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Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA, MERCED

**Adapting in Times of Stress:
Community Responses in Monkeyflowers, Microbes, and Classrooms**

A dissertation submitted in partial satisfaction of the requirements for the degree of
Doctor of Philosophy

in

Quantitative and Systems Biology

by

Jackie E. Shay

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Professor Gordon M. Bennett
Professor Clarissa J. Nobile
Professor Brian A. Perry

2021

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To my family, friends, mentors, and students.

o

*To my husband, Dave,
for being an adventurous field partner,
a steady rock, and a warm embrace.
You got me here.*

o

*To my son, Marty,
for pushing me after I pushed you.
You are my reason.*

o

*To Heather,
for igniting the fire in me.
This is for you.*

o

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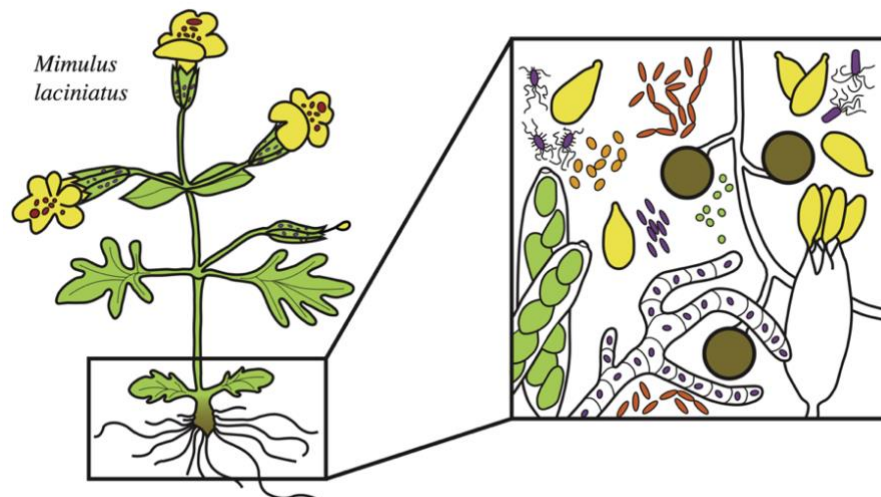
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Let the journey continue...



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EMPLOYMENT & PROFESSIONAL EXPERIENCE

Associate Director, Center for Engaged Teaching and Learning 2020–present

Department of Undergraduate Education, University of California, Merced

- Design and implement new programs in professional development in pedagogy and effective communication practices
- Collaborate with campus partners to foster a culture of learning and teaching
- Develop and deliver services and programs including orientations for new hires, workshops on course design and assessment, and inclusive teaching strategies
- Serve as a contact for individual instructors seeking advice on matters related to teaching, including creating hybrid and/or online courses, individual faculty coaching, and effective delivery of class material

Graduate Student Researcher, Co-Instructor, and Teaching Assistant 2016–2021

School of Natural Sciences, University of California, Merced

- Investigating the endophytic communities of native endemic flowering plants in light of drought from climate change using molecular, bioinformatics, and field-based techniques
- Co-creating course learning outcomes and curriculum for flipped/hybrid course environments
- Facilitating engaging active learning strategies in microbiology and general biology classes
- Responsible for lab safety in two labs as dual lab safety coordinator
- Mentor to four underrepresented undergraduate student women in science

Graduate Student Researcher, Harry D. Thiers Herbarium Assistant Curator, and Teaching Assistant 2012–2016

Department of Biology, San Francisco State University

- Studied systematics and phylogeny of wood-decomposing fungi from tropics using molecular, morphological, and field-based techniques
- Performed herbarium curating duties including accessioning, labeling, digitizing, organizing, and confirming stored specimens
- Taught botany, biology, and active learning strategies with mostly first and second year students

Youth Development and Health Educator 2010–2012

Peace Corps, Demnat and Tiznit, Morocco, Africa

- Built peer health education program with local nurses and teacher
- Established and maintained volunteer support network
- Created and demonstrated engaging hygiene education course for local schools

