

## ***How does the canopy microbiome impact forest health and can we harness those effects in reforestation projects?***

### Facilitating reforestation through the plant microbiome: Perspectives from the Phyllosphere

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### **Can reforestation outcomes be improved by simultaneously introducing endophytes and other canopy microbes with tree plantings?**

Planting trees is a common way people attempt to mitigate the effects of climate change and to counteract the loss of native forest from land use change and plantings are more successful when soils are inoculated with symbiotic microorganisms. However, little research has been done to determine the impact of co-introducing beneficial canopy microbes such as fungal leaf endophytes. These endophytes can impact resilience, growth, development, and defense against pathogens. This paper attempts to determine whether enough evidence exists to incorporate aboveground and belowground microbiota into reforestation projects.

### **Do all tree species have pathogens and what was the range of pathogens associated with species?**

- Almost half, 48%, of the 1312 tree species in the U.S. did not have any known pathogens. Species may have escaped pathogens through long-distance dispersal. Some evidence for this is that most of the tree species without known pathogens are native or endemic to Hawaii.
- The number of known pathogens was higher for tree species with broad ranges and wide distributions. For example, the highest number of fungal and oomycetous pathogens, 876, were associated with Douglas fir, which is dominant in forests of the Pacific Northwest.

### **Are there beneficial microorganisms that could be included in reforestation projects?**

- Leaf endophytes hosted by trees provide benefits through disease protection and disease modification and can provide as much disease defense as genetic factors. Some disease resistance mechanisms are competition with pathogens and stimulating the tree's immune response.
- Future reforestation and tree-planting efforts may benefit from endophyte integration, but more research is needed to understand when they should be introduced and if order of introduction is important. There is evidence that increased microbiome diversity is associated with increased disease protection.

### **Are there large scale studies that have investigated the role of canopy microbes and success of tree plantings or reforestation efforts?**

- There were no published studies that investigated feedback between the microbiome of the phyllosphere, the entire above ground plant surface, and experimental plantings of tree species.

The authors argue that future studies should use phyllosphere microbes taken from leaf litter, but not leaf litter itself to isolate effects of the microbiome.

- Studies on other plant types suggest that plants interact differently with microbe communities at different life stages and that plant species may be impacted differently by microbes from heterospecifics than from conspecifics.

### **What do we know about how the canopy microbiome impacts forest health and what further information is needed to understand how to incorporate microbial communities into reforestation?**

- A diverse native microbiome is an integral part of a healthy forest. Through symbiotic and pathogenic processes, microbes contribute to tree species' success by influencing competition and growth. Pathogenic processes also contribute to animal habitat creation.
- To conserve and rebuild the microbiomes of global forests, inoculations should be locally-sourced and mirror the natural diversity and complexity of microbial communities.
- Future studies must replicate the context in which they will be used, in order to be efficacious in designing projects that include microbiome inoculation in tree plantings. For example, in a greenhouse experiment the authors found that the response of coastal Douglas fir to microbes sourced from leaf litter differed between tree groups sourced at different elevations.

### **What research questions should be prioritized in future projects?**

- Researchers need to investigate how a diverse microbiome influences disease resistance and whether diversity of the phyllosphere microbiome impacts its functional role in the forest community. Can endophytic communities be considered a part of the defense system comprised of soil microbes?
- Does transplanting of diverse leaf litter successfully inoculate new plantings and provide the benefits observed in mature forests? Leaf litter transplantation is easily carried out and does not require specialized equipment because microbes do not need to be isolated and applied.
- For forest regeneration, retention of live trees, which preserves their microbiomes, necrobiomes, and mycorrhizal networks, is important. Future research should determine critical forest microbes that should also be prioritized for conservation and used in forest regeneration.
- How do forest microbe communities interact with greenhouse gasses other than CO<sub>2</sub>, to influence emission or capture by forests? Do certain microbes have a greater impact on specific greenhouse gasses?

### **Research Approach/Methods**

- The authors undertook a literature review to investigate whether current knowledge was extensive enough to build comprehensive strategies to improve defensive symbiosis between microbiomes and tree species during forest restorations.
- They hypothesized that including a robust and representative microbial community in tree plantings and reforestations will improve the survival and success of tree seedlings, improve biodiversity, and increase resilience, as well as enhance the ability of replanted forests to capture carbon.
- The researchers additionally argued that to make the strategies feasible on large scales, they also need to be low-tech, low-cost, and easily performed.

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

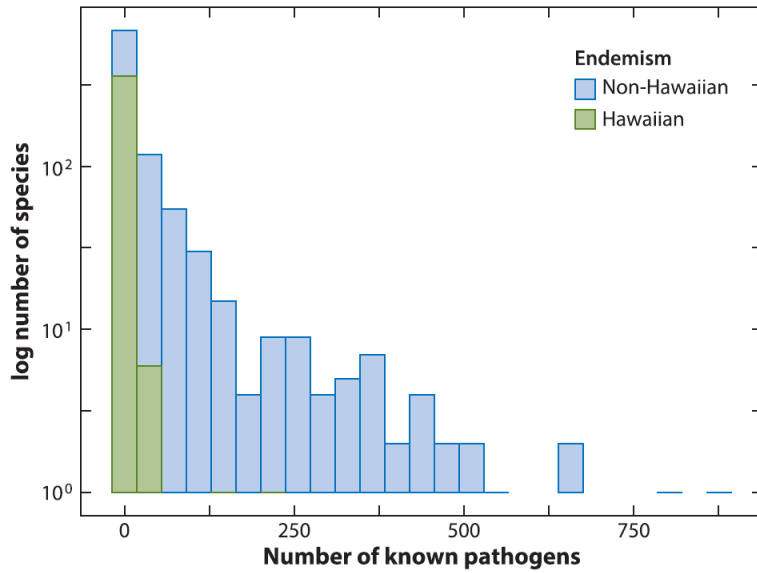
## Images

### RANK 1



**Figure 2 in Busby et al. 2022.** 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.

### RANK 2



**Figure 1 in Busby et al. 2022.** 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.