How will climate change influence how endophytes decompose plant litter?

INTRODUCTION

Predicting whether ecosystems will release more carbon than they store is a major challenge in the face of global climate change [1–4]. These predictions are contingent on understanding how changes in climate influence microbial communities that release carbon from decaying plant litter through decomposition [5–7]. Decomposition is a key pathway in the terrestrial carbon cycle that releases eight times more carbon dioxide than human activities [6] but can be highly variable depending on the species composition of the microbial community and its response to a changing climate [8–10].

Much work relating changes in climate to decomposition focuses on the responses of free-living fungi in the soil [11–16] because they produce an array of enzymes that degrade plant cell walls [17], **causing litter to lose mass.** However, my dissertation work revealed that foliar fungal **endophytes** (fungi that live inside plants [18]) **caused pine litter to lose almost as much mass as litter decomposed by soil fungi** over nine months (34.6% by endophytes *vs*. 41.7% by soil fungi), supporting evidence that endophytes significantly decompose plant litter [19–22]. While changes in precipitation and temperature have been shown to alter soil microbial communities and the amount of carbon they release [8–10], models that predict how climate affects endophyte-induced decomposition remain undescribed.

To address this, **I will transplant endophyte communities from two pine species across a precipitation and temperature gradient (Figure 1) to investigate the following question: How do changes in climate influence how endophytes decompose plant litter?**

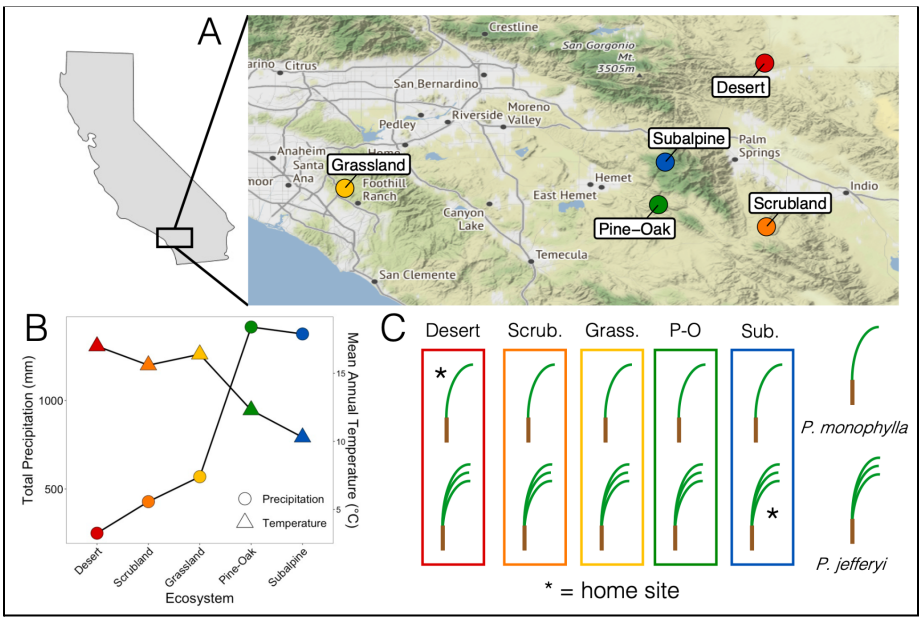

Figure 1. Diagram of Transplant Study. **(A)** Map of study sites. **(B)** Precipitation and temperature are inversely related. The subalpine site tends to be the coldest and wettest site because half of its precipitation is snow [23]. **(C)** Endophytes in *P. monophylla* (one needle per fascicle) and P. *jefferyi* (three needles per fascicle) will decompose litter *in situ* at their home site (denoted by *) and four other sites.

RESEARCH OBJECTIVES

I will identify which of three candidate mechanisms explain litter decomposition rate by endophytes. To do this, I will evaluate how climate and the endophyte community affect litter decomposition by comparing how endophytes induce mass loss *in situ* at their "home site" and in response to a precipitation gradient (**Figure 1**). While temperature can increase microbial activity, fungal biomass and enzyme activity are limited by moisture in arid environments [24,25]. Thus, **I will test three hypotheses that predict how precipitation, the endophyte community and their interaction influence litter mass loss**,

a measure of decomposition (**Figure 2**). For **Hypotheses 2 and 3, I will also test whether endophyte functional responses can be predicted from endophyte transcriptomes**.