Undergraduate Teaching Assistant for Evolution and Biology Tutor 2005–2009

University of California, Merced

- Facilitated small group discussions in anatomy, genetics, evolution, and biology
- Co-authored student tutor teaching manual with learning staff
- Developed course curriculum and materials with faculty advisors

FUNDING

- 2021 UC Merced QSB Remote Teaching and Research Fellowship (\$250)
- 2020 UC Merced Academic Senate Faculty Research Grant; Sexton & Frank (\$10,000)
- 2020 UC Merced Outstanding Teacher Award (\$1000)
- 2019 Sonoma County Mycological Society Graduate Scholarship (\$1,500)
- 2019 Northern California Botanist Research Scholarship (\$1,000)
- 2019 Southern California Edison Fellowship (\$13,065)
- 2018 Sonoma County Mycological Society Graduate Scholarship (\$2,500)
- 2018 Mycological Society of America Translational Research Award (\$1,250)
- 2018 California Native Plant Society Bristlecone Chapter Mary DeDecker Grant (\$1,000)
- 2016 UC Merced Quantitative and Systems Biology Recruitment Award (\$20,106)
- 2015 Sonoma County Mycological Association Graduate Scholarship (\$2,500)
- 2015 Mushrooms of Madagascar *Kickstarter* Campaign (\$7,682)
- 2014 Mycological Society of San Francisco Graduate Scholarship (\$1,000)
- 2014 SFSU College of Science and Engineering Scholarship (\$2,500)
- 2013 Robert W. Maxwell Scholarship (\$4,000)

AWARDS

- 2020 UC President's Award for Outstanding Student Leadership (awarded to RadioBio)
- 2020 UC Merced QSB Dissertation Incentive Award (\$580)
- 2019 UC Merced Outstanding Graduate Student Leadership Award
- 2019 Northern California Botanist Symposium Poster Competition First Place Award (\$200)
- 2016 UC Merced School of Natural Sciences Dean's Relocation Award (\$400)
- 2009 UC Merced Distinguished Leadership Award
- 2007 UC Merced Distinguished Student Volunteer Award

INVITED TALKS & REVIEWS

- 2020 Shay, J.E. "Symbiosis and Climate Change". *Biology Seminar*, Invited Talk, San Francisco State University, San Francisco, CA
- 2020 Shay, J.E., Brooks, L., Kaze, M., Frank, C.A., Sexton, J.P., Sistrom, M. "Endophyte communities in the cut-lead monkeyflower (*Erythranthe laciniata*) respond differently to drought conditions above and belowground" *Northern California Botanist Symposium*, Invited talk and session organizer for Plant-Biotic Interactions. Chico, California.
- 2019 Invited as reviewer for the journal of Plant Systematics and Evolution
- 2019 Shay, J.E. "Fungus Among Us", Invited Talk, *Camp Trip Music Festival*, Weldon, CA
- 2019 Shay, J.E., Brooks, L., Kaze, M., Frank, C.A., Sexton, J.P., Sistrom, M. "Endophytes shift in response to drought in monkeyflowers (*Erythranthe laciniata*) from native soil" *Symbiosis*, Yosemite National Park, CA
- 2017 Shay, J.E., Newman, D.S., Grace, C., Perry, B.A., Desjardins, D. "Biodiversity and Phylogeny of *Marasmius* (Basidiomycota, Agaricales) from Madagascar" *Marin County Mycological Society*, CA
- 2017 Shay, J.E., Newman, D.S., Grace, C., Perry, B.A., Desjardins, D. "Biodiversity and Phylogeny of *Marasmius* (Basidiomycota, Agaricales) from Madagascar" *Mycological Society of San Francisco*, CA
- 2015 Shay, J.E. "A Mushroom Adventure Collecting in Madagascar." *Mycological Society of San Francisco*, CA

