



ANNUAL MEETING OF THE CALIFORNIA FOREST PEST COUNCIL - 2020

THE FOREST MICROBIOME AND FOREST HEALTH

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Research Focus Areas

- Detecting and identifying forest pathogens and associated microbes by developing techniques of **molecular diagnostics**
- Determining origin and migratory routes of invasive forest pathogens using **population genetics/genomics**
- Discovering and developing **novel disease management tools** using unconventional approaches (e.g., metagenomics and bioclimatic modelling)
- Determining complex **interactions among biotic and abiotic ecosystem components** that influence outbreaks of forest diseases



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Paradigm shift in Forest Pathology?

Shifting the paradigm from pathogens to pathobiome

- The forest pathology paradigm that one disease is caused by one pathogen is shifting toward a consideration of the complex ecological interactions among pathogens, microbial communities, tree host, and environment.

What are microbiomes, phytobiomes, and pathobiomes?

- **Microbiome:** entire microbial communities associated with animals, plants, soils, air, etc.
- **Phytobiome:** entire microbial communities associated with plants (in, on, and adjacent to)
 - *Rhizosphere, phyllosphere, leaves, stem, endophytic compartments*
- **Pathobiome:** the set of host-associated organisms (encompassing prokaryotes, eukaryotes, and viruses) associated with reduced (or potentially reduced) health status, as a result of interactions among members of that ecological community and the host (Bass et al. 2019).
- Includes bacterial, fungal, nematode, and virus communities

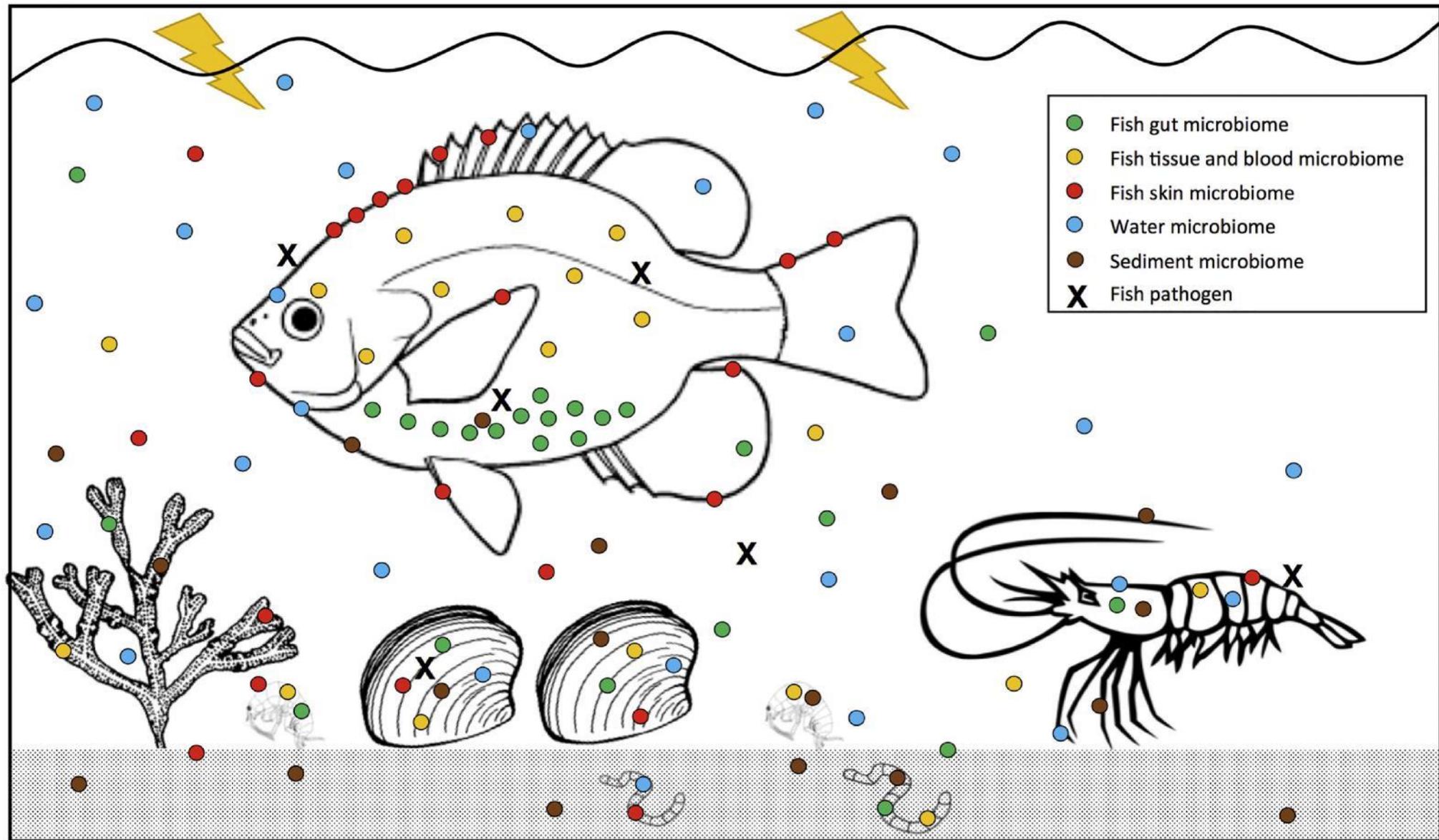
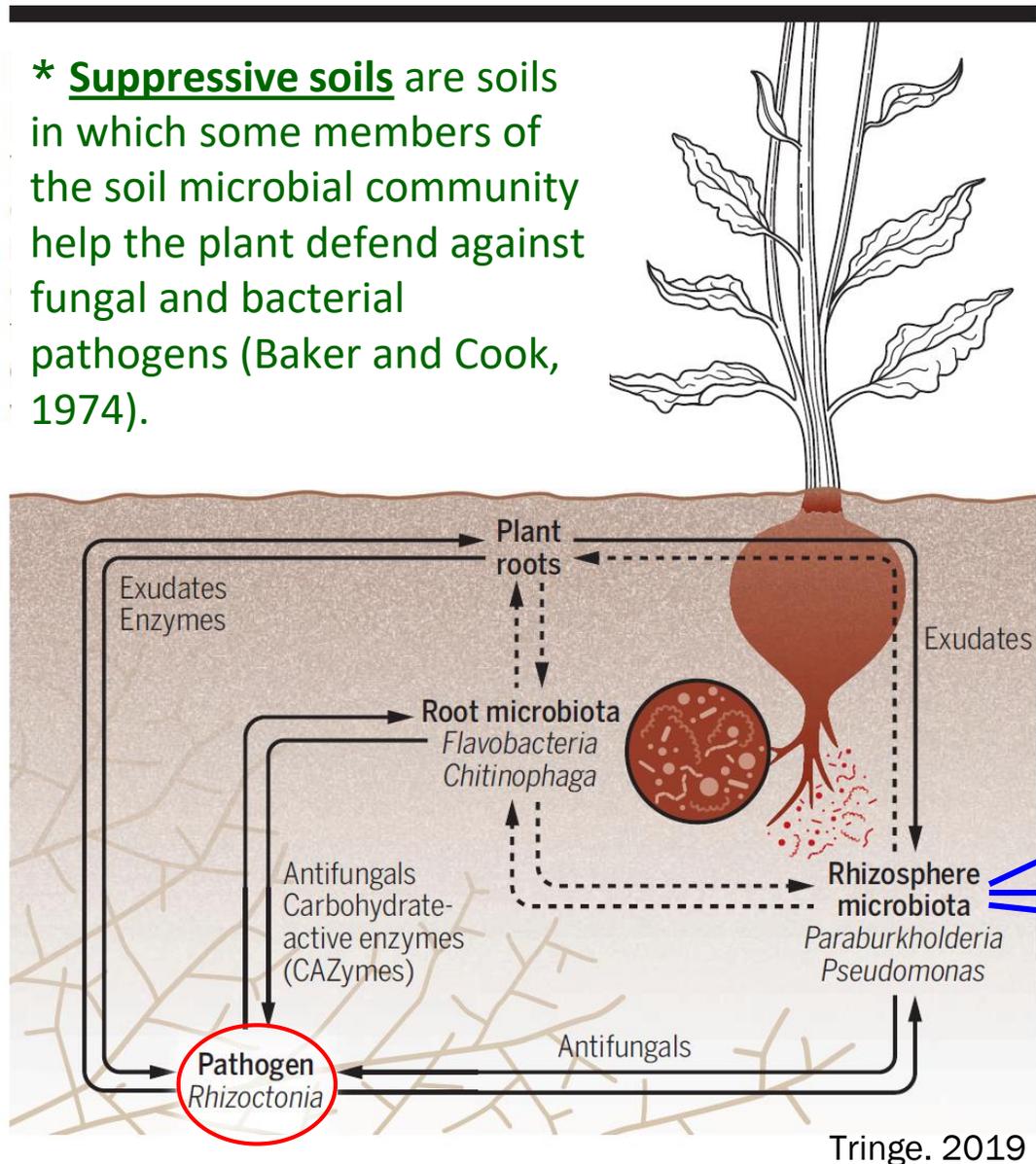


