

Ecological processes and keystone drivers of forest root-microbiome assembly

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Chapter 1. General introduction

Root-associated microbiomes, primarily composed of diverse fungi and bacteria, drive forest ecosystem dynamics through their effects on host fitness. Previous studies have mainly focused on functional groups with clear mutualistic roles, such as ectomycorrhizal fungi, and the ecological processes underlying their root colonization. However, the multitude of microbes interacting within roots can directly or indirectly affect host physiology. Therefore, understanding the assembly of the entire microbiome is indispensable for explaining the outcomes of complex interactions in forest belowground ecosystems. Progress on such integrative understanding has been hindered by two major challenges: the need for large-scale community datasets capturing multivariate microbial interactions, and the lack of frameworks capable of interpreting the underlying processes. In this thesis, I integrated high-throughput sequencing of over 1,000 root tips with a new analytical framework grounded in community ecology and statistical physics to overcome these challenges. This data-driven approach enabled me to identify the ecological processes shaping root microbiomes and to detect keystone microbes with critical roles in their organization.

Chapter 2. Mycorrhizal and endophytic fungi structure forest below-ground symbiosis through contrasting but interdependent assembly processes

Background

Interactions between plants and diverse root-associated fungi are potentially influenced by multiple ecological factors and processes, including host–symbiont specificity, the background soil microbiome, inter-root dispersal of symbionts, and within-root fungus–fungus interactions. However, disentangling how these multiple processes collectively determine fungal community assembly has remained a major challenge.

Methods

Using a joint species distribution model, I analyzed 1,615 root fungal communities in a cool-temperate forest to disentangle the relative importance of host filtering, soil fungal communities as a species pool, spatial autocorrelation, and fungus–fungus interactions within root tips. Networks of direct fungal associations were further inferred by co-occurrence network analysis that accounted for unmeasured environmental variation.

Results

The best model included all four ecological processes. Among individual effects, background soil fungal composition and within-root fungal associations most strongly explained fine-scale community structure. Ectomycorrhizal fungi were closely linked to soil fungal communities and spatial patterns, whereas root-endophytic fungi were mainly associated with biotic associations. Network analyses revealed certain endophytes, such as *Phialocephala* and *Leptodontidium*, as central hubs connecting diverse fungal taxa.

Conclusion

These findings demonstrate that root-associated fungal communities are shaped by the combined effects of recruitments from soil, host identity, spatial structure, and within-root biotic associations. Moreover, the fundamental assembly rules differed between mycorrhizal and endophytic fungi, both of which are key components of forest ecosystems.

Chapter 3. Statistical inference of keystone taxa reshaping the assembly rules of forest root microbiomes

Background

Root-associated fungi and prokaryotes aggregate within fine root tips forming complex microbial communities. Previous efforts have sought to identify “hub” microbes in the microbial interaction webs. However, because network analyses based on co-occurrence patterns cannot quantify the community-wide influence of each taxon, identifying keystone microbes that govern community assembly remains a major challenge.

Methods

Here, drawing on statistical physics and community ecology, I developed a framework to identify microbes that govern the assembly rules of complex plant microbiomes. Using the datasets comprising 1,215 fungal and prokaryotic communities, “assembly landscapes” that

represent probability distributions of their community states were reconstructed. By simulating community assembly under varying abundances of individual taxa based on the inferred landscapes, I quantified their influence as “keystoneness.”

Results

Several endophytic fungi (e.g. *Oidiodendron*, *Cladophialophora*, *Hyaloscypha*, and *Meliniomyces*) were shown to reorganize the topography of assembly landscapes, potentially driving shifts in mycorrhizal symbiotic types of host plants such as *Populus*, *Acer*, and *Juglans*, which associated with both ectomycorrhizal and arbuscular mycorrhizal fungi in the study site. Cross-kingdom effects were also evident: uncharacterized bacterial taxa (e.g. *Thermosporothrix*) influenced fungal community organization. Prokaryotic communities underwent transitions among states dominated by core taxa, *Pseudomonadota*, and *Actinomycetota*, under the disproportionate influence of *Candidatus* Udaeobacter and several endophytic fungi.

Conclusion

This statistical framework thus identified previously overlooked microbes with strong, system-level impacts on root microbiome dynamics. The results highlight that key ecological functions in plant–microbe systems may be maintained by microbial taxa not recognized as principal symbionts. Data-driven inference of assembly landscapes offers a promising tool for uncovering hidden drivers governing ecosystem-scale microbial organization.

Chapter 4. General discussions

In this thesis, I revealed that the root microbiome assembly is jointly driven by soil-mediated colonization of ectomycorrhizal fungi and biotic filtering within roots through microbe–microbe associations. The results also emphasized the ecological importance of diverse, previously overlooked microbial taxa in shaping belowground communities. Because this framework can be extended to other complex microbiomes, it provides a powerful approach for elucidating the ecological processes underlying community organization beyond descriptive pattern analysis. While the present study primarily examined taxonomic composition, microbial dynamics ultimately depend on the physiological behaviors and functional capacities of community members. Therefore, integrating genomic and transcriptomic analyses with ecological theory will be essential for constructing a bottom-up, multiscale understanding of microbial ecosystems.