PRESENTATIONS & POSTERS

- 2020 Garcia-Ojeda, M., Shay, J.E., Zimmerman, J., Schuchardt, A., Durazo, A. "Underrepresented women students close the achievement gap in a flipped class"

- delivered in a TEAL classroom” *Society for the Advancement of Biology Education Research*, UC Irvine, CA
- 2019 Shay, J.E., Brooks, L., Kaze, M., Frank, C.A., Sexton, J.P., Siström, M. “Endophytes shift in response to drought in monkeyflowers (*Erythranthe laciniata*) from native soil” *Mycological Society of America*, Minneapolis, MN
- 2019 Shay, J.E., Brooks, L., Kaze, M., Frank, C.A., Sexton, J.P., Siström, M. “Endophytes shift in response to drought in monkeyflowers (*Erythranthe laciniata*) from native soil” *Northern California Botanists Symposium*, Chico, CA
- 2018 Shay, J.E., Pennington, L., Toews, D., Sexton, J.P. “Climate adaptation and fitness variation is greatest near the leading edge of a species range” *Climate Change: Ranges and Phenology*, *Ecological Society of America*, New Orleans, LA
- 2017 Shay, J.E., Newman, D.S., Grace, C., Perry, B.A., Desjardins, D. “Biodiversity and Phylogeny of *Marasmius* (Basidiomycota, Agaricales) from Madagascar” *Fungal Genetics*, Asilomar, Pacific Grove, CA
- 2016 Shay, J.E. “Do microbiomes influence host evolution?” Quantitative and Systems Biology Topics Final Project. *University of California Merced*
- 2016 Shay, J.E., Newman, D.S., Grace, C., Perry, B.A., Desjardins, D. “Biodiversity and Phylogeny of *Marasmius* (Basidiomycota, Agaricales) from Madagascar” M.Sc. Thesis Defense. *San Francisco State University*
- 2015 Shay, J.E., Newman, D.S., Desjardin, D. “A preliminary monograph of *Marasmius* (Basidiomycota, Agaricales) from Madagascar.” Conference Research Poster. *BOTANY 2015*, Edmonton, Alberta, Canada
- 2013 Shay, J.E. “Termitomyces and Macrotermitinae: Masters of Efficiency, an Exploration of Fungal Symbioses.” *San Francisco State University*
- 2013 Shay, J.E. “The Understory Story: Mycoheterotrophy of Ericacea.” *San Francisco State University*

TECHNICAL SKILLS & WORKSHOPS

- 2020 UC Merced Graduate Division Dissertation Boot Camp
- 2019 Regional Summer Institute on Scientific Teaching, UC San Diego
- 2019 Collaborative Institutional Training Initiative Social-Behavioral-Educational Course
- 2019 Illumina Metagenomic Sequencing Workshop, UC Merced
- 2018 Data Carpentry Workshop, UC Merced
- 2018 Introduction to Computation Workshop, UC Merced
- 2018 Botanical Illustration Workshop, California Native Plant Society Meeting
- 2017 NSF Interdisciplinary Computational Graduate Education Program, UC Merced
- 2017 Joint Genome Institute Summer Internship Program
- 2015 Science Education Partnership and Assessment Laboratory Summer Institute, SFSU
- 2015 Macro nature photography summer course, SFSU Sierra Nevada Field Campus
- 2014 Fungi of Sierra Nevada summer course, SFSU Sierra Nevada Field Campus

TEACHING EXPERIENCE

General Microbiology BIO 120

Fall 2018–Spring 2020

University of California, Merced

- Co-instructor with resident faculty
- Developing flipped classroom curriculum and recording lecture content
- Engaging students with active learning teaching strategies both online and in-class
- Trained on Learning Glass™ technology
- Trained on Camtasia lecture video creation and editing

- Created higher order learning outcomes aligned with course goals

CBEST Summer Bridge Program for Elementary School

Summer 2019

University of California, Merced

- Developed an interactive class discussing plant biology for 3rd and 4th grade students
- Worked closely with undergraduate interns to facilitate this summer class
- Shared materials for future class activities