EXPERIMENTAL DESIGN

I will allow endophytes to decompose litter at their "home site" and across a 1165.8 mm precipitation and 6.7°C temperature gradient over the course of one year (**Figure 1**). Climatic conditions at my proposed field sites have been monitored for over 10 years as part of the Loma Ridge Global Change Experiment managed by Dr. Kathleen Treseder, my proposed lead mentor.

***Transplant Experiment*:** Endophytes colonize the interior of healthy leaf tissues and may be particularly important in early decomposition stages [20], depleting litter of easily metabolizable carbons sources (e.g., sugars). Thus, I will transplant healthy, green needles across the climate gradient. I will sample and homogenize 100 needles from each of 10 trees at the subalpine and desert site and surface sterilize them to remove non-endophytic microbes from the leaf surface [26]. I will distribute 2,000 needles across 200 litter bags (10 needles/bag) and enclose them in mesh bags with 0.22-µm openings that allow endophytes to experience the climatic conditions of each site while limiting microbial movement into or out of the litter bag [as in 27]. Precipitation will be monitored from precipitation gauges and temperature will be measured with iButton sensors. Five litter bags replicated across four collection time points (0, 4, 8, 12 months) will be deployed across five sites for endophyte communities from two pine species (5 replicates x 4 timepoints x 5 sites x 2 pine species = 200 litter bags) that belong to a plant genus of well-studied litter systems [28].

***Characterization of Endophyte Communities*:** I will extract RNA from litter to characterize the endophyte community in two different ways. I will use high-throughput sequencing of the reverse-transcribed internal transcribed spacer 1 region, a common marker for fungal identification, to characterize the species composition of metabolically-active endophytes [29,30] at each collection time point. In addition, I will use RNA-sequencing to assess changes in the expression of endophyte genes associated with enzymatic activity and stress tolerance [11]. I will quantify endophyte biomass with qPCR by amplifying the 18S rRNA region [as in 31].

***Nutrient and Enzymatic Assays*:** The activity of four enzymes (cellobiohydrolase, β-glucosidase, β-xylosidase and laccase) will be measured on a subsample of fresh litter [21,32,33]. I will oven-dry a separate subsample of litter to quantify mass loss and specific carbon compounds (sugars, cellulose, hemicellulose, lignin) that these enzymes can degrade [34] along with nitrogen. I will complement these coarse measurements of litter chemistry with Fourier Transform Infrared (FTIR) Spectroscopy, a high-throughput method to characterize a broad spectrum of carbon compounds in litter [as in 35].

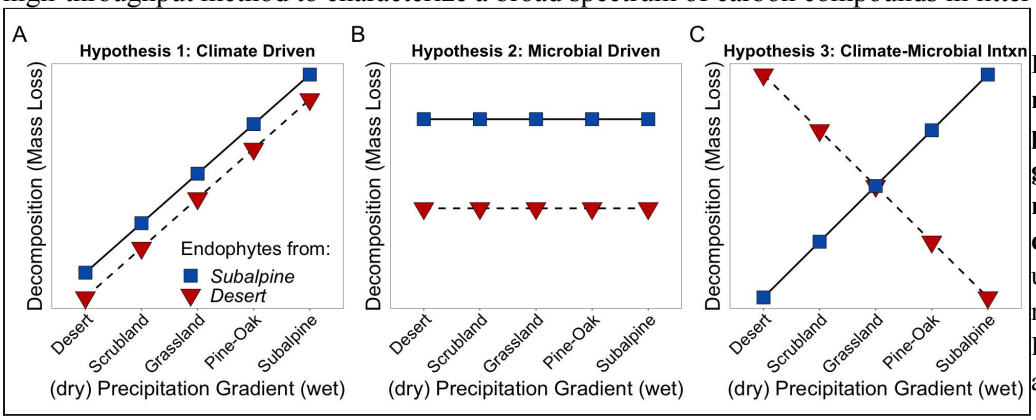

Figure 2. Predicted mass loss across a precipitation gradient after 12 months of decomposition using theoretical models from [36]. Legend in panel A applies to all panels.

HYPOTHESES

Hypothesis 1: Litter decomposition rates are driven by differences in precipitation. Drier climates have been associated with slower decomposition rates attributed to shifts in litter chemistry and reduced microbial activity [37–40]. Thus, ***I predict that endophyte communities in both pine species will have greater biomass and enzymatic activity in wetter sites, inducing greater mass loss with increasing precipitation (Figure 2A).*** I expect the slopes of these responses to be positive but vary depending on differences in the chemical composition of litter between pine species. For each of the two litter types , I will correlate endophyte biomass, enzymatic activity or mass loss to precipitation and include the relative abundance of carbon compounds and nitrogen (e.g., C:N, lignin:N, cellulose:lignin as in [41]) and principal components of carbon compounds (using FTIR spectra) as covariates with linear regression.

Hypothesis 2: The endophyte community controls the rate of litter decomposition. The biomass or species composition of microbial communities has often been associated with litter decomposition rates [23,27,42,43]. If the endophyte community controls litter decomposition, ***I predict that endophyte communities in both pine species will induce the same mass loss regardless of precipitation level (Figure 2B)***. I expect that the decomposition of each litter type will depend on endophyte resource use and litter chemistry. For example, in early stages of decomposition endophytes may capitalize on readily available soluble carbon compounds (e.g., sugars) as leaves senesce to invest in reproduction (e.g., fruiting bodies). At later stages, endophyte persistence may depend on the ability to degrade lignified carbohydrates that are tough to decompose. To assess how endophytes cause mass loss, I will correlate endophyte enzymatic activity or composition to standardized units of carbon and nitrogen (e.g., C:N, lignin:N, cellulose:lignin) and principal components of FTIR spectra with linear or multivariate models. I also will use RNA-seq to link endophyte gene expression to function. ***I predict that carbohydrate active enzyme (CAZy) genes associated with decomposition*** *[11]* ***will be correlated with the loss of specific carbon compounds*** (e.g., expression of cellobiohydrolase should be associated with cellulose depletion).

Hypothesis 3: Endophyte communities decompose the most litter at their "home site" because they are adapted to local environmental conditions. Recent work has shown that leaf microbial communities decompose litter faster in their home environment than in different environments [44], suggesting endophytes are locally adapted to historical climate conditions. ***I predict that endophytes in both pine species will decompose the most litter at their home site with decreasing endophyte biomass, enzymatic activity and mass loss as the precipitation regime becomes more dissimilar (Figure 2C).*** I will assess these relationships with linear models as in H1 and H2 and expect that these responses can be explained by how endophytes experience the local environment in non-home sites. For example, increasing moisture limitation for wet-adapted endophytes could shift the endophyte community to species with stress-tolerant traits, a costly metabolic investment that reduces growth and enzyme synthesis needed to induce mass loss [45], resulting in a positive correlation between mass loss and precipitation (blue squares; Fig. 2C). Thus, I also hypothesize that endophytes allocate resources to survival (stress tolerance) when transplanted to a non-home site at the expense of decomposition ability. Using RNA-seq data, ***I predict that gene expression associated with decomposition (e.g., CAZy genes) will be greatest at "home" for endophyte communities in both pines species while genes expected to confer stress tolerance (e.g., osmolytes, cold-induced RNA helicase or heat shock proteins as in*** *[46])* ***will be greatest at the non-home site with the most dissimilar precipitation regime.***

SIGNIFICANCE: Ninety percent of all plant biomass on land is decomposed by microbial communities [47] but much of the literature ignores how endophytes contribute to carbon cycling by moving carbon from terrestrial environments to the atmosphere [6]. Endophytes occur within the tissues of all major plant lineages [48] and have been shown to contribute to litter degradation and mass loss at multiple stages of decomposition [21,49–51]. Yet it is not clear how this function may change under future environmental scenarios. Here, I propose to link changes in the endophyte community (e.g., biomass, composition, gene expression) and litter chemistry with changes in function (e.g., mass loss, enzymatic activity) across a precipitation gradient. Under the mentorship of Dr. Treseder, the NSF Postdoctoral Fellowship will allow me to scale up basic lab findings from my dissertation to an ecosystem-level experimental manipulation to test ecological hypotheses that remain unexplored in endophytes.

BROADER IMPACTS

My lived experiences as an undergraduate who **overcame economic inequities and as a Filipino male within the LGBTQ+ community will enhance diversity at the postdoctoral level.** As an undergraduate, I worked at multiple service industry jobs for 30 hours a week to support my basic needs - often finishing shifts at midnight followed by an hour bike commute to and from work. Therefore, **I have worked throughout my entire graduate career to provide equitable access to higher education and address barriers to inclusion.** For example, **I initiated a program to address economic barriers that can exclude undergraduates from research opportunities** (much like myself during my undergraduate career) as representative for the UC Santa Barbara Coastal Fund **where undergraduates in financial need can apply for funding** ($1,000-$5,000 per quarter) for internships that would otherwise be unpaid.

I have found that it is rare to meet colleagues and mentors with similarities in culture and lived experiences which, at times, has contributed to feelings of imposter syndrome and lack of inclusion, a major challenge to broadening participation in ecology and evolution [52,53]. This has shaped **my commitment to engage in mentorship practices that seek to include and retain underrepresented scholars in the scientific community.** As a graduate student, I collaborated with an undergraduate who identifies as a first-generation Latina in STEM who was **awarded admission into the *McNair Scholars Program*** and another undergraduate within the LGBTQ+ community who was **awarded the *Undergraduate Research and Creative Activities Grant* and the *Worster Award*** to investigate how fungi that live inside leaves decompose plant litter.

Broadening Participation Among Underrepresented Groups: I will continue similar efforts as a postdoctoral scholar at UC Irvine, a Hispanic serving institution, by (1) mentoring undergraduate students from underrepresented backgrounds, (2) leading coding workshops that engage underrepresented students and (3) teaching climate science to middle school students.

(1) Broadening Participation through Direct Mentorship: I will **recruit five undergraduates from underrepresented groups** with specific hiring outreach to the following UC Irvine organizations: California Alliance for Minority Participation, the Black Student Union, Queer and Trans in STEM, Society for Advancement of Chicanos/Hispanics & Native Americans, Transfer Student Center and the First-Generation Student Center. Students will leverage their training in ecological fieldwork, molecular biology and data science skills in this proposed work **to develop their own research interests**. I will encourage them to apply for the UCI Undergraduate Research Opportunities Program to fund their projects and present their work at the Undergraduate Research Symposium. We will set goals at the beginning of each quarter and meet weekly to discuss progress and ensure **each student has the support to develop their science and professional development skills** (e.g., presentations at conferences). I will

encourage them to participate in Treseder lab meetings, a weekly journal club and will make concerted efforts to connect them with other PIs, postdocs and graduate students with similar research interests.

(2) Broadening Participation in Computer Programming: As a graduate student, **I co-developed the Data Analysis & Coding Club at UC Santa Barbara, an undergraduate-focused group (~20 undergraduates) that seeks to increase programming skills in the R language,** and have shared how this work advances diversity, equity and inclusion (DEI) as an invited panelist at the 2021 UCSB Data Science Summit. I will continue similar efforts by collaborating with Dr. Pheather Harris, director of UC Irvine's California Alliance for Minority Participation (CAMP), an NSF-funded program that supports underrepresented students in STEM. **I will lead a coding workshop for 15 students** in the CAMP computer lab that is equipped with R software, using a teaching model I have already implemented at UCSB. Over 10 weeks for 2 hours per week (a reasonable learning period for first-time coders, in my experience), students will learn how to navigate RStudio, manipulate data, visualize data and write functions with the intent of creating an interactive web application they will present to the group. I will teach this program every other academic quarter and **will recruit other postdoctoral scholars and graduate students from underrepresented groups** to co-lead this workshop.

(3) Broadening Participation in Global Change Biology: Although climate change is taught at 90% of all US public middle schools, prior training in climate science is limited with only 43% of science teachers surveyed by the National Center for Science Education reporting college-level instruction in climate science [54]. To support climate science outside of academia, I will partner with Climate, Literacy, Empowerment And iNquiry (CLEAN) Education, an organization run by UC Irvine graduate students at my host department, to teach climate science at local 6th grade classrooms (~30 students per classroom). I will **introduce students to the carbon cycle using interactive exercises that focus on fungal decomposition**. Students will formulate hypotheses about the rate of decomposition for litter that varies in recalcitrance (herbaceous leaf litter, pine litter and wood), fungal abundance (autoclaved vs. unaltered litter) and environment (wet vs. dry). Students will record weights for substrates that have been decomposing for 0, 1, 2 and 3 months and observe the morphology (via microscopy) of litter fungi *in situ* and in culture. I will ask students to explain why differences in decomposition rates exist among litter that varies in substrate type, fungal abundance and environment to encourage them to draw upon the taught curriculum and to gauge the effectiveness of my approach to teaching.

TRAINING OBJECTIVES

A central goal of this work is to build a pathway towards a faculty position that integrates microbial community ecology, functional genomics and global change biology. My proposed work under the guidance of Dr. Treseder will allow me to (1) continue my training in fungal and molecular ecology, (2) learn new high-throughput techniques (RNA-seq, FTIR spectroscopy) and (3) further develop my mentoring and teaching skills to support broader participation in ecology and evolution. I will broaden my current training in fungal ecology by conducting an ecosystem-level manipulation to understand how endophytes respond to changes in climate, a research avenue distinct from my dissertation research that focused on fungal biogeography. I will receive training from Dr. Alejandra Vázquez-Lobo at the Universidad Autónoma del Estado de Morelos and from Dr. Bruno Chávez-Vergara at the Universidad Nacional Autónoma de México (a 1 hour drive apart) to analyze RNA-seq or generate FTIR spectroscopy data, respectively. This fellowship will allow me to further develop my mentoring skills through one-on-one undergraduate mentorship, my leadership skills through running coding workshops and my science communication skills through outreach to middle school students.

Career Development Plan

Under the guidance of Dr. Treseder, this fellowship will be instrumental in supporting my career development towards an academic position and as a mentor that is guided by and advocates for diversity, equity and inclusion. My proposed work focuses on the functional response of endophytes in response to changes in climate by linking fungal community ecology, metatranscriptomics and biogeochemistry. This distinguishes itself from my dissertation research, which was primarily grounded in the biogeography of endophyte communities across latitudinal gradients. My existing small-scale and lab-based work on the role of endophytes as decomposers provides a baseline for the ecosystem-level manipulation with high-throughput approaches I have proposed here. I will have the opportunity to work with Dr. Kathleen Treseder, a leader in fungal ecology, functional genomics and global change biology, and broaden my collaborative network with Dr. Alejandra Vázquez-Lobo, an expert in RNA-seq, and Dr. Bruno Chávez-Vergara, an expert in biogeochemistry. Through this fellowship, I will have the opportunity to engage with underrepresented students in the scientific community and hone skills I will need as a future faculty member by teaching and collaborating with them in field, lab and classroom settings.

Justification of Sponsoring Scientists and Host Institutions

Dr. Kathleen Treseder's expertise in fungal ecology, functional genomics and commitment to broader participation among underrepresented groups makes her an ideal sponsoring scientist for my proposed work. The Treseder lab at UC Irvine has the established infrastructure required to support the ecosystem-level manipulation I have proposed. I will have full access to field sites at the Loma Ridge Global Change Experiment. The Treseder lab has established protocols to transplant fungal communities with "microbial cages" [42], run enzymatic and carbon fractionation assays and prepare cDNA and RNA sequencing libraries that will be sequenced at the UC Irvine Genomics High Throughput Facility. In addition, UC Irvine supports prominent microbial ecologists (Drs. S. Allison, J. Martiny, A. Martiny, K. Whiteson and A. Rodriguez-Verdugo) that will provide valuable perspectives on my proposed work and broaden my collaborative network.

RNA-seq and litter chemistry analyses are central to understanding how endophyte communities function and respond to changes in climate. Dr. Alejandra Vázquez-Lobo and Dr. Bruno Chávez-Vergara have published papers using RNA-seq [55] or FTIR spectroscopy [35], respectively, and will advise on aspects of the experimental design related to their areas of expertise. Visiting these international scholars will be a valuable opportunity to acquire hands-on experience with transcriptomic pipelines and generating FTIR spectra data, two skills that I hope to utilize in future work. Funding from this fellowship will expand my collaborative network internationally and offer me the opportunity to implement an ecosystem-level manipulation with high-throughput approaches to understand how endophytes - ubiquitous symbionts of the plant kingdom - function and respond to changes in climate.

Timetable Including Broader Impacts

Su = Summer; Fa = Fall; Win = Winter; Spr = Spring	Su. 2022	Fa. 2022	Win. 2023	Spr. 2023	Su. 2023	Fa. 2023	Win. 2024	Spr. 2024	Su. 2024
Research									
Collect litter and deploy litter bags across field sites	■								
Run qPCR, prepare cDNA and RNA-seq libraries (all timepoints)		■	■	■	■	■			
Enzymatic and nutrient (carbon, nitrogen) assays (all timepoints)		■	■	■	■	■			
Travel to Mexico to run FTIR spectroscopy and analyze RNA-seq							■	■	
Prepare research for publication and dissemination at conferences							■	■	■
Broader Impacts									
Recruit and train undergraduate mentees	■	■	■						
Undergraduate presentations at UCI Research Symposium				■				■	
Develop cirricula for coding and climate science workshops	■								
Coding workshop with underrepresented students		■		■		■			
Climate science instruction at local middle schools			■		■		■		