globally.

At the other end of the spectrum, an absence of beneficial microbes can also lead to plantation failure. This is because when we plant forests, we rarely think to simultaneously "plant" a tree's symbionts. Although a tree's pathogens can be found in its leaves and soil, so are its key mutualists. Early efforts to establish pine plantations in the Southern Hemisphere failed until scientists thought to co-introduce associated mycorrhizal fungi, critical members of the forest microbiome (81). More generally, the importance of actively introducing symbiotic soil organisms to facilitate plant growth in novel landscapes has been known for more than a century (24, 62), and recent work has discovered the importance of soil microbiome restoration for facilitating ecosystem restoration. Native grasses in restored American Midwest prairies grow more vigorously and are more likely to survive when inoculated with the soil microbiome inhabiting intact prairie remnants (17, 71). Grassland restoration on abandoned Estonian mining landscapes could only be achieved by cointroducing native AM fungi (133). Restoration of high-latitude grassland communities could only achieve plant communities similar to those of intact remnants if soil microbial communities were cointroduced at the time of planting (142). Taken together, this body of work suggests an incredible, and largely unrealized, opportunity to enable and accelerate the restoration of Earth's forests by reintroducing tree microbiomes.

The potential for belowground microbiome restoration to facilitate ecosystem restoration suggests an analogous opportunity within the aboveground forest microbiome given the potential for dispersal of immigrant inoculum from plants or litter nearby. Indeed, beneficial tree symbionts are also found in the phyllosphere but have received far less attention than their belowground

counterparts (86). In particular, fungal leaf endophytes found in all plants can contribute to plant defense against pathogens (29, 107), in addition to influencing various other aspects of plant growth, development, and resilience (102, 113). The mechanisms by which endophytes increase or decrease disease severity in their hosts are complex (e.g., parasitism, antibiosis, host-triggered immunity), but perhaps most simply, by occupying space and competing for resources, these commensal symbionts can exclude pathogens. Once leaves senesce and drop, they form a layer of dead leaves (litter) containing live endophytes beneath the tree. Endophytes complete their life cycle in the leaf litter; they sporulate and send their spores upward to reinfect newly emerging leaves. Thus, leaf litter, like soil, is an important reservoir of beneficial microbes (97, 131).

Given the ambitious goal of restoring Earth's forests, and the importance of microbial symbionts for tree seedling defense and survival, a key question is what knowledge is needed to integrate the tree microbiome into forest restoration efforts. In this review, we focus on whether our current understanding of interactions between tree pathogens and leaf endophytes can inform low-cost, low-tech ways to promote aboveground defensive symbiosis in reforestation. We also discuss burgeoning efforts to integrate belowground symbionts into planting efforts as well as what success means in terms of carbon and diversity for the long-term. For example, by tolerating slow, initial growth we may successfully reduce the magnitude of the otherwise climate-unfriendly tradeoff between fast initial tree growth and tree longevity (101). Thus, our approach is distinct from the plantation forestry paradigm, which selects for fast, initial growth of single tree species. And our approach is distinct from the agricultural paradigm, which seeks to eliminate pathogens from crop monocultures. Instead, we explore approaches that integrate diverse tree microbiomes, which include pathogens, thereby supporting forest biodiversity, carbon capture, and long-term forest resilience.

CHARACTERIZING PATHOGEN COMMUNITIES OF TREE SPECIES

Understanding the pathogen communities of tree species used in tree-planting campaigns is a first step in managing disease during the vulnerable seedling stage. A common assumption is that all plants are affected by pathogens (79). In fact, a general introduction to plant pathology states that each species of plant appears to be attacked by 50–100 pathogens (3). If this is true, characterizing the pathogens of the world's 60,065 tree species is a daunting place for reforestation efforts to begin. To probe our knowledge of tree species affected by pathogens, we used the databases of the US National Fungus Collections (BPI) to search for the fungal and oomycetous pathogens of the 1,312 tree species in the United States, given that fungi and oomycetes are the two major classes of plant pathogens (13). These trees were identified using a geographically constrained search of the 60,065 tree species in GlobalTreeSearch (14), where a tree species is defined as "a woody plant with usually a single stem growing to a height of at least two meters, or if multi-stemmed, then at least one vertical stem five centimeters in diameter at breast height."

Our search of the BPI databases for fungal and oomycetous pathogens of the 1,312 tree species found in the United States showed that pathogen richness varied greatly among tree species, ranging from 0 to 876 known fungal and oomycetous pathogens per US tree species), with nearly half (48%) of tree species lacking known pathogens (**Figure 1**). In other words, the assumption that all plants, including trees, have pathogens may not be true. Of course, there are some tree species for which pathogens have simply not been studied. But for many of the US tree species without fungal/oomycetous pathogens, evolutionary escape following long-distance dispersal is a plausible explanation (27). For example, most species lacking pathogens are Hawaiian endemics, whose mainland relatives host a diverse array of pathogens. The Hawaiian flora has long been understood as the product of many long-distance dispersal events (103). Those founding events, leading to the

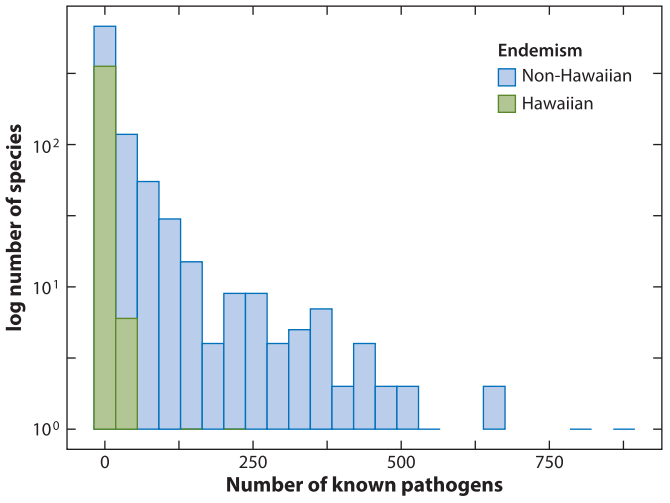

Figure 1

Results of a US National Fungus Collections database search for known pathogens of all tree species found in the United States. Almost half of tree species in the United States lack known fungal and oomycete pathogens, and a significant portion of these are endemic to Hawaii. *Pseudotsuga menziesii* (Douglas fir) has 876 pathogens, which is the most reported of any US tree species. The x-axis indicates the number of known pathogens for each tree species, and the y-axis indicates the quantity of species per pathogen count. The frequency (y-axis) has been log transformed and the bars are overlaid. The blue bars represent species found in the United States overall, except for the Hawaiian endemics. The green bars represent species endemic to Hawaii.

speciation of Hawaiian endemics, have similarities to anthropogenic enemy release expected for introduced plants and their pathogens (88).

Of the 363 tree species native or endemic to Hawaii, 312 (i.e., 86%) may have lost their pathogens, as there are no BPI pathogen records. Many Hawaiian endemics have been studied for pathogens because the diseases they cause threaten small populations. For example, *Cyanea* comprises 85 species endemic to Kauai (114). One such endangered species, *Cyanea kuhihewa*, is closely monitored and traps are currently being used to control rats that might damage the remaining two mature individuals, two juveniles, and 11 seedlings (114); we found only one pathogen of *Cyanea* species overall in our BPI search, and it was not affecting *C. kuhihewa*. Of the many studies of the ecology and genetics of five species of *Metrosideros* endemic to Hawaii (98), pathology has usually been included (11). In BPI records for *Metrosideros*, we found pathogen richness varied from 0 pathogens for *Metrosideros rugosa* and *Metrosideros waialealae* to 150 pathogens for *Metrosideros polymorpha*, which occurs across the Hawaiian Islands (11).