Figure I. Microbial Complexity in a Typical Host–Symbiont–Environment System. Trends in Ecology & Evolution (Bass et al. 2019)

Why we care about the microbiome?

- Human microbiota play key roles in human health and disease progression
- Plant microbiota are important for plant growth, health, and stress resilience (Compant *et al.* 2019)
 - Members of the plant microbiota engage in highly co-evolved interactions with the plant and with one other microbiota under changing environments.
 - Their interactions can result in significant consequences for plant health and productivity in crop and forest systems.
 - Some of the forest microbiota are known to aid in key functions across the entire forest ecosystems, e.g., water and nutrient cycling, soil carbon sequestration.

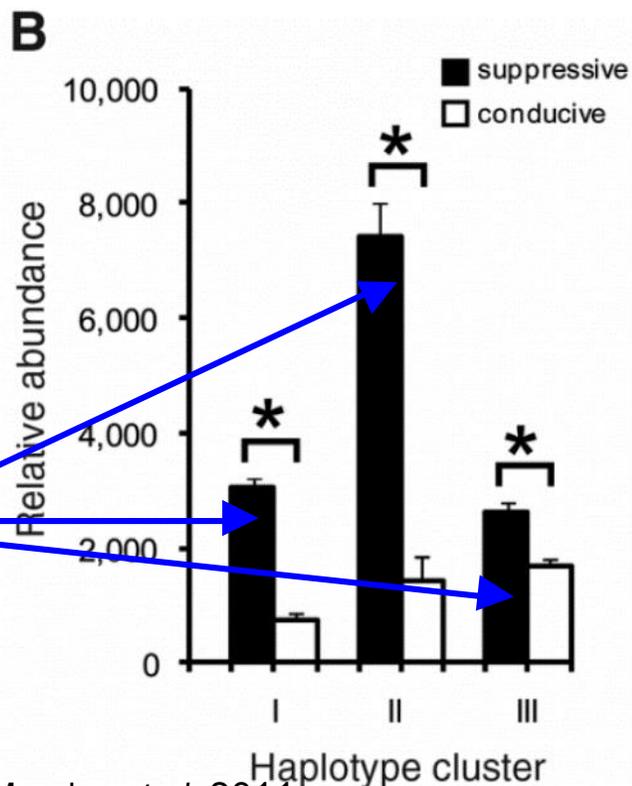
* **Suppressive soils** are soils in which some members of the soil microbial community help the plant defend against fungal and bacterial pathogens (Baker and Cook, 1974).