Contemporary Biology BIO 001

Fall 2016, 2017

University of California, Merced

- Guest lectured for fungal and plant sections of the course
- Facilitated weekly discussion with original activities, learning outcomes, and reviews
- Submitted dozens of activities, assessments, and review sessions for future course use

Flora of California BIO/ESS 133

Spring 2017

University of California, Merced

- Guided students through plant identification, anatomy, and field trip excursions
- Demonstrated correct use of the Jepson Guide to California Flora
- Collected plant material for identification based on plant family
- Guest lectured for soil ecology and symbiosis lectures

General Botany BIO 220

Fall 2015

College of San Mateo

- Co-instructed students with resident faculty
- Developed assessment-based approach to active learning
- Collected and presented data from course to SEPAL Summer Institute

Introduction to Biology II BIO 240

Spring 2014–Spring 2016

San Francisco State University

- Taught discussion, lab, and supplemental courses
- Assisted students in lab and discussion sections component of class
- Facilitated mini-lectures, lab activities, and discussions for deeper engagement
- Supplemented learning for a portion of students by working strictly on active learning strategies, growth mindset, and high order learning

World of Plants BIO 150

Fall 2012–Fall 20

San Francisco State University

- Guided students through lab experiments and field trips
- Created mini lectures to pair with lab activities
- Shared materials with future teaching assistants for this course

SERVICE & SYNERGISTIC EXPERIENCES

COVID-19 Emergency Remote Learning Transition Training for GTAs

2020

University of California, Merced

- Developed and lead emergency remote training sessions to assist fellow graduate teaching assistants as they transition their classes to online learning
- Coordinated with campus resources to schedule and advertise trainings
- Taught 30 graduate students and 3 faculty in six different hour-long sessions

- Interviewed about increasing engagement in emergency remote learning
<https://cetl.ucmerced.edu/node/14081>

Session Chair for Northern California Botanist Symposium Meeting 2020
California State University, Chico

- Invited by board members to chair the session
- Coordinated speakers for the *Plant-Biotic Interactions* Session
- Established a growing interest in microbe-associated plant interactions

RadioBio Podcast 2017–2021
University of California, Merced

- Pioneered RadioBio, a graduate student-led biology-themed podcast that discusses biology from molecules to ecosystems
- Develop and execute all major events associated with the RadioBio podcast including community outreach events, science showcases, and art exhibitions
- Facilitate interviews, editing, and production podcasts every two weeks

Teaching Intern, Center for Engaged Teaching and Learning 2017–2018
University of California, Merced

- Encouraged the university community to adopt active teaching strategies
- Participated in teacher training workshops to enhance my practice
- Lectured to peers on teaching strategies for large classrooms

University Committees and Other Services

- Executive Committee of the Quantitative Systems Biology Group 2017–2020
- Research Week Planning Committee 2017–2020
- Search Committee for the Executive Vice Chancellor and Provost 2018
- Vice President and Board Member UC Merced Alumni Association 2012–2018
- Search Committee for Dean of School of Natural Sciences 2017
- Review Board for the Office of the Vice Chancellor of Student Services 2017
- UC Merced W-STEM Member and Mentorship Program Participant 2016–2017
- Vice President and Chair of Annual Fungus Fair 2014–2017
Mycological Society of San Francisco, San Francisco

Acting and Stage Presence

- Performed in, created, or directed over a dozen plays and musicals 1999–2016
- Induction to the International Thespian Society 2004
- Meisner Technique Acting Classes 2002–2003
The Ruskin School of Acting, Santa Monica, California

CURRENT PROFESSIONAL MEMBERSHIPS

2019	Society for the Advancement of Biology Education Research
2018	Ecological Society of America
2015	Mycological Society of America
2013	California Native Plant Society

RESEARCH INTERESTS

Biodiversity | Fungi | Microbiome | Ecology | Evolution | Taxonomy | Systematics Endophytes
Climate Change | Herbaria | Symbiosis | Pedagogy | Graduate Student Professional Development