A further 324 (of 949, or 34%) non-Hawaiian tree species in the United States are also unaccompanied by pathogen records, suggesting that the phenomenon of natural enemy release is not restricted to Hawaii. Although an extensive analysis of range size relative to pathogen diversity is needed to clarify why some tree species may have escaped or lost their pathogens, it appears that the most widely distributed tree species are accompanied by many pathogens: 180 for *Prunus serotina*, which ranges through eastern North America, 108 for the wide-ranging *Prunus americana*, and 876 for *Pseudotsuga menziesii*, the dominant conifer of the Pacific Northwest. Such widely distributed trees are likely to be included in reforestation efforts, and thus defense against pathogens is crucial for their survival. In contrast, the most isolated tree species with the smallest populations are without pathogens: *Prunus eremophila*, a critically endangered species of perhaps

2,000 individuals native to the Mojave Desert (104), and *Prunus fremontii*, desert apricot of California and contiguous Mexico.

Disease-protective fungal leaf endophytes might seem to be of little value or concern for trees without pathogens, but pathogen reunions following the introduction of exotic pathogens are always a possibility. Some threatened Hawaiian endemics have been rescued from introduced, but perhaps ancient, pathogens, with endophytes from related plants with more robust populations (144). Indeed, endophyte-mediated approaches in conservation have become a frontier in endophyte ecology, with additional examples from Hawaii (32, 41). Hawaii has also pledged to add 100 million trees by 2030 through a combination of conservation, restoration, and tree planting. Thus, even in an environment where many tree species appear to have escaped their pathogens, endophytes could serve as insurance against pathogen reunions or encounters with novel pathogens (67).

FOLIAR ENDOPHYTES AS DEFENSIVE SYMBIONTS

Low-cost, low-tech solutions are needed in global tree-planting efforts to boost seedling defense against pathogens (21, 111, 123). Here, we explore whether defensive symbionts of the phyllosphere can fill this need, yet we recognize that a plant's primary mechanisms of defense are genetic. Nonhost resistance, or complete resistance, is conferred by several genetic factors (48). Pathogens cannot infect nonhosts. For known host–pathogen combinations, the next best defense against pathogens is major gene resistance (i.e., gene-for-gene relationship) (47). Plants containing R genes are often selected for cultivation given their protection against disease. This practice has been successful for the few tree species that are cultivated in plantation forestry. But for most of the world's tree species, breeding programs do not exist and are challenging and expensive to implement given long generation times. Seed for planting efforts could be selected from tree populations where R genes are likely to occur. Indeed, selecting genetically appropriate plant material (within a species) is one strategy for enhancing resilience to biotic and abiotic stressors in tree-planting efforts (125).

When pathogen infection does occur, ubiquitous fungal leaf endophytes can produce robust defensive responses (reviewed by 29, 107), rivaling the contributions of quantitative genetic resistance (108). This body of work suggests that harnessing these defensive symbionts could indeed bolster defense in regenerating forests. Diverse fungal leaf endophytes communities are found in all tree species; they are dominated by Ascomycetes, including ubiquitous taxa like *Alternaria* and *Cladosporium*, but also include basidiomycetous yeasts and many rare taxa (9, 60, 99, 132, 148). The incredible fungal diversity found within individual leaves was first discovered using culture-based methods (4, 50) and has been confirmed with culture-free methods (25). The consequences of hosting diverse endophytic communities are an active area of research in plant and fungal ecology. But an early (5) and commonly reported benefit of hosting endophytes is disease protection (reviewed by 29, 107).

Plant disease modification is a central function of many fungal leaf endophytes. These defensive symbionts occur in diverse fungal lineages (145) and have been studied in several tree species (5, 28, 33, 52). *Populus* has become a model in the study of plant–endophyte–pathogen interactions, with several studies showing that common fungal leaf endophytes reduce the severity of the tree's major leaf rust pathogen (28, 76, 108). Several of the taxa identified as reducing leaf rust disease, like fungi such as *Eudarluca*, *Cladosporium*, and *Trichoderma*, or bacteria such as *Bacillus*, are known to antagonize pathogens via mycoparasitism (10, 95) and/or by inducing plant genetic resistance (43). Communities are also protective, with the arrival order of endophytes into the community modulating the level of disease protection (76). At face value, priority effects in community

assembly may seem to suggest that early integration of endophytes into tree-planting efforts will boost their abundance and their efficacy in reducing disease. However, we urge caution before drawing general conclusions, as much remains to be learned about how endophyte interactions impact the functional influence of the microbiome on plant health (61). For example, Leopold & Busby (76) found that downstream differences in plant disease modification resulting from varying endophyte arrival order were not the result of the differential relative abundance of endophytes or shifts in community composition. Instead, early arriving endophytes may have interacted with the plant's immune system to reduce disease. In another study, simply changing the arrival order of endophytes into a community resulted in a switch from a protective interaction to a pathogenic interaction (1). Together, this work highlights how the composition of the plant microbiome can be decoupled from its functional influence on plant disease, and the challenge of precisely predicting the impact of the tree microbiome in reforestation.

The complex interactions between plants and endophyte communities that result in disease protection are not known for the vast majority of global tree species. However, a variety of mechanisms have been identified. Endophytes can exclude pathogens via competition for space and resources (82). These interactions are likely to occur between endophytes and necrotrophic pathogens given their competition for space and nutrients in the apoplast. However, direct pathogen antagonism via parasitism or antibiosis may be more likely between endophytes and biotrophic pathogens. Endophytes can also trigger host immunity and thereby reduce disease (42, 83). Given the complexity of mechanistic interactions underlying endophyte-mediated disease modification, one might conclude that designing applications that are generalizable across the world's 60,065 tree species is futile. To the contrary, given that endophyte communities are taxonomically diverse and that endophytes capable of modifying disease occur across diverse fungal lineages, we expect that promoting endophyte symbiosis will result in a diversity of interactions that protect tree seedlings from their pathogens. Such a boost by inoculation may be needed to eliminate a deficit in endophyte diversity in nursery-grown seedlings compared to their naturally regenerated counterparts (51). A positive relationship between endophyte diversity and disease protection could result from complementarity among the effects of many species or a greater chance that the community includes a particularly strong pathogen antagonist (72, 127). Indeed, diversity within phyllosphere bacteria communities was previously shown to be associated with greater disease protection in *Arabidopsis* (31). Beyond diverse fungal leaf endophyte communities, plants deploy a variety of other above- and belowground defensive symbionts to aid in pathogen defense—e.g., bacterial endophytes (34, 106, 138), viruses (115), mycorrhizae (66, 80), and nematodes (70)—further suggesting that conserving diversity in the microbiome will protect this associated function.

PHYLLOSPHERE FEEDBACK CAN BE POSITIVE

To explore the potential for fungal leaf endophyte communities to serve as defensive symbionts in regenerating forests, we searched the literature for studies of plant phyllosphere feedback. This is an emerging area of research that adapts the experimental framework from plant soil feedback, builds on considerable work with nurse plants and mulch in forest restoration (19), and asks questions about how foliar microbes may either support or hinder the performance (growth, defense, etc.) of conspecific seedlings (140). These feedbacks can be tested by inoculating aboveground plant tissues with microbes sourced from the phyllosphere (synthetic communities or bulked from litter) and comparing plant performance relative to either uninoculated controls or those inoculated with microbes sourced from heterospecific litter. Likewise, plant soil feedback experiments investigate these interactions with soil-inhabiting microbial communities by

examining the extent to which belowground communities facilitate or inhibit growth of conspecific seedlings (18). Past research has shown that host-specific soil pathogens can accumulate and suppress seedling growth in conspecific soils (16, 35, 68, 78), whereas mycorrhizae facilitate positive feedbacks in conspecific soils (35). In some cases, endophytic bacteria (*Pseudomonas* spp.) in roots directly antagonize root pathogens of forest trees linked by the mycorrhizal network (39). However, rarely are phyllosphere microbes considered in feedback studies.

In a strict sense, a plant phyllosphere feedback study is one in which microbes sourced from the phyllosphere, rather than the leaf litter in its entirety, are applied to experimental plants. This distinction ensures that any treatment effect on plant performance can be attributed to the microbes rather than to other factors associated with leaf litter that have been shown to influence seedling fitness, such as metabolites or shading (136, 137). Unfortunately, our literature search identified very few published plant phyllosphere feedback studies that fit our definition, and no studies focusing on tree species or pathogen protection (**Table 1**). All experiments used Asteraceae plant hosts and found that microbes sourced from conspecific litter reduced seedling fitness relative to heterospecific litter (45, 140, 146). Plants that are phylogenetically or physiologically different from those used in these studies, like tree species, may exhibit a different feedback strength or type.

Other plant phyllosphere feedback studies, although not strictly fitting our definition as stated above, used low-tech protocols for applying phyllosphere microbes and could thus serve as models for reforestation efforts. Such studies diverged from our definition in that the inoculum source was litter in its entirety (rather than microbes sourced from litter), or the experiment did not include conspecific inoculum. Although several of these studies have demonstrated conspecific litter inhibiting the growth of seedlings (6, 77), others suggest circumstances in which plant phyllosphere feedback is positive. For example, a recent study found that conspecific litter of the common ragwort, *Jacobaea vulgaris*, reduced seed germination rates yet increased growth for established seedlings (90). These findings imply that the way plants interact with microbial communities varies by life stage. Litter microbes have also been found to provide plant hosts with disease protection. In one example, the host was a conspecific tree seedling that had been inoculated with conspecific litter (33), whereas, in another, the host was an agricultural crop and the litter inoculum had been sourced from a nearby pine forest (110). Overall, these studies highlight context dependency in plant phyllosphere feedback. Experiments have the potential to play an integral role in informing how to best harness the aboveground microbiome in planting efforts. However, to do so effectively, these experiments will need to replicate the context in which the litter microbes are to be applied.

Douglas fir (*P. menziesii*), a dominant conifer of the Pacific Northwest of the United States (109), will be a focal species used in a reforestation project in Willamette National Forest, Oregon. This ready application makes *P. menziesii* an ideal candidate for a plant phyllosphere feedback experimental case study that could inform the use of leaf litter as a source of microbial inoculum for seedlings in this tree-planting project. In a greenhouse inoculation experiment, we found that coastal Douglas fir (*P. menziesii* var. *menziesii*) seedlings inoculated with microbes sourced from Douglas fir litter (moist-incubated to enhance fungal sporulation) and applied directly to needles displayed greater growth than uninoculated control plants (A.S. Neat, F.A. Jones, J. LaManna, K.A. Gervers, P.E. Busby, unpublished data). However, feedback varied between two tree populations: the upper-elevation population benefited from phyllosphere microbes, whereas the lower-elevation population was unaffected. Similarly, the forestry literature reports coastal Douglas fir (*P. menziesii* var. *menziesii*) seedlings perform best on bare mineral soils, whereas Rocky Mountain Douglas fir (*P. menziesii* var. *glauca*) seedlings benefit from a litter layer (65). Although these examples illustrate the benefit of litter microbes for seedling growth and their inexpensive and easy

Table 1 Summary of methods and findings for published phyllosphere feedback studies

Plant species	Plant type	Life stage	Experimental design	Inoculum	Inoculum source	Response to inoculum	Reference[a]
Triticum aestivum	Grass (crop)	Seedling	Greenhouse	Foliar microbes	Nearby pine forest	Pathogen protection	110
Ageratina adenophora	Herbaceous	Seed, seedling	Laboratory	Foliar microbes	Conspecific plant material	Reduced germination rate, reduced growth	45
Jacobaea vulgaris	Herbaceous	Seed, seedling	Greenhouse	Direct litter exposure	Conspecific or heterospecific plant material	Reduced germination rate, increased growth from conspecific exposure	90
Asteraceae spp.	Herbaceous	Seedling	Greenhouse	Foliar microbes	Conspecific or heterospecific plant material	Reduced growth from conspecific exposure	140
Asteraceae spp.	Herbaceous	Seedling	Greenhouse	Foliar microbes	Conspecific or heterospecific plant material	Reduced growth from conspecific exposure	146
Theobroma cacao	Tree	Seedling	Field	Direct litter exposure	Conspecific plant material	Pathogen protection	33
Fagus sylvatica, *Picea abies*	Tree	Seed	Field	Direct litter exposure	Conspecific or heterospecific plant material	Reduced germination rate from conspecific or heterospecific exposure	6
Machilus leptophylla, *Litsea elongata*, *Acer pubinerve*, *Distylium myricoides*	Tree	Seedling	Field survey	Direct litter exposure	Conspecific or heterospecific plant material	Reduced seedling survival from conspecific exposure	77
Pseudotsuga menziesii	Tree	Seedling	Greenhouse	Foliar microbes	Conspecific or heterospecific plant material	Increased growth from conspecific exposure	A.S. Neat, F.A. Jones, J. LaManna, K.A. Gervers, P. Busby, unpublished data

[a]These studies were selected because the host plants were inoculated with microbes sourced from litter or directly exposed to litter in an experiment designed specifically to focus on the influence of litter microbes on host fitness (rather than other qualities of litter, such as shading).

application, they also highlight complexities in outcomes that are not currently understood. Thus, even for one of the most well-studied tree species in forestry, knowledge gaps prevent us from answering the question of when, where, and how adding conspecific leaf litter to planted seedlings promotes defensive symbiosis. Below, we highlight research priorities that can accelerate our understanding of the role of diverse microbiomes in tree performance, defense, and resilience as well as our ability to optimize these benefits in global forest restoration.

THE IMPORTANCE OF INTEGRATING PLANT AND MICROBIAL DIVERSITY INTO GLOBAL FOREST RESTORATION

Given the urgency in responding to the climate and biodiversity crises, and the known problems associated with planting tree species outside their native ranges, it will be necessary to expand tree populations in situ where their pathogens commonly occur. An alternative to the plantation forestry paradigm is needed, one that integrates both local tree species diversity and microbial diversity. The importance of tree diversity (e.g., plantations of species mixtures) has received some attention in forestry and conservation (2, 68, 106). In this review, we have focused on how tree microbiome diversity can promote pathogen defense in regenerating forests. In addition, transplanting the tree microbiome should also promote forest biodiversity broadly (86). For example, at the scale of a forest ecosystem, tree pathogens are not necessarily negative. Specialized, host-specific pathogens diversify plant communities by attacking only their hosts, thus providing a local advantage to their hosts' competitors (15). Pathogens can also diversify forest communities via habitat formation, e.g., by creating witches' brooms and stem cavities for birds and mammals (126). Forest endophytes can promote plant diversity by acting as commensal symbionts on their host plants but pathogens on their hosts' competitors (i.e., apparent competition) (56, 92). Thus, just as a tree seedling will suffer if it is not transplanted with its associated microbes, a forest cannot be truly restored without its microbiome.

Plantations are well known to lack the biodiversity found in nearby forests (22). Similarly, arboretums illustrate how tree planting without consideration for microbial diversity can fail to become forests. For example, the University of Idaho's 100-year-old arboretum is superficially like the natural forests of Moscow Mountain 16 kilometers away—both contain a mixture of mature coniferous and deciduous tree species. The founding goal of the arboretum was to introduce and observe exotic tree species. The pervasive consequences of that goal are striking. The obvious difference is that there are non-native tree species in the arboretum and native trees in the natural forest; less obvious is the recapitulation of this pattern by understory flora (30). In addition, the mycofloral diversity of the arboretum is lacking compared to the natural forest. Common pathogens of native trees such as dwarf mistletoe of Douglas fir and decay fungi such as species of *Fomitopsis* are also absent from the arboretum. In contrast, non-native pathogens of non-native trees (e.g., powdery mildew of *Acer platanoides*) and herbaceous plants (e.g., an oomycetous pathogen of *Lunaria annua*) are present. The contrast between plantings and forests reminds us that tree plantings should not only remove carbon from the atmosphere but also conserve biodiversity. Symbionts, with their interactions and processes, make plantings into forests.

The idea of promoting biodiversity to benefit ecosystem health and productivity is not new. In the mid-nineteenth century, Darwin hypothesized that diverse plant communities were more productive than species individually (37). The relationship between plant species diversity and ecosystem productivity was later confirmed (128) and then shown to be driven by microbes (116). In forestry, muvuca de sementes (Portuguese for mixture of seeds) in the Amazon and the Japanese method developed by the late Akira Miyawaki are two examples in which tree diversity seeks to enhance forest biodiversity (89). Miyawaki was also an early advocate of integrating mycorrhizae

Figure 2

A tree-planting site. Plant-for-the-Planet trial, Yucatán Peninsula of México. (*a*) 16,000 seedlings were planted in a randomized block design situated on an abandoned cattle pasture in a Mexican semi-evergreen tropical forest in 2020. The blocks were paired with natural regeneration control plots. Survival and growth rates are measured to track response. (*b*) Planting ~50-cm tall seedlings of eight native, arbuscular mycorrhizal fungi–associated tree species. Half of the planting holes were inoculated with 500 mL of soil from a nearby forest in which the same species frequently occur to accelerate restoration by reintroducing symbionts. The other half of the seedlings were planted without inoculum.

into forest restoration to enhance seedling growth and survival, a practice now recognized in grassland restoration (17, 71, 142). Also, ongoing, large-scale field trials in Wales, UK and the Yucatán Peninsula of México are testing the idea that restoration of both trees and a complex soil microbiome from nearby intact forests can facilitate tree growth and survival. For example, in a Plant-for-the-Planet trial in the Yucatán, seedlings of eight native, AM fungi–associated tree species are being planted with or without 500 mL of soil from a nearby forest in which the same species occur (**Figure 2**). This treatment aims to accelerate restoration by reintroducing fungal symbionts. If positive microbial restoration–ecosystem function outcomes are strong, they could form the foundation of a microbiome restoration industry built on top of emerging forest carbon and biodiversity markets. However, as we work to incorporate the microbiome into a more holistic picture of ecosystem restoration, it is critical that introduced microbes are complex, diverse, and sourced from local populations. Doing so represents the best chance to rebuild and maintain the incredible biodiversity of microbial life inhabiting Earth's forests.

RESEARCH PRIORITIES FOR FACILITATING REFORESTATION THROUGH THE PLANT MICROBIOME

We have illustrated knowledge gaps in our understanding of tree pathogens and endophytes that raise questions about how to best promote microbiome-mediated defense against pathogens during a tree's most vulnerable stage of development and beyond, as trees age and face an unpredictable array of abiotic and biotic challenges (101). These gaps exist because plant–microbiome interactions have traditionally been studied in model systems (e.g., *Arabidopsis*) and crop plants, with objectives that are about neither conservation of biodiversity nor carbon removals from the atmosphere. Thus, tree-planting and natural forest regeneration projects must pioneer new opportunities to study tree microbiomes as they broadly relate to host health across a diverse array of tree species. Our overarching hypothesis is that conserving the tree microbiome in planting

efforts will enhance tree seedling defense and survival and ultimately the success of forest restoration in terms of carbon capture, forest biodiversity, and long-term forest resilience. The research priorities described below provide a framework for testing this hypothesis.

Identifying Causal Factors that Give Rise to Variation in Tree Microbiome Composition and Its Functional Influence on Tree Seedlings

How do the benefits of hosting a diverse microbiome vary among tree species and environments? Many studies have documented variation in the plant microbiome across host species, guilds (e.g., EM versus AM, evergreen versus deciduous), developmental stages, trait values, and along complex environmental gradients (e.g., temperature, moisture, elevational, latitudinal gradients) (38). For example, variation among tree hosts (73, 134) and along climatic gradients (9, 55, 148) influences microbial composition in the phyllosphere. Yet how such variation, in turn, influences the functional influence of the microbiome on its host is poorly understood. Functional convergence among compositionally distinct soil microbial communities has been shown at continental scales in *Pinus* forests (124). However, plant–soil feedback can vary along environmental gradients (74), with particular microbes enabling plant survival in extreme environments (i.e., habitat-adapted symbiosis) (112). Given the urgency in global forest restoration, we must learn as we go, experimentally manipulating hosts and microbiomes across regenerating forests to determine optimal parameters for maximizing success. At the same time, we must also continue to learn from intact forests, asking questions such as whether endophytic communities of tree species vary along gradients of forest age and diversity. Formally recognizing thousands of undescribed species in this effort may be necessary for consideration in conservation. Do communities of endophytic bacteria and fungi constitute an extension of the tree defense system provided by mycorrhizal networks (120) or a separate system? Collaboration among forest ecologists, microbiologists, pathologists, and tree planters is essential for making progress on this and other research priorities.

Determining How Planting and Transplanting Techniques for Both Seedlings and Associated Microbes Influence the Benefit of the Microbiome to its Host

What planting and transplanting practices best promote above- and belowground tree symbionts? How well do transplanted microbes establish and persist in forest restoration sites? How do the age of the intact forest and its distance to planted or naturally regenerated tree seedlings influence benefits derived from the forest's mycorrhizal network? In native prairie restoration, the benefit of inoculating plants with AM fungi extended to uninoculated plants two meters away (84, 85). Does it make a difference if the network is EM or AM (118)? Comparisons of agricultural fields and intact forests have revealed little diversity of Glomales (AM forming) in arable land and more diversity in the forest soil (64). If older forest soil is used as inoculum, spores and other propagules must be present and viable; fortunately, fungal propagules remain viable for years (94). As with the first research priority, these questions can be addressed in the context of ongoing restoration efforts with effective collaboration between scientists and restorationists.

Transplanting the phyllosphere microbiome involves additional considerations. Can we transplant the phyllosphere microbiome as readily as we transplant the soil microbiome? Fungal leaf endophytes have been applied individually to bolster plant defense. For example, seedlings are inoculated with *Beauveria bassiana*, an endophyte of *Pinus monticola* (51) and an entomopathogenic member of Cordycipitaceae, to protect against insects. And seedlings are commonly inoculated with species of *Trichoderma*, common antagonistic endophytes of many tree species, to protect against a variety of pathogens (26). However, even in the few well-studied systems in which good candidates for inoculation are known, there are logistical obstacles to generating and applying

inoculum. Isolating and applying an individual endophyte require technical skills and access to specialized equipment, whereas diverse leaf litter can be applied by anyone given simple instructions. Some evidence for disease protection in tree seedlings treated with conspecific litter (33), in combination with the benefits of mulching (19), suggests that planting tree seedlings with conspecific litter is a tractable method for conserving phyllosphere microbial diversity in tree-planting efforts. Litter can be transplanted to naturally regenerating tree seedlings, which may help to accelerate a process that is often viewed as passive (143). Yet these are hypotheses that require testing in ongoing reforestation efforts.

Identifying Keystone Microbes in the Forest Microbiome

The tree microbiome is a part of the broader forest microbiome, i.e., microbes associated with trees but also with other forest habitats like soil, snags, rocks, insects, mammals, birds, lichens, etc. Successfully restoring forests, with their diverse organisms, processes, and interactions, hinges on restoring the forest microbiome as a whole, including pathogens. But how do we do this? The renowned forest restorationist Payeng, or "Forest Man" (54), swore by his practice of transplanting tree seedlings with worms, ants, termites, and other soil builders. Starting on barren ground and with no professional credentials, Payeng single-handedly built a forest on his island in the Brahmaputra River, India, that now attracts diverse megafauna like tigers and elephants. On the other side of the world, in the US Pacific Northwest, live Douglas fir trees and snags, and therefore their associated microbiomes and necrobiomes, are retained after cutting to accelerate forest development (49). Retaining live trees and their associated mycorrhizal networks is now well recognized as benefiting forest regeneration (117). But what are other keystone forest microbes? Identifying critical forest microbes and associated processes will allow us to prioritize elements of the forest microbiome for conservation.

Determining the Relationship Between Forest Microbiomes and Greenhouse Gasses Beyond Carbon Dioxide

Forest microbes play a critical role in carbon capture. Yet we know little about how forest microbes influence greenhouse gasses other than carbon dioxide, e.g., nitrous oxide (a greenhouse gas 300 times more potent than carbon dioxide). Urea is applied in agriculture to compensate for limited nitrogen, but this can lead to an increase in atmospheric nitrous oxide. An early study showed that excluding EM fungi increased nitrous oxide emissions (44). More recent work, with both EM and AM fungi, has shown that these symbionts can reduce emissions of nitrous oxide (123, 125). Additional studies on both nitrous oxide and methane emissions from forest trees are needed, with the contributions of specific microbes addressed (105).

CONCLUSION

A growing body of work supports the notion that the functional influence of the microbiome on its tree host is beneficial and protective. Yet the full extent of microbial interactions and their potential contributions to global reforestation are not clearly understood (86). We identified major knowledge gaps by reviewing the pathogen status of US tree species, and the functionality of phyllosphere microbes. Even for the world's most simple forest—Pando, the 47,000-stem aspen monoculture in central Utah that is the world's largest and oldest organism (40)—the tree microbiome is largely unstudied. Forest microbiologists and ecologists will play an important role in advancing the science of forest microbiomes as we begin the Decade of Ecosystem Restoration called for by the United Nations. With variation in new forests ranging from monoclonal

plantations to highly diverse Miyawaki forests and naturally regenerated or surviving old forests, there is ample variation to study and understand. From tree species without pathogens and trees whose pathogens have yet to be described to the functions of phyllosphere microbes in widely ranging species with hundreds of known pathogens, we have a great deal to learn. We will need to consider the presence or absence of genes for resistance, particular symbionts that protect their hosts, the functions of endophytes in trees lacking pathogens, and the overall contribution of the forest microbiome to ecosystem function. We may discover ways to better protect seedlings and young saplings with low-tech, cost-effective exposures to leaf litter. We may find that greater initial diversity shortens the period that new plantings spend as carbon sources before becoming carbon sinks. Above all, we must engage in this urgent effort to restore Earth's forests.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We are grateful to Devin Leopold and Andy Jones for comments on an earlier version of this manuscript. This work was supported by the National Science Foundation under the LTER Grant LTER8 DEB-2025755 (2020–2026), the US Department of Energy's Office of Biological and Environmental Research (DE-SC0019435), and the US Department of Agriculture National Institute of Food and Agriculture (2020-67013-31797). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation.

LITERATURE CITED

1. Adame-Álvarez R-M, Mendiola-Soto J, Heil M. 2014. Order of arrival shifts endophyte-pathogen interactions in bean from resistance induction to disease facilitation. *FEMS Microbiol. Lett.* 355(2):100–7
2. Aerts R, Honnay O. 2011. Forest restoration, biodiversity and ecosystem functioning. *BMC Ecol.* 11(1):29
3. Agrios GN. 2005. *Plant Pathology*. Cambridge, MA: Acad. Press. 5th ed.
4. Arnold AE, Lutzoni F. 2007. Diversity and host range of foliar fungal endophytes: Are tropical leaves biodiversity hotspots? *Ecology* 88(3):541–49
5. Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, et al. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *PNAS* 100(26):15649–54
6. Asplund J, Hustoft E, Nybakken L, Ohlson M, Lie MH. 2018. Litter impair spruce seedling emergence in beech forests: a litter manipulation experiment. *Scand. J. For. Res.* 33(4):332–37
7. Averill C, Turner BL, Finzi AC. 2014. Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature* 505(7484):543–45
8. Baldrian P. 2016. Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiol. Rev.* 41(2):109–30
9. Barge EG, Leopold DR, Peay KG, Newcombe G, Busby PE. 2019. Differentiating spatial from environmental effects on foliar fungal communities of *Populus trichocarpa*. *J. Biogeogr.* 46(9):2001–11
10. Barge EG, Leopold DR, Rojas A, Vilgalys R, Busby PE. 2022. Phylogenetic conservatism of mycoparasitism and its contribution to pathogen antagonism. *Mol. Ecol.* 31(10):3018–30
11. Barnes I, Fourie A, Wingfield MJ, Harrington TC, McNew DL, et al. 2018. New *Ceratocystis* species associated with rapid death of *Metrosideros polymorpha* in Hawai'i. *Persoonia* 40(1):154–81
12. Baskin CC, Baskin JM. 1998. *Seeds*: *Ecology*, *Biogeography*, *and Evolution of Dormancy and Germination*. San Diego: Academic
13. Bebber DP, Gurr SJ. 2015. Crop-destroying fungal and oomycete pathogens challenge food security. *Fungal Genet. Biol.* 74:62–64

14. Beech E, Rivers M, Oldfield S, Smith PP. 2017. GlobalTreeSearch: the first complete global database of tree species and country distributions. *J. Sustain. For.* 36(5):454–89
15. Benítez M-S, Hersh MH, Vilgalys R, Clark JS. 2013. Pathogen regulation of plant diversity via effective specialization. *Trends Ecol. Evol.* 28(12):705–11
16. Bever JD, Mangan SA, Alexander HM. 2015. Maintenance of plant species diversity by pathogens. *Annu. Rev. Ecol. Evol. Syst.* 46:305–25
17. Bever JD, Schultz PA, Miller RM, Gades L, Jastrow JD. 2003. Prairie mycorrhizal fungi inoculant may increase native plant diversity on restored sites (Illinois). *Ecol. Restor.* 21:311–12
18. Bever JD, Westover KM, Antonovics J. 1997. Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *J. Ecol.* 85(5):561–73
19. Blanco-García A, Sáenz-Romero C, Martorell C, Alvarado-Sosa P, Lindig-Cisneros R. 2011. Nurse-plant and mulching effects on three conifer species in a Mexican temperate forest. *Ecol. Eng.* 37(6):994–98
20. Bogdanski BEC, Cruickshank M, Mario Di Lucca C, Becker E. 2018. Stumping out tree root disease: an economic analysis of controlling root disease, including its effects on carbon storage in southern British Columbia. *For. Ecol. Manag.* 409:129–47
21. Brasier CM. 2008. The biosecurity threat to the UK and global environment from international trade in plants. *Plant Pathol.* 57(5):792–808
22. Bremer LL, Farley KA. 2010. Does plantation forestry restore biodiversity or create green deserts? A synthesis of the effects of land-use transitions on plant species richness. *Biodivers. Conserv.* 19(14):3893–915
23. Brinck K, Fischer R, Groeneveld J, Lehmann S, Dantas De Paula M, et al. 2017. High resolution analysis of tropical forest fragmentation and its impact on the global carbon cycle. *Nat. Commun.* 8(1):14855
24. Brown PE. 1918. *Soil inoculation*. Rep. 43, Agric. Exp. Stn. Iowa State Coll. Agric. Mech. Arts, Ames, IA
25. Brown SP, Leopold DR, Busby PE. 2018. Protocols for investigating the leaf mycobiome using high-throughput DNA sequencing. *Methods Mol. Biol.* 1848:39–51
26. Brunner K, Zeilinger S, Ciliento R, Woo SL, Lorito M, et al. 2005. Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance. *Appl. Environ. Microbiol.* 71(7):3959–65
27. Busby PE, Aime MC, Newcombe G. 2012. Foliar pathogens of *Populus angustifolia* are consistent with a hypothesis of Beringian migration into North America. *Fungal Biol.* 116(7):792–801
28. Busby PE, Peay KG, Newcombe G. 2016. Common foliar fungi of *Populus trichocarpa* modify Melampsora rust disease severity. *New Phytol.* 209(4):1681–92
29. Busby PE, Ridout M, Newcombe G. 2016. Fungal endophytes: modifiers of plant disease. *Plant Mol. Biol.* 90(6):645–55
30. Cervantes D, Ridout M, Nischwitz C, Newcombe G. 2021. Adult plant resistance to white rust in *Lunaria annua*. *Phytopathol. Mediterr.* 60(2):381–85
31. Chen T, Nomura K, Wang X, Sohrabi R, Xu J, et al. 2020. A plant genetic network for preventing dysbiosis in the phyllosphere. *Nature* 580(7805):653–57
32. Chock MK, Hoyt BK, Amend AS. 2021. Mycobiome transplant increases resistance to *Austropuccinia psidii* in an endangered Hawaiian plant. *Phytobiomes J.* 5(3):326–34
33. Christian N, Herre EA, Mejia LC, Clay K. 2017. Exposure to the leaf litter microbiome of healthy adults protects seedlings from pathogen damage. *Proc. R. Soc. B* 284(1858):20170641
34. Compant S, Duffy B, Nowak J, Clément C, Barka EA. 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.* 71(9):4951–59
35. Crawford KM, Bauer JT, Comita LS, Eppinga MB, Johnson DJ, et al. 2019. When and where plant-soil feedback may promote plant coexistence: a meta-analysis. *Ecol. Lett.* 22(8):1274–84
36. Crowther TW, Glick HB, Covey KR, Bettigole C, Maynard DS, et al. 2015. Mapping tree density at a global scale. *Nature* 525(7568):201–5
37. Darwin C. 2008. *On the Origin of Species*. New York: Oxford Univ. Press
38. Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A. 2020. Plant microbiome: an account of the factors that shape community composition and diversity. *Curr. Plant Biol.* 23:100161

39. DeLong RL, Lewis KJ, Simard SW, Gibson S. 2002. Fluorescent pseudomonad population sizes baited from soils under pure birch, pure Douglas-fir, and mixed forest stands and their antagonism toward *Armillaria ostoyae* in vitro. *Can. J. For. Res.* 32(12):2146–59
40. DeWoody J, Rowe CA, Hipkins VD, Mock KE. 2008. "Pando" lives: molecular genetic evidence of a giant aspen clone in central Utah. *West. N. Am. Nat.* 68(4):493–97
41. Egan CP, Koko JH, Muir CD, Zahn G, Swift SOI, et al. 2021. Restoration of the mycobiome of the endangered Hawaiian mint *Phyllostegia kaalaensis* increases its resistance to a common powdery mildew. *Fungal Ecol.* 52:101070
42. Eitzen K, Sengupta P, Kroll S, Kemen E, Doehlemann G. 2021. A fungal member of the *Arabidopsis thaliana* phyllosphere antagonizes *Albugo laibachii* via a GH25 lysozyme. *eLife* 10:e65306
43. Enebak SA, Carey WA. 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. *Plant Dis.* 84(3):306–8
44. Ernfors M, Rütting T, Klemedtsson L. 2011. Increased nitrous oxide emissions from a drained organic forest soil after exclusion of ectomycorrhizal mycelia. *Plant Soil* 343(1–2):161–70
45. Feng X, Chen L, Zhou J, Yang Z-P, Dong X-F, Zhang H-B. 2019. Plant–soil–foliage feedbacks on seed germination and seedling growth of the invasive plant *Ageratina adenophora*. *Proc. R. Soc. B.* 286:1917
46. Fenner M, Thompson K. 2005. *The Ecology of Seeds*. New York: Cambridge Univ. Press
47. Flor HH. 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9:275–96
48. Fonseca JP, Mysore KS. 2019. Genes involved in nonhost disease resistance as a key to engineer durable resistance in crops. *Plant Sci.* 279:108–16
49. Franklin JF, Donato DC. 2020. Variable retention harvesting in the Douglas-fir region. *Ecol. Process.* 9(1):8
50. Ganley RJ, Brunsfeld SJ, Newcombe G. 2004. A community of unknown, endophytic fungi in western white pine. *PNAS* 101(27):10107–12
51. Ganley RJ, Newcombe G. 2006. Fungal endophytes in seeds and needles of *Pinus monticola*. *Mycol. Res.* 110(3):318–27
52. Ganley RJ, Sniezko RA, Newcombe G. 2008. Endophyte-mediated resistance against white pine blister rust in *Pinus monticola*. *For. Ecol. Manag.* 255(7):2751–60
53. Gatti LV, Basso LS, Miller JB, Gloor M, Gatti Domingues L, et al. 2021. Amazonia as a carbon source linked to deforestation and climate change. *Nature* 595(7867):388–93
54. Gautam H, Sharma I, Kumar R. 2014. Jadav Molai Payeng: the "Forest Man of India." *Curr. Sci.* 106(4):499
55. Gomes T, Pereira JA, Benhadi J, Lino-Neto T, Baptista P. 2018. Endophytic and epiphytic phyllosphere fungal communities are shaped by different environmental factors in a Mediterranean ecosystem. *Microb. Ecol.* 76(3):668–79
56. Griffin EA, Carson WP. 2018. Tree endophytes: cryptic drivers of tropical forest diversity. In *Endophytes of Forest Trees*, ed. AM Pirttilä, AC Frank, pp. 63–103. Cham, Switz.: Springer
57. Hansen AJ, Burns P, Ervin J, Goetz SJ, Hansen M, et al. 2020. A policy-driven framework for conserving the best of Earth's remaining moist tropical forests. *Nat. Ecol. Evol.* 4(10):1377–84
58. Hansen AJ, Noble BP, Veneros J, East A, Goetz SJ, et al. 2021. Toward monitoring forest ecosystem integrity within the post-2020 Global Biodiversity Framework. *Conserv. Lett.* 14(4):e12822
59. Harper JL. 2010. *Population Biology of Plants*. London: Acad. Press
60. Harrison JG, Forister ML, Parchman TL, Koch GW. 2016. Vertical stratification of the foliar fungal community in the world's tallest trees. *Am. J. Bot.* 103(12):2087–95
61. Hassani MA, Durán P, Hacquard S. 2018. Microbial interactions within the plant holobiont. *Microbiome* 6(1):58
62. Hatch AB. 1936. The role of mycorrhizae in afforestation. *J. For.* 34(1):22–29
63. Heijden MGA, Martin FM, Selosse M, Sanders IR. 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytol.* 205(4):1406–23
64. Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW. 1998. Ploughing up the wood-wide web? *Nature* 394(6692):431
65. Hermann RK, Lavender DP. 1990. *Pseudotsuga menziesii* (Mirb.) Franco, Douglas-fir. *Silv. N. Am.* 1:527–40

66. Jung SC, Martinez-Medina A, Lopez-Raez JA, Pozo MJ. 2012. Mycorrhiza-induced resistance and priming of plant defenses. *J. Chem. Ecol.* 38(6):651–64
67. Keith LM, Hughes RF, Sugiyama LS, Heller WP, Bushe BC, Friday JB. 2015. First report of *Ceratocystis* wilt on `Ōhi`a (*Metrosideros polymorpha*). *Plant Dis.* 99(9):1276
68. Klironomos JN. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* 417(6884):67–70
69. Kolbert E. 2014. *The Sixth Extinction: An Unnatural History*. New York: Henry Holt
70. Kosaka H, Aikawa T, Ogura N, Tabata K, Kiyohara T. 2001. Pine wilt disease caused by the pine wood nematode: induced resistance of pine trees by the avirulent isolates of nematode. *Eur. J. Plant Pathol.* 107(7):667–75
71. Koziol L, Bauer JT, Duell EB, Hickman K, House GL, et al. 2021. Manipulating plant microbiomes in the field: Native mycorrhizae advance plant succession and improve native plant restoration. *J. Appl. Ecol.* In press
72. Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. 2017. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* 546(7656):145–47
73. Lajoie G, Kembel SW. 2019. Making the most of trait-based approaches for microbial ecology. *Trends Microbiol.* 27(10):814–23
74. LaManna JA, Mangan SA, Alonso A, Bourg NA, Brockelman WY, et al. 2017. Plant diversity increases with the strength of negative density dependence at the global scale. *Science* 356(6345):1389–92
75. Leopold A. 1972. *A Sand County Almanac and Sketches Here and There*. New York: Oxford Univ. Press
76. Leopold DR, Busby PE. 2020. Host genotype and colonist arrival order jointly govern plant microbiome composition and function. *Curr. Biol.* 30(16):3260–66.e5
77. Liu H, Shen G, Ma Z, Yang Q, Xia J, et al. 2016. Conspecific leaf litter-mediated effect of conspecific adult neighborhood on early-stage seedling survival in a subtropical forest. *Sci. Rep.* 6(1):37830
78. Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, et al. 2010. Negative plant-soil feedback predicts tree-species relative abundance in a tropical forest. *Nature* 466(7307):752–55
79. Martinez-Vilalta J. 2014. Carbon storage in trees: pathogens have their say. *Tree Physiol.* 34(3):215–17
80. Marx DH. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. II. Production, identification, and biological activity of antibiotics produced by *Leucopaxillus cerealis* var. *piceina*. *Phytopathology* 59(4):411–17
81. Marx DH. 1991. The practical significance of ectomycorrhizae in forest establishment. *Marcus Wallenberg Found. Symp. Proc.* 7:54–90
82. McLaren MR, Callahan BJ. 2020. Pathogen resistance may be the principal evolutionary advantage provided by the microbiome. *Philos. Trans. R. Soc. B* 375(1808):20190592
83. Mejía LC, Herre EA, Sparks JP, Winter K, García MN, et al. 2014. Pervasive effects of a dominant foliar endophytic fungus on host genetic and phenotypic expression in a tropical tree. *Front. Microbiol.* 5:479
84. Middleton EL, Bever JD. 2012. Inoculation with a native soil community advances succession in a grassland restoration. *Restor. Ecol.* 20(2):218–26
85. Middleton EL, Richardson S, Koziol L, Palmer CE, Yermakov Z, et al. 2015. Locally adapted arbuscular mycorrhizal fungi improve vigor and resistance to herbivory of native prairie plant species. *Ecosphere* 6(12):art276
86. Mishra S, Hättenschwiler S, Yang X. 2020. The plant microbiome: a missing link for the understanding of community dynamics and multifunctionality in forest ecosystems. *Appl. Soil Ecol.* 145:103345
87. Mitchard ETA. 2018. The tropical forest carbon cycle and climate change. *Nature* 559(7715):527–34
88. Mitchell CE, Power AG. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* 421(6923):625–27
89. Miyawaki A, Box EO. 2006. *The Healing Power of Forests: The Philosophy Behind Restoring Earth's Balance with Native Trees*. Tokyo: Kosei Publ.
90. Möhler H, Diekötter T, Bauer GM, Donath TW. 2021. Conspecific and heterospecific grass litter effects on seedling emergence and growth in ragwort (*Jacobaea vulgaris*). *PLOS ONE* 16(2):e0246459
91. Mora C, Dousset B, Caldwell IR, Powell FE, Geronimo RC, et al. 2017. Global risk of deadly heat. *Nat. Clim. Change* 7(7):501–6

92. Newcombe G, Fraser SJ, Ridout M, Busby PE. 2020. Leaf endophytes of *Populus trichocarpa* act as pathogens of neighboring plant species. *Front. Microbiol.* 11:573056
93. Newton P, Kinzer AT, Miller DC, Oldekop JA, Agrawal A. 2020. The number and spatial distribution of forest-proximate people globally. *One Earth* 3(3):363–70
94. Nguyen NH, Hynson NA, Bruns TD. 2012. Stayin' alive: survival of mycorrhizal fungal propagules from 6-yr-old forest soil. *Fungal Ecol.* 5(6):741–46
95. Nischwitz C, Newcombe G, Anderson CL. 2005. Host specialization of the mycoparasite *Eudarluca caricis* and its evolutionary relationship to *Ampelomyces*. *Mycol. Res.* 109(4):421–28
96. O'Connor B, Bojinski S, Röösli C, Schaepman ME. 2020. Monitoring global changes in biodiversity and climate essential as ecological crisis intensifies. *Ecol. Inform.* 55:101033
97. Osono T. 2006. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Can. J. Microbiol.* 52(8):701–16
98. Percy DM, Garver AM, Wagner WL, James HF, Cunningham CW, et al. 2008. Progressive island colonization and ancient origin of Hawaiian *Metrosideros* (Myrtaceae). *Proc. R. Soc. B* 275(1642):1479–90
99. Petrini O. 1991. Fungal endophytes of tree leaves. In *Microbial Ecology of Leaves*, ed. JH Andrews, SS Hirano, pp. 179–97. New York: Springer
100. Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM. 2014. Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.* 52:347–75
101. Piovesan G, Biondi F. 2021. On tree longevity. *New Phytol.* 231(4):1318–37
102. Porras-Alfaro A, Bayman P. 2011. Hidden fungi, emergent properties: endophytes and microbiomes. *Annu. Rev. Phytopathol.* 49:291–315
103. Price JP, Wagner WL. 2018. Origins of the Hawaiian flora: Phylogenies and biogeography reveal patterns of long-distance dispersal. *J. Syst. Evol.* 56(6):600–20
104. Prigge BA. 2002. A new species of *Prunus* (Rosaceae) from the Mojave Desert of California. *Madroño* 49(4):285–88
105. Putkinen A, Siljanen HMP, Laihonen A, Paasisalo I, Porkka K, et al. 2021. New insight to the role of microbes in the methane exchange in trees: evidence from metagenomic sequencing. *New Phytol.* 231(2):524–36
106. Raaijmakers JM, Vlami M, de Souza JT. 2002. Antibiotic production by bacterial bioconrol agenets. *Antonie Van Leeuwenhoek* 81(1–4):537–47
107. Rabiey M, Hailey LE, Roy SR, Grenz K, Al-Zadjali MAS, et al. 2019. Endophytes versus tree pathogens and pests: Can they be used as biological control agents to improve tree health? *Eur. J. Plant Pathol.* 155(3):711–29
108. Raghavendra AKH, Newcombe G. 2013. The contribution of foliar endophytes to quantitative resistance to *Melampsora* rust. *New Phytol.* 197(3):909–18
109. Rehfeldt GE. 1989. Ecological adaptations in Douglas-Fir (*Pseudotsuga menziesii* var. *glauca*): a synthesis. *For. Ecol. Manag.* 28(3–4):203–15
110. Ridout M, Newcombe G. 2016. Disease suppression in winter wheat from novel symbiosis with forest fungi. *Fungal Ecol.* 20:40–48
111. Roberts M, Gilligan CA, Kleczkowski A, Hanley N, Whalley AE, Healey JR. 2020. The effect of forest management options on forest resilience to pathogens. *Front. For. Glob. Change* 3:7
112. Rodriguez RJ, Henson J, Van Volkenburgh E, Hoy M, Wright L, et al. 2008. Stress tolerance in plants via habitat-adapted symbiosis. *ISME J.* 2(4):404–16
113. Rodriguez RJ, White JF Jr., Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional roles. *New Phytol.* 182(2):314–30
114. Rønsted N, Wood KR. 2020. *Cyanea kuhihewa*: rediscovering one of Hawai'i's rarest trees. *Plants People Planet* 2(2):107–10
115. Ross AF. 1961. Systemic acquired resistance induced by localized virus infections in plants. *Virology* 14(3):340–58
116. Schnitzer SA, Klironomos JN, HilleRisLambers J, Kinkel LL, Reich PB, et al. 2011. Soil microbes drive the classic plant diversity-productivity pattern. *Ecology* 92(2):296–303
117. Simard S. 2021. *Finding the Mother Tree: Discovering the Wisdom of the Forest*. New York: Knopf

118. Simard SW, Perry DA, Jones MD, Myrold DD, Durall DM, Molina R. 1997. Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* 388(6642):579–82
119. Song XP, Richards D, Edwards P, Tan PY. 2017. Benefits of trees in tropical cities. *Science* 356(6344):1241
120. Song YY, Simard SW, Carroll A, Mohn WW, Zeng RS. 2015. Defoliation of interior Douglas-fir elicits carbon transfer and stress signalling to ponderosa pine neighbors through ectomycorrhizal networks. *Sci. Rep.* 5(1):8495
121. Stanke H, Finley AO, Domke GM, Weed AS, MacFarlane DW. 2021. Over half of western United States' most abundant tree species in decline. *Nat. Commun.* 12(1):451
122. Steidinger BS, Crowther TW, Liang J, Van Nuland ME, Werner GDA, et al. 2019. Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature* 569(7756):404–8
123. Sturrock RN, Frankel SJ, Brown AV, Hennon PE, Kliejunas JT, et al. 2011. Climate change and forest diseases: climate change and forest diseases. *Plant Pathol.* 60(1):133–49
124. Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, et al. 2014. Endemism and functional convergence across the North American soil mycobiome. *PNAS* 111(17):6341–46
125. Thomas E, Jalonen R, Loo J, Boshier D, Gallo L, et al. 2014. Genetic considerations in ecosystem restoration using native tree species. *For. Ecol. Manag.* 333:66–75
126. Thomsen MS, Wernberg T. 2014. On the generality of cascading habitat-formation. *Proc. R. Soc. B* 281(1777):20131994
127. Tilman D, Lehman CL, Thomson KT. 1997. Plant diversity and ecosystem productivity: theoretical considerations. *PNAS* 94(5):1857–61
128. Tilman D, Wedin D, Knops J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 379(6567):718–20
129. Tinya F, Kovács B, Bidló A, Dima B, Király I, et al. 2021. Environmental drivers of forest biodiversity in temperate mixed forests: a multi-taxon approach. *Sci. Total Environ.* 795:148720
130. Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant-microbiome interactions: from community assembly to plant health. *Nat. Rev. Microbiol.* 18(11):607–21
131. U'Ren JM, Arnold AE. 2016. Diversity, taxonomic composition, and functional aspects of fungal communities in living, senesced, and fallen leaves at five sites across North America. *PeerJ* 4:e2768
132. U'Ren JM, Lutzoni F, Miadlikowska J, Laetsch AD, Arnold AE. 2012. Host and geographic structure of endophytic and endolichenic fungi at a continental scale. *Am. J. Bot.* 99(5):898–914
133. Vahter T, Bueno CG, Davison J, Herodes K, Hiiesalu I, et al. 2020. Co-introduction of native mycorrhizal fungi and plant seeds accelerates restoration of post-mining landscapes. *J. Appl. Ecol.* 57(9):1741–51
134. Van Bael SA, Estrada C, Arnold AE. 2017. Foliar endophyte communities and leaf traits in tropical trees. In *The Fungal Community: Its Organization and Role in the Ecosystem*, ed. J Dighton, JF White, pp. 79–94. Boca Raton, FL: CRC Press. 4th ed.
135. Vannier N, Agler M, Hacquard S. 2019. Microbiota-mediated disease resistance in plants. *PLOS Pathog.* 15(6):e1007740
136. Vazquez-Yanes C, Orozco-Segovia A. 1992. Effects of litter from a tropical rainforest on tree seed germination and establishment under controlled conditions. *Tree Physiol.* 11(4):391–400
137. Veen GF, Fry EL, ten Hooven FC, Kardol P, Morriën E, De Long JR. 2019. The role of plant litter in driving plant-soil feedbacks. *Front. Environ. Sci.* 7:168
138. Weller DM. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* 26:379–407
139. Werner D. 1992. *Symbiosis of Plants and Microbes*. New York: Chapman & Hall
140. Whitaker BK, Bauer JT, Bever JD, Clay K. 2017. Negative plant-phyllosphere feedbacks in native Asteraceae hosts: a novel extension of the plant-soil feedback framework. *Ecol. Lett.* 20(8):1064–73
141. Woodcock P, Cottrell JE, Buggs RJA, Quine CP. 2018. Mitigating pest and pathogen impacts using resistant trees: a framework and overview to inform development and deployment in Europe and North America. *For. Int. J. For. Res.* 91(1):1–16
142. Wubs ERJ, Putten WH, Mortimer SR, Korthals GW, Duyts H, et al. 2019. Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. *Ecol. Lett.* 22(7):1145–51

143. Zahawi RA, Reid JL, Holl KD. 2014. Hidden costs of passive restoration: passive restoration. *Restor. Ecol.* 22(3):284–87
144. Zahn G, Amend AS. 2017. Foliar microbiome transplants confer disease resistance in a critically-endangered plant. *PeerJ* 5:e4020
145. Zanne AE, Abarenkov K, Afkhami ME, Aguilar-Trigueros CA, Bates S, et al. 2020. Fungal functional ecology: bringing a trait-based approach to plant-associated fungi. *Biol. Rev.* 95(2):409–33
146. Zaret MM, Bauer JT, Clay K, Whitaker BK. 2021. Conspecific leaf litter induces negative feedbacks in Asteraceae seedlings. *Ecology* 102(12):e03557
147. Zilber-Rosenberg I, Rosenberg E. 2008. Role of microorganisms in the evolution of animals and plants: the hologenome theory of evolution. *FEMS Microbiol. Rev.* 32(5):723–35
148. Zimmerman NB, Vitousek PM. 2012. Fungal endophyte communities reflect environmental structuring across a Hawaiian landscape. *PNAS* 109(32):13022–27

Annual Review of Phytopathology

Volume 60, 2022

Contents

Errata

An online log of corrections to *Annual Review of Phytopathology* articles may be found at http://www.annualreviews.org/errata/phyto