Plant-pathogen-microbe interactions in suppressive soils*

Rhizosphere microbiota

Relative abundance of *Pseudomonas* in suppressive and conducive soils

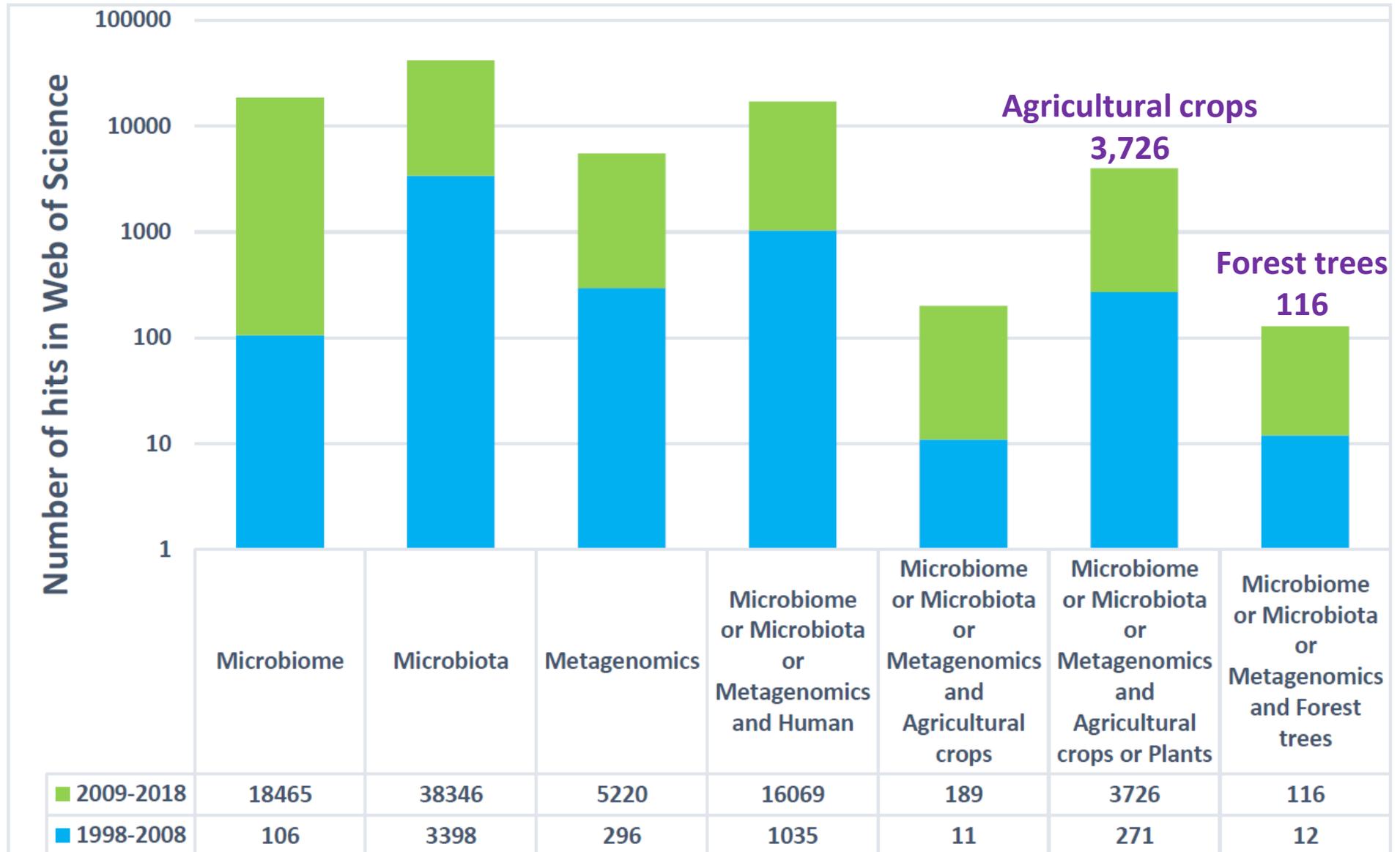


Fungal infection (*Rhizoctonia*) of plant roots is suppressed with certain *Paraburkholderia* and *Pseudomonas* are abundant.