DISSERTATION ABSTRACT

Adapting in Times of Stress:
Community Responses in Monkeyflowers, Microbes, and Classrooms

Jackie E. Shay

Doctor of Philosophy in Quantitative and Systems Biology
Concentration in Ecology and Evolutionary Biology
University of California, Merced
2021

Dr. David H. Ardell, Chair
Dr. A. Carolin Frank, Research Advisor
Dr. Jason P. Sexton, Research Advisor

Life on planet Earth is inundated with stress. In the following studies, I explore how stress affects the structure of communities, from plant population response to climate change, to microbial response to drought, and to student responses to active learning and resilient teaching amidst the COVID-19 pandemic. To understand how species are responding to climate change, it is vital to recognize patterns of fitness and adaptation as they respond to these stressors. In shifting montane regions, high elevation edges are areas of range expansion, where populations have low levels of genetic variation and little to no adaptation. Yet, few studies have simultaneously determined how fitness and adaptive patterns vary among populations between climate edges. In chapter one, I discuss the lifetime fitness response of the cutleaf monkeyflower, *Mimulus laciniatus*, an annual plant endemic to the western slopes of the California Sierra Nevada, to environmental variation in a common garden experiment. Contrary to expectation, the high garden hosted the greatest expression of genetic variation and climate adaptation across the species range. Eco-evolutionary patterns in plant species range studies, such as these, can inform our understanding of such processes as they respond to climate change. In chapter two, I synthesize general biogeographic “rules” and how they can be used to develop adaptive conservation strategies of native plant species across their ranges. I conclude this review by outlining areas of research to better our understanding of the adaptive capacity of plants under environmental change and the properties that govern species ranges. In chapter three, I take a deeper look into *M. laciniatus* and report the first investigation of its native microbiome and how these communities respond to a drought experiment. I found that that spatial, temporal, and environmental variation structured the fungal and bacterial endophytic community. I conclude with an initial report of the root endophyte community, their general functional implications, and ecological associations with their host. In chapters four and five, I focus on evidence-based teaching strategies in STEM curricula. In chapter five, I discuss the impact of flipping an upper-division microbiology course on student learning. I also outline the effect of a student-centered, problem-based activity on student performance. In chapter six, I list a series of pedagogical strategies for developing a resilient online classroom. I conclude with feedback from faculty from the COVID-19 remote instruction experience and suggest the most effective techniques and technologies for teaching online.

CHAPTER 1

Is the edge of a species range becoming a climate refuge?

1.1. Abstract

Recognizing patterns of fitness and adaptation across species ranges is a vital step towards understanding how ranges evolve and respond to climate changes. In shifting montane ranges, high elevations are areas of range expansion, the leading-edge, where founder effects are expected to lead to low genetic variation and little to no adaptation. Yet, few studies have simultaneously determined how fitness and adaptive patterns vary among populations between climate edges. We conducted a common garden experiment among 23 populations at three sites across the species range of *Mimulus laciniatus*, an annual plant endemic to the western slopes of the California Sierra Nevada. We regressed lifetime fitness against spatial, genetic, and climate distances to each garden and tested for local adaptation within three adaptive contexts for their role in explaining fitness variation. We found evidence that suggests fitness and local adaptation are suppressed at lower elevations, possibly signaling a future range shift, and that climate of origin predicted fitness only at the high elevation garden. Contrary to expectation, the high garden hosted the greatest expression of genetic variation and climate adaptation across the species range. This work encourages consideration of leading-edge areas for both current and future refugia as ranges shift in response to climate change. Moreover, this work highlights the importance of inter-population genetic variation in maintaining climate niche breadth and demonstrates the use of multiple adaptive contexts to predict patterns of adaptation in response to shifting climates.

1.2. Introduction

Globally, populations are at risk of rapidly declining from climate change with particular impact on species fitness and distribution (Anderson 2016; