PPath 315: Plant Microbiomes

Spring 2017, 4 credits

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Lectures: MWF, 11:00-11:50 AM, Rm. 187 Russell Laboratories

Laboratory: W, 1:20-4:20 PM, Rm. 187 Russell Laboratories

Office hours: by appointment (arrange via email)

Course description: Explore plant associated microbial communities (the plant microbiome), methods used to study them, and how we can use them to improve plant and ecosystem health in the face of challenges to agricultural and natural systems. Examples will be drawn from annual crop, grassland, and forested ecosystems. The course will consist of lecture/discussion as well as a hands-on laboratory. In the laboratory section, we will use current molecular, bioinformatic, and statistical approaches to characterize rhizosphere microbiomes from samples collected as part of on-going research projects.

The course will cover 1) the biology of major microbial components of root and leaf associated microbiomes and their impacts on plant growth, nutrient acquisition, drought tolerance, and disease suppression, 2) past, current, and emerging methods of studying microbiomes, and 3) strategies for conserving and manipulating microbiomes in natural and agricultural systems. In the laboratory section, we will characterize rhizosphere microbiomes from forest tree root samples collected as part of a citizen scientist led project to map the distribution of major rhizosphere microbial species across the eastern temperate forests of the United States.

Learning outcomes: Upon completing this course, students will be able to:

- Describe the major members of plant microbiomes and their functions
- Compare and contrast current and past approaches to studying plant microbiomes
- Use evidence to critically evaluate claims and test hypotheses
- Propose potential ways in which plant microbiomes may be utilized or manipulated to achieve societal goals
- Perform laboratory and bioinformatic protocols to characterize microbiome composition
- Propose, test, and interpret hypotheses about the biogeographic patterning of root associated microbial communities

Additional Learning outcomes: Graduate students

- Propose, test, and interpret hypotheses about plant microbiome assembly using multivariate statistical approaches
- Critically evaluate primary scientific literature regarding plant microbiomes

Assessments: Course grades will be determined by three exams covering the lecture material (50%), and by several assignments and an individual project based on the laboratory section (50%).

Exam 1 100	Laboratory – 300 total
Exam 2 100	Lab notebook: 50
Exam 3 100	Assignment 1: Plant PCR gel results 10
	Assignment 2: Stress tolerance case study 10
	Assignment 3: Plant identification write-up 15
	Assignment 4: NGS quality control results write-up 15
	Assignment 5: Comparison of microbial communities 50
	Individual Project: Distribution mapping of rhizosphere fungi 150

Letter Grade Assignments: A: 100-92.5%, AB: 92.4-87.5%, B: 87.4-82.5%, BC: 82.4-77.5%, C: 77.4-69.5%, D: 69.4-59.5%, F:59.4-0% out of 600 total points. Percentages will be rounded to the nearest 0.1%.

Undergraduate and graduate students will be assessed separately. Assignment 5 and the individual project will include additional components for graduate student (specifically, additional literature review and presentation in a formal scientific journal style).

Course Requirements: Attendance at both lecture/discussion meetings and laboratory sessions is expected. If you must miss either meeting, contact me prior to your absence to arrange to make up the missed work or material. Because our work in the laboratory sections builds on previous weeks, attendance at lab sections is vital. Students will be allowed one, *excused* absence from a lab section, which must be arranged prior to the absence OR be validated with a doctor's note or other documentation. Absences beyond this will result in a 10% reduction in your final course grade per absence unless adequate alternative plans are made. Please meet with me to discuss any known issues that may affect your ability to attend all laboratory sections.

Class meetings will be heavily geared toward small and large group discussions. You will be responsible for all material covered, whether presented in a lecture format, in the laboratory sections, or in a class discussion. Thus, class attendance is vital to accessing the complete complement of course materials that will be covered on the three exams.

Expect to spend 6 hours in class (lecture plus laboratory) per week, and an additional 6 hours per week outside of class for assigned reading, homework assignments, laboratory reports, and exam preparation.

Prerequisites: BIOLOGY 151, ZOOLOGY 101 & 102, BOTANY 130, BIOCORE 381, and STAT 301 or 371; or graduate or professional standing

Text/References: There is no main text for this course; instead, we will read examples of the current scientific literature

UNIVERSITY OF WISCONSIN POLICIES AND EXPECTATIONS

Academic Integrity Academic integrity is critical to maintaining learning at UW-Madison. Academic dishonesty is a serious violation: it undermines the bonds of trust and honesty between members of our academic community, degrades the value of your degree, and defrauds those who may eventually depend upon your knowledge and integrity. Examples of academic misconduct include, but are not limited to: cheating on an exam (copying from another student's sheet, continuing to work on an exam after the time has expired, turning in an exam for re-grading after making changes to the exam), copying the homework of someone else, submitting work done by someone else, stealing examinations or course materials, tampering with the grade records or with another student's work, or knowingly and intentionally assisting another student in any of the above. If you present the words or ides of others without giving them proper credit, you are guilty of plagiarism. It is your responsibility to learn what constitutes plagiarism and the correct rules for citing sources. Please refer to the Dean of Students' webpage for further information.

Disability Access All students are welcome in this class, regardless of disability status. In order to receive disability-related academic accommodations, students must first be registered with the McBurney Disability Resource Center. Students who have or think they may have a disability are invited to contact the McBurney Center for a confidential discussion at 608-263-2741 (phone), 608-225-7956 (text), or by email at mcburney@studentlife.wisc.edu. If you have already registered with the McBurney Center, and if you wish to request any accommodations on the basis of disability, you should schedule an office appointment me within the first two weeks of the semester (by Feb. 1). You should bring a copy of your service plan/VISA to your meeting. Please note: I am unlikely to honor an exam accommodation request made within 72 hours of any exam. Advanced planning on your part will help me to create an accessible classroom/laboratory environment.

Laboratory Section: Mapping geographic distribution of forest rhizosphere fungi

Forests in the eastern United States are currently under threat from a number of stresses, including changing climates. We have a very detailed understanding of the geographic distributions and climate tolerances of forest tree species, which has been used to predict how forests are likely to change in future conditions. However, we know very little about the distribution and climate tolerances of the members of tree microbiomes. Since rhizosphere communities, especially symbiotic fungi, can alter plant responses to climate, predicting how tree species will respond to warming or drying conditions may require understanding how their rhizosphere microbiome will shift in these future climates. In this course, students will learn state-of-the-science approaches to studying microbial communities while simultaneously producing valuable data on a pressing scientific question.

To gain a better understanding of the geographic distribution and climatic niches of the major rhizosphere fungal species in eastern US forests, the Lankau research group has initiated a citizen-science led sampling effort to collect root material from eight common tree species from sites across the eastern US. To date we have received several hundred samples from dozens of locations, with more samples arriving on a continuous basis. In this course, we will use modern molecular and bioinformatics techniques to identify the fungal taxa present in these root samples. In the lab, students will work in small groups to extract DNA, amplify plant and fungal barcoding genes with polymerase chain reactions (PCR), and prepare the resulting products for next-generation sequencing. Then, we will switch to the computer lab where the students will use bioinformatics approaches to cluster sequence reads into operational taxonomic units and identify these "species" by comparison to databases of known specimens.

Each laboratory group will perform the molecular and computational procedures on a unique set of samples, and write a report on the quantity and quality of data they generated. Then, we will combine the data from all groups to test how the rhizosphere fungal communities differ between sites, tree species, and along climatic gradients.

Finally, each student will choose one of the major fungal species identified in our sample set, and using the data from all the groups, map the geographic distribution of the chosen species. Students will use publically available climatic data to determine the climate niche of their chosen species, and use predictions of future climates to predict how their species is likely to respond to climate change. Student reports will be published on our project website (www.forestfungiproject.org) to build a publically available resource for mycologists and forest ecologists studying forest responses to climate change.

The laboratory component of this course is designed to address six key learning goals of the College of Agricultural and Life Sciences:

1. *Develop problem-solving skills*: Anytime we are working with real, unknown samples, problems or unexpected results are likely to arise. One key component of the course will

be troubleshooting problems, individually and as a group, that arise in our molecular procedures, bioinformatics analyses, or statistical analyses.

2. *Expose students to multidisciplinary approach:* The research in this course will include a mix of traditional molecular biology techniques (e.g. PCR), computational approaches, and large-scale ecological analyses.

3. *Develop teamwork and interpersonal skills, including the ability to communicate effectively to multiple audiences*: You will work in teams throughout the semester, and will be required to collaborate on various laboratory reports and analyses. Your final reports will require you to write about scientific results in a manner accessible to our citizen science participants, which include adult hobbyist but also school and youth groups of various ages

4. *Develop skills in accessing and using information resources:* You will work with several genomic databases in order to match sequence reads to fungal taxonomic identities, as well as climatic, soil type, and other environmental databases to test their biogeographic hypotheses.

5. *Address societal, economic, ethical, scientific, and profession issues:* Working with authentic research samples, you will be directly addressing a pressing need for scientific data on microbial distributions with respect to climates. By the nature of the project, you will become more familiar with climate change and the predicted consequences for the eastern US.

6. *Communicate and extend the capstone experience via written, oral, and/or multimedia reports by each student:* You will produce multiple written reports on various stages of your project, which will culminate in an oral presentation to the class and webpage to be posted on our project website, open to the public and geared toward our citizen scientist participants.

Month	Day	Week day	Theme	Lecture/Discussion Topic	Laboratory Topic	Reading	Assignments due
Jan	18	W		Introduction	no meeting		
			Biology of microbiome members				
	20	F		The plant as habitat		Philpott et al. 2013 OR Vorholt 2012	
	23	M		Introduction to microbial lifestyles			
	25	W		Mycorrhizae 1 - types and structures	DNA extraction		
	27	F		Mycorrhizae 2 - evolutionary and ecological issues		Kiers et al. 2011	
	30	M		Nitrogen fixing symbioses			
Feb	1	W		Endophytes	Plant DNA PCR		
	3	F		Pathogens			
			Microbiome functions for plants				
	6	M		Nutrient acquisition			
	8	W		Drought tolerance	Fungal PCR 1	Lau and Lennon 2012	
	10	F		Abiotic stress tolerance			Assignment 2: Stress tolerance case study assignment
	13	M		Foliar disease suppression			
	15	W		Soil disease suppression	Fungal PCR 2	Mendes et al. 2011	Assignment 1. Plant DNA gel results
	17	F		Host growth promotion			
	20	**M**		**Exam 1**			
			Methods to study microbiomes				
	22	W		Culture dependent approches	Plant DNA sequence editing and BLAST		Assignment 3. Fungal DNA gel results
	24	F		Biomarkers and DNA barcoding			
	27	M		Sequencing technologies			
Mar	1	W		Bioinformatics 1: Clustering and species concepts	Intro to QIIME		Assignment 4: Plant idenfication results
	3	F		Bioinformatics 2: Taxonomic and phylogenetic assignment			

	6	M		Targeting active microbes			
				Ecology of plant microbiomes			
	8	W		Niches and distributions	Exploring OTU tables		Assignment 5: NGS quality control results
	10	F		Competition and coexistence			
	13	M		Community assembly theory			
	15	W		Metacommunities	Multivariate tests		
	17	F		Diversity and ecosystem function		Mahrali and Klironomos 2007	
	20	M		*Spring Recess*			
	22	W		*Spring Recess*			
	24	F		*Spring Recess*			
	27	M		Biogeography of microbes			
	29	W		Plant-soil feedbacks and control of plant communities	Brainstorm geographic hypotheses		Assignment 6: Comparison of microbial communities
April	1	F		**Exam 2**			
				Metagenome concepts and methods			
	3	M		Holobionts and the metagenome concept		Bordenstein and Theis 2015	
	5	W		Studying metagenomes 1	Individual projects: linear modeling		
	7	F		Shotgun metagenomics			
	10	M		Meta-omics		Ofek-Lalzar et al. 2014 or Delmonte et al. 2009	Independent Project Step 1 due
				Applications of microbiome science			
	12	W		Farming systems and microbial diversity	Individual projects: species distribution modeling		
	14	F		Microbial response to farm management			Independent Project Step 2 due
	17	M		Phyllosphere communities and human health			

	19	W		Bio-control and bioinoculant industry	Individual projects: species distribution modeling		
	21	F		Plant breeding and microbiomes			Independent Project Step 3 due
	24	M		Plant breeding and microbiomes 2			
	26	W		Microbial responses to climate change	Presentations		
	28	F		Microbial communities and the carbon cycle			
May	1	M		Microbial communities and restoration			Individual Project Final Report due
	3	W		**Exam 3**			

Independent Project: Climate change response of forest rhizosphere fungi

We will spend the last six weeks of the semester working on your independent project. The goal of these independent projects is to increase our basic scientific knowledge about some of the fungi we detected in our root communities. Each of you will chose two fungal species from different functional guilds (i.e. mycorrhizal fungi, putative pathogen, endophyte, etc.). Using the data we have collected, as well as other information you can gather from literature searches, each of you will prepare a written report on the biology of these species in eastern temperate forests. High quality reports will be posted on our citizen science project website (www.forestfungiproject.org), to provide information for our participants as well as serve as a resource for fungal biologists and forest ecologists generally.

The project will proceed through a series of intermediate steps, which we will work through together during our laboratory meetings. You will turn in outputs and preliminary interpretations after each lab section, and then use these results and your interpretations to prepare your final reports.

Step 1. Chose two target fungal species, and map their detection in our samples. I have curated our data set down to a list of fungal species to choose from, based on 1) their representation (present in at least 20 samples), 2) resolved taxonomy (identified to the species level), and 3) assigned functional group (for now, we are only using species that have been putatively assigned to be mycorrhizal, potentially pathogenic, or known endophytes).

Use the statistical modeling approaches to compare the presence/absence of your fungal species to host genus, and separately to soil order. Interpret the outputs of these models to answer these two questions.

A) Does this species show any significant associations with particular host genera?
B) Does this species show any significant associations with particular soil types?

Output: Statistical table, appropriate figure, and preliminary interpretation of results for the two models. Due April 7

Step 2. Using the pattern of presence and absence among our sites along with climatic variables to make a map of the probability of occurrence for your species across the eastern US based on current climate conditions. Evaluate the validity of your species distribution model using cross-validation, and list the relative importance of the climatic variables in shaping the current distribution

Output: Maps of predicted occurrence, model evaluation statistics, and relative importance table for both species. Due April 14.

Step 3. Map the predicted future distribution of the fungal species by applying the species distribution model you developed in step 2, and predicting where the suitable climatic conditions will occur in 100 years based on climate change projections. Compare with the maps produced in step 2 to identify areas of predicted range expansion and/or contraction.

Output: Maps of predicted future occurrence for both species, and short discussion of predicted range changes. Due April 21.

Format for final reports.

The goal of your final reports will be to briefly summarize what is currently known about the ecology and distribution of your target species, and illustrate the new knowledge gleaned from our class samples. Ultimately, we will use the information in your reports to create species pages on our project website, www.forestfungiproject.org. Please write a separate report for your two species. Each report should contain the following five sections: Literature review, Tests of ecological hypotheses (step 1), modeling of current distribution (step 2), modeling of potential future distribution (step 3), and conclusions. Write 1-2 paragraphs per section, in addition to whatever tables and figures are necessary. For our purposes, it is important that all of your reports follow the same format to make a coherent product for our project website.

Some brief guidance on what to include in each section:

Literature review: Is there currently anything reported in the scientific literature about the species' 1) ecological role/lifestyle, 2) taxonomic placement, 3) host distribution, 3) geographic distribution? All of your species should at least have information on taxonomy and basic lifestyle (pathogen, mycorrhizal, endophyte, etc.), as this is available in the data that I gave you. Cite peer-reviewed literature to support any information you provide (one or two sources per question is sufficient). If you can find a photo of your species that is licensed under a Creative Commons license, please include it (with appropriate attribution!).

Tests of ecological hypotheses: Summarize the results of the statistical modeling you did for step 3, presenting the statistics in a table and figures illustrating any interesting relationships. Explain how you interpret these statistics to answer the two questions posed above for step 1.

Modeling of current distribution. Show the interpolated map of the species distribution model you fit in step 2. Discuss the reliability of the patterns displayed in your map and which climatic variables are most important in determining the species' geographic distribution.

Modeling of future distribution. Show the map of predicted future suitable climate that we created in our last lab. Compare this with the map of current distribution, and discuss areas where this species is predicted to decline/disappear (if any), and areas where it is expect to expand.

Conclusions. Based on your previous three sections, write a short summary statement indicating your conclusion on how you expect this species to respond to changing climates. Based on your statistical models, discuss how ecological relationships with host trees or soil types may affect the validity of the species distribution model that is based only on climate. Determine whether you expect this species to show a strong direct response to warming climates, if this species' distribution will instead be constrained by soil types, which are not changing, or finally, if the species' future distribution will be determined by climatic responses of host trees.

Rubric for Independent Project

Each species report will be graded separately.
Each project will be worth a total of 150 points, 75 for each analyzed species
Total points will reflect scores on the three intermediary outputs and the final report

	Points	Low	Mid	High
Step 1 Output: Statistical modeling of occurrence	10	Tests performed incorrectly, important results missing	Tests performed correctly, but interpretation insufficient or inconsistent with results	Tests performed and interpreted correctly
Step 2 Output: Species distribution model and map	10	Some components missing, metrics interpreted incorrectly	All components present, but evaluation insufficient or inconsistent with results	All components present and evaluated appropriately
Step 3 Output: Predicted future distribution	5	Future prediction missing or performed incorrectly	Future prediction performed correctly but interpretation of patterns insufficient or inconsistent with results	Future prediction performed correctly and interpretation follows logically from results

Report	**50 pts total**			
Literature Review	5	Literature review absent or lacking support	Literature review addresses some but not all relevant topics, some sources cited	Literature review address all relevant topics to the degree possible; all statements supported with appropriate citations
Tests of ecological hypotheses	10	Biological interpretation missing or inconsistent with evidence	Biological interpretation is correct, but presentation is unclear or not suitable for a lay audience	Biological interpretation is correct and is presented in compelling and accessible way with both text and displays
Modeling of current distribution	10	Maps presented without context or interpretation	Maps presented with appropriate interpretation and model evaluation, but presentation is unclear or not suitable for a lay audience	Maps presented with appropriate interpretation and model evaluation in a compelling and accessible way

Modeling of future distribution	10	Predicted maps presented without context or interpretation	Predicted maps presented with appropriate discussion of expected range shifts, but presentation is unclear or not suitable for a lay audience	Predicted maps presented with appropriate discussion of expected range shifts in a compelling and accessible way
Conclusions	15	Conclusions fail to address some or all of the key questions, and/or are not supported with the available evidence	Conclusions are partially supported with available evidence, and/or reasoning is not well explained	Conclusions are fully consistent with available evidence and alternative explanations are considered. Reasoning is presented in a clear and accessible way