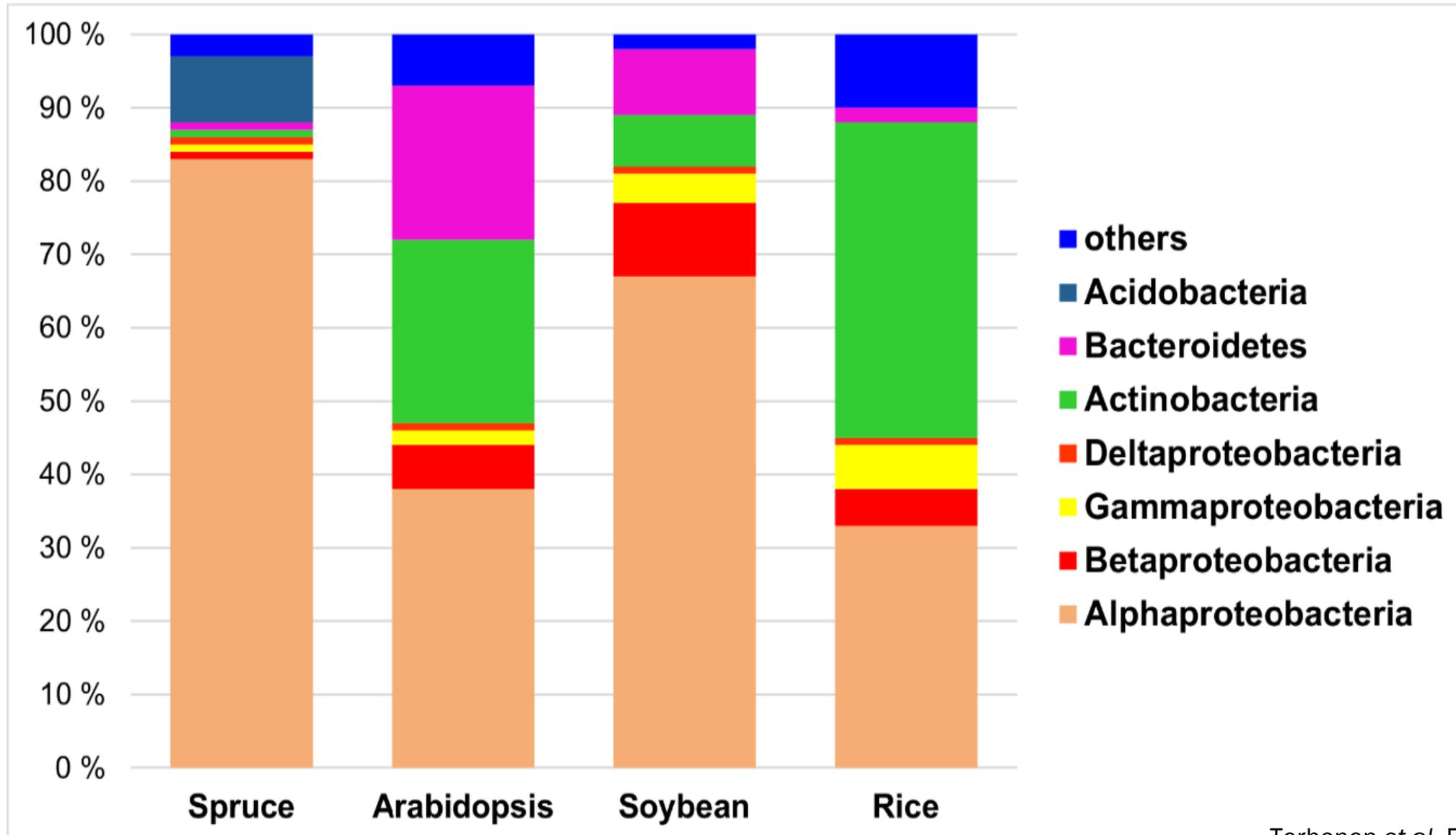
Questions addressed by microbiome research?

- What microbes are present in the microbiomes within and on plant surfaces? (who is there?)
- Function (what can they do?)
- Activity (how do they do what they do?)
- What factors are important for their survival, colonization, and distribution?
- What is their ability to cause/enhance disease or induce resistance?
- How do they communicate with each other?

Increases in microbiome research



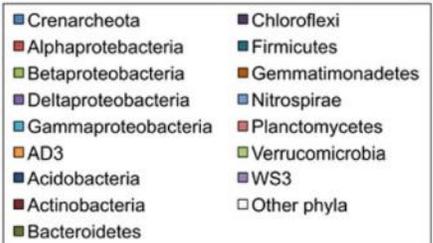
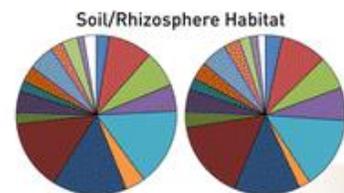
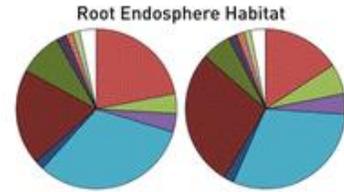
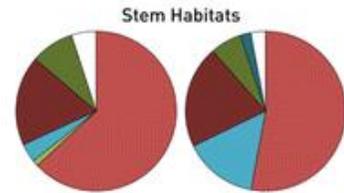
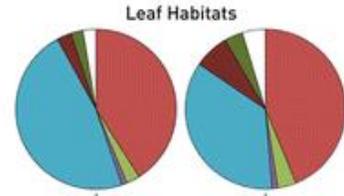
Plant species affects microbe community



- Host tree genotype affects bacterial and fungal communities across different tissues
- Differences in host species, cultivar type within a species, soil type, physiological status of host, and pathogen presence can influence variation in microbial communities.

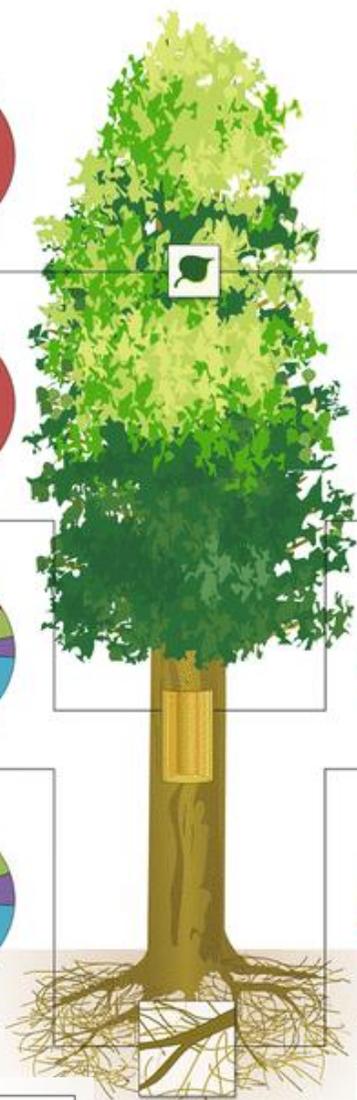
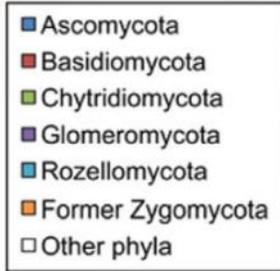
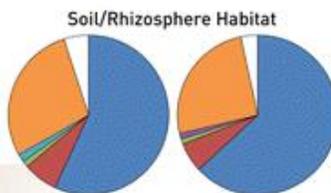
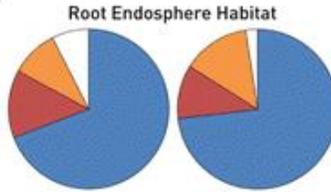
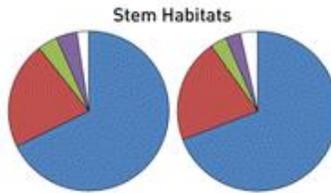
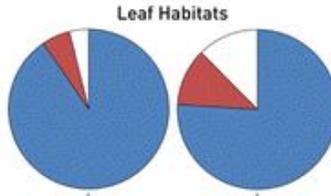
The Archaeal/Bacterial Phytobiome

P. trichocarpa x deltooides P. deltooides



The Fungal Phytobiome

P. trichocarpa x deltooides P. deltooides



Three methods to examine microbial communities

■ **Meta-barcoding:**

- PCR–amplicon-based sequencing
- Typically at the 16S and ITS regions of rDNA (Bacteria and Fungi)
- ID based on curated databases

■ **Metagenomics:**

- Shotgun approach
- Sequencing DNA without enrichment
- Potentially obtains whole genomes of some microbes
- ID based on genomes

■ **Metatranscriptomics:**

- Shotgun approach
- Sequencing RNA (expressed transcripts)
- Whole transcriptomes of many organisms shows expressed genes and their function
- ID based on genomes

Meta-barcoding ribosomal genetic regions for species identification

Fungi: Ribosomal internal transcribed spacer region: ITS



Bacterial: Ribosomal 16s genetic region



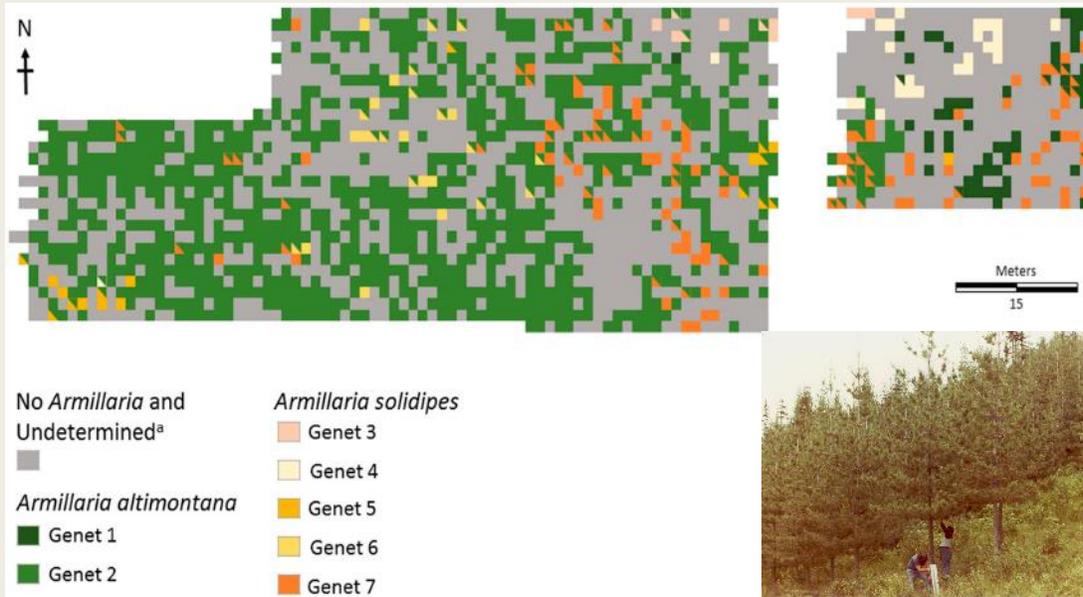
Example

Fungal and bacterial communities associated with Armillaria root diseases

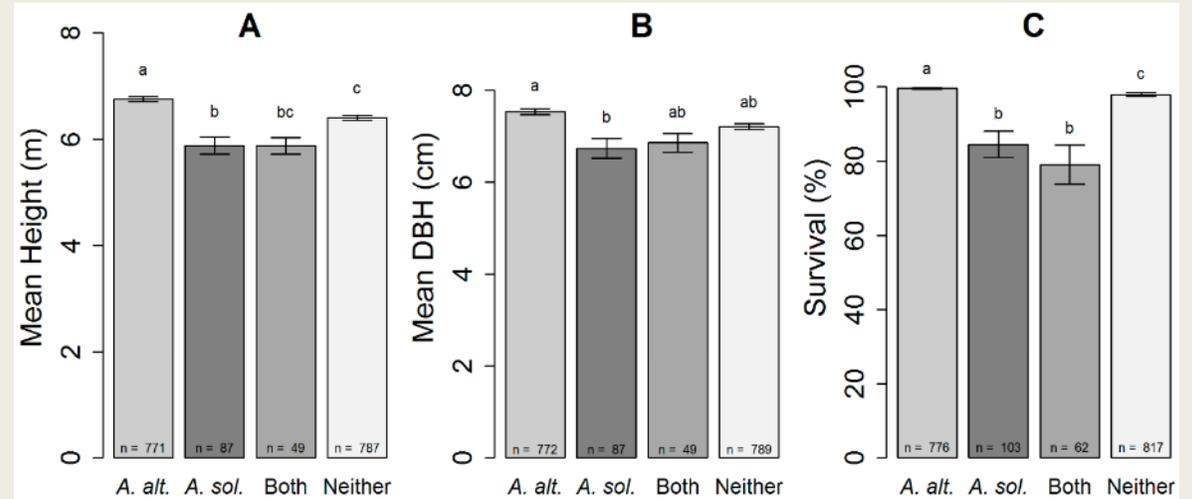


J. Hanna

Armillaria altimontana can exhibit biological control of *Armillaria* root disease on western white pine



Armillaria spp. and genet distribution within western white pine plantation in northern Idaho. Each square represents 1.2 m² (tree location). Colored pixels indicate trees associated with *A. altimontana*, *A. solidipes*, both, or neither.



A: The mean height, **B:** mean diameter at breast height (DBH), and **C:** percent survival among western white pine growing in association with *Armillaria altimontana* (*A. alt.*), *A. solidipes* (*A. sol.*), *A. alt.* and *A. sol.* (Both), or no *Armillaria* (Neither) at 16 years post-planting. Means sharing a lower case letter within each bar graph are not significantly different ($p < 0.05$) by Tukey-adjusted means separation.

From: Warwell, M.V.; McDonald, G.I.; Hanna, J.W.; Kim, M.-S.; Lalande, B.M.; Stewart, J.E.; Hudak, A.T.; Klopfenstein, N.B. 2019. *Armillaria altimontana* is associated with healthy western white pine (*Pinus monticola*): Potential *in situ* biological control of the *Armillaria* root disease pathogen, *A. solidipes*. *Forests* 10: 294.

Samples collected for soil and microbial communities analyses

- DBH & tree health status
- Bulk density of soil samples, duff and litter measurements
- *A. solidipes* and *A. altimontana* Meta-barcoding
 - ITS2, 16s - V4



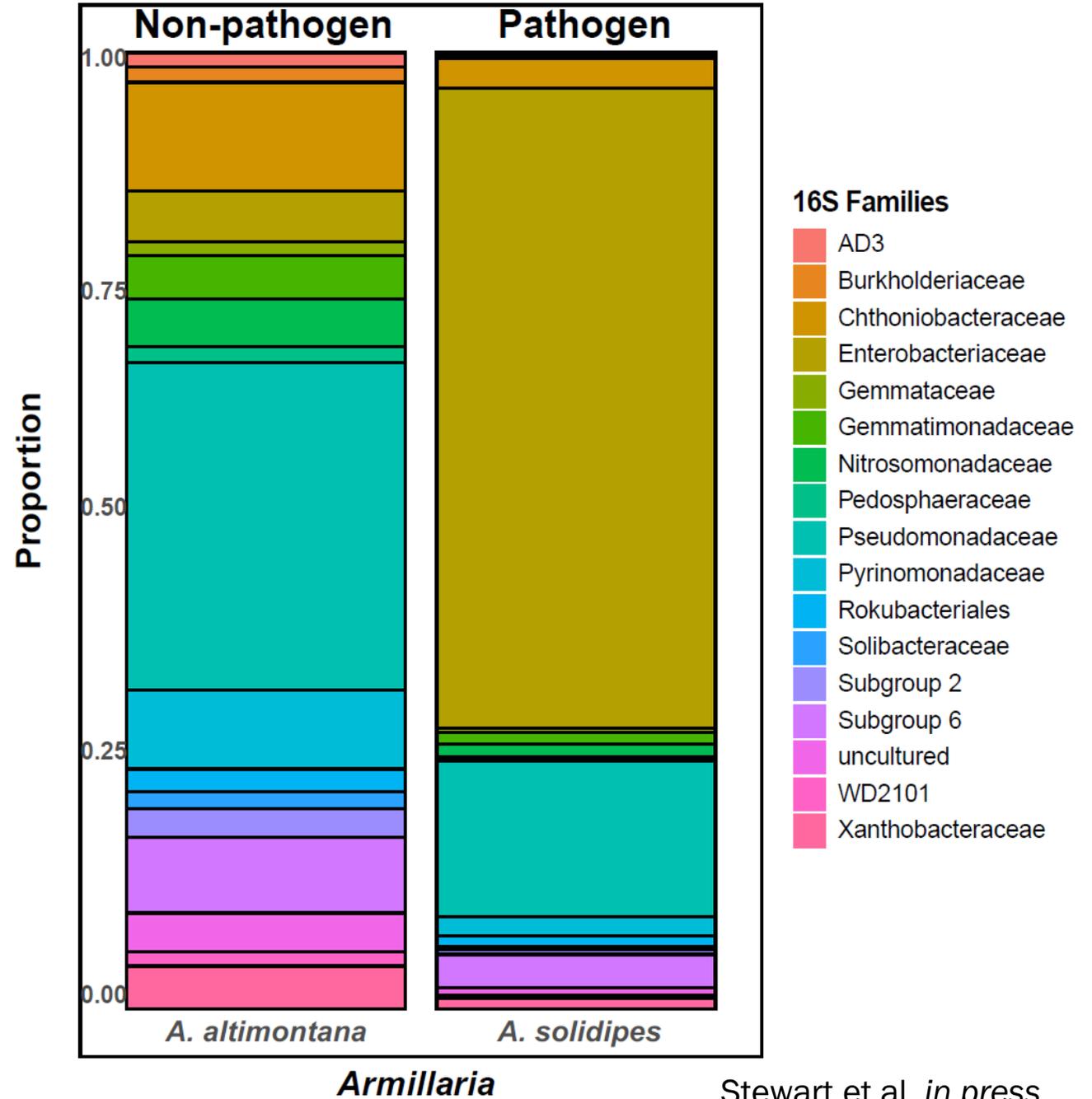
Bacterial communities associated with *Armillaria* spp. in forest soils

A. altimontana

- Pseudomonadaceae – Ubiquitous in soil, water, plants, animals. Pathogens and beneficials – suppressive soils

A. solidipes

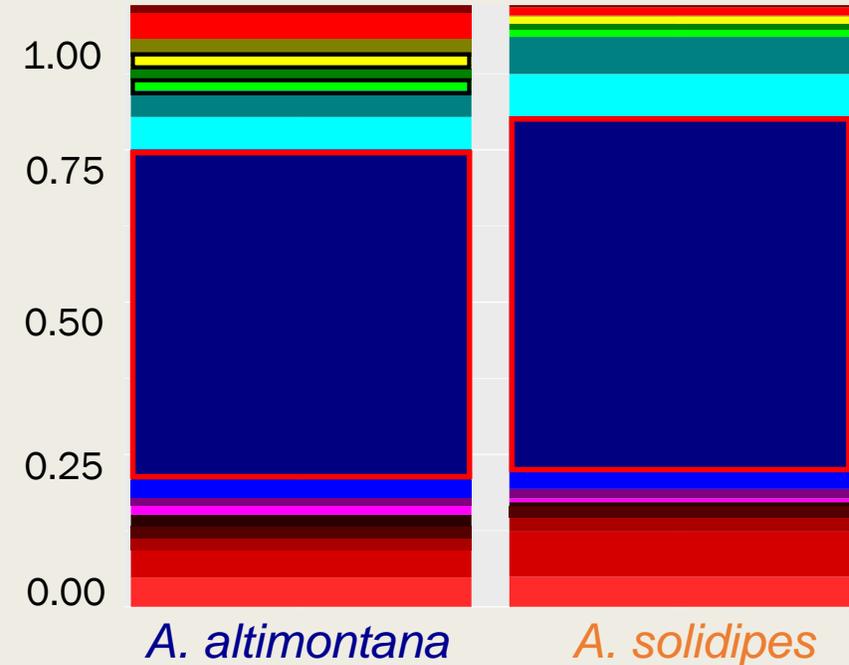
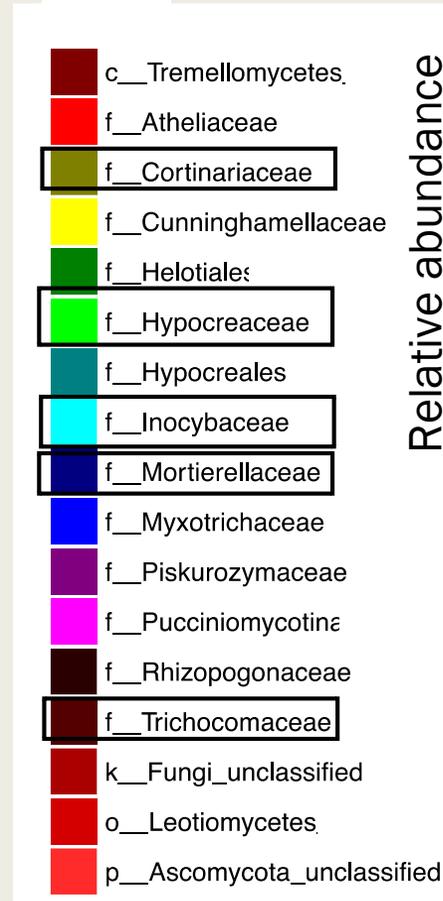
- Enterobacteriaceae – found in soil, water, and decaying plant materials, plant and animal pathogen



Fungal communities associated with *Armillaria* spp. in forest soils

- Armillaria altimontana*:**
Increased levels:
 Trichocomaceae,
 Cortinariaceae,
 Rhizopogonaceae,
 Hypocreaceae
 (e.g., *Trichoderma*)
- A. solidipes*:**
Increased levels:
 Mortierellaceae

OTUs
(Optional Taxonomical Units)



3,383 unique OTUs identified

Stewart et al.,
in preparation

Role of secondary metabolites on plant disease development and suppression

- Secondary metabolites, also called natural products, are organic compounds of low molecular mass that are produced by bacteria (e.g., *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp.), fungi (e.g., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp.), and plants of certain taxonomic groups.
- These metabolites often act as key factors that either enhance and suppress other organisms (e.g., bacteria, fungi, amoebae, plants, insects, and large animals), which can increase the survival of the organisms that produce them.
- It has become increasingly clear that these natural secondary metabolites can alter the structure, composition, and ecological function of the pathobiome, resulting in the increase or suppression of plant disease.

Secondary metabolites shaping the microbiome

Specific interactions between the organisms under specific circumstances

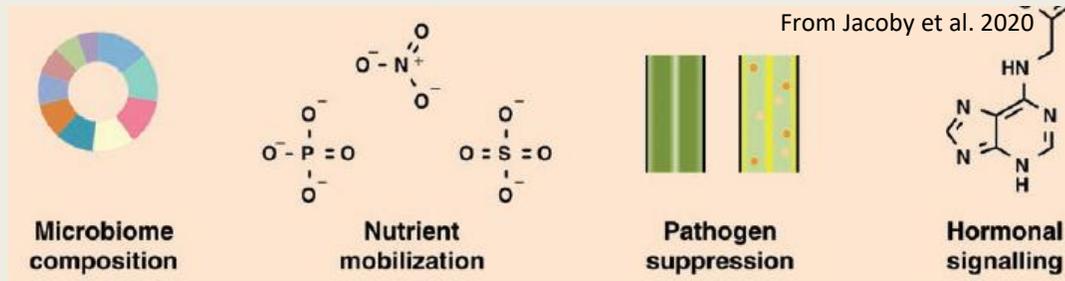
Plants – prokaryotes, eukaryotes, and viruses microorganisms – microorganisms



SMs volatile organic compounds
hydrogen cyanide, alkaloids, and bacteriocins
coumarins, benzoxazinoids, camalexin, and triterpenes



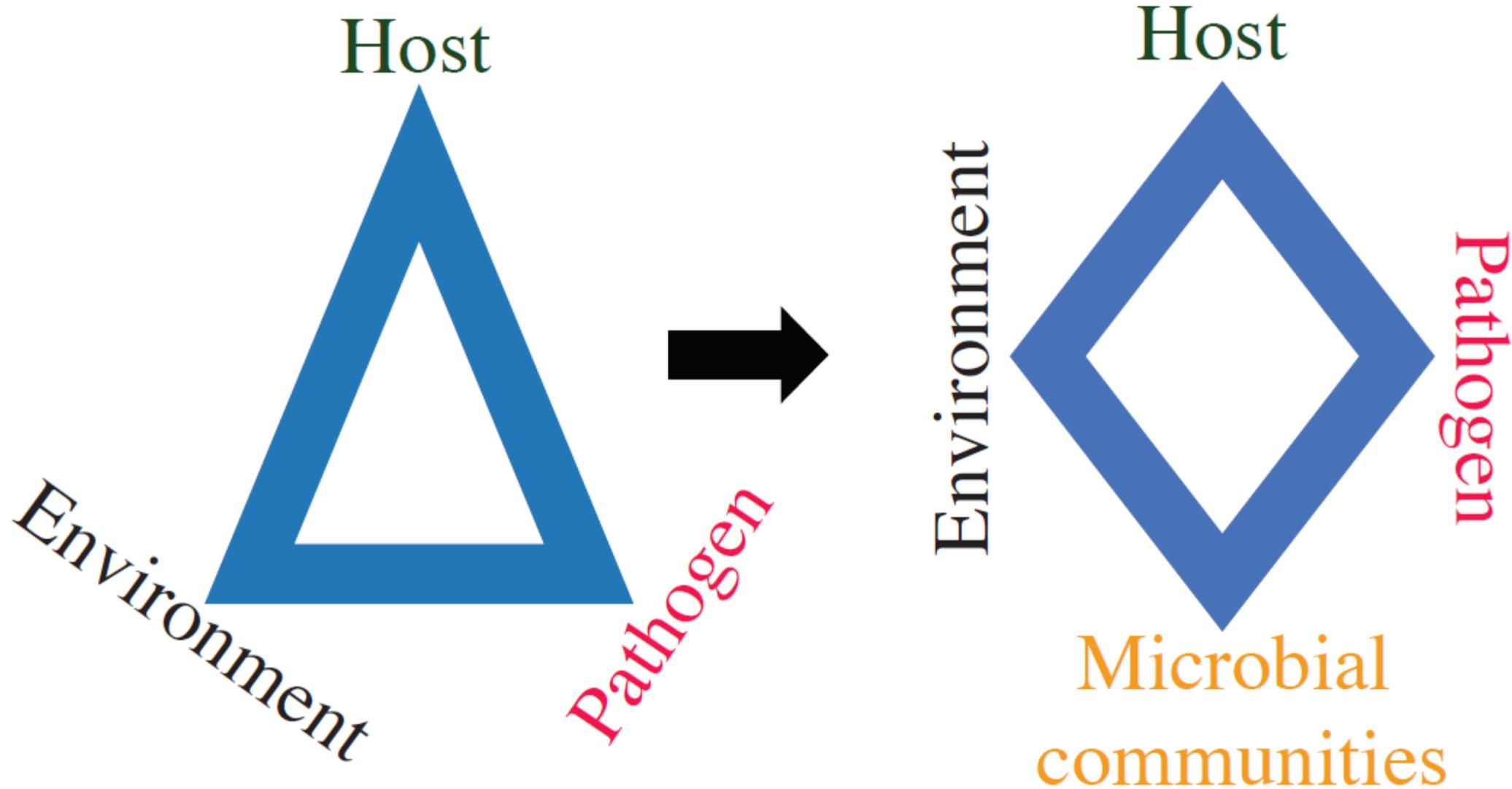
Microbial responses



Forest microbiome and forest health

- Innovative approaches are essential for assessing the interactions and functions of the microbial communities in forest disease processes, while integrating the influences of other environmental factors.
 - *'omics' approaches including metagenomics, metatranscriptomics, metabolomics*
- Results from such integrated information will help develop novel approaches to manage forest disease and improve forest health by promoting conditions that suppress disease or enhance beneficial processes.
 - *'biocontrol' approaches including co-cultivation of phytopathogens with mixed bacteria-fungi may provide effective strategies for discovering antimicrobial agents with roles in reducing plant disease*

Adapting the disease triangle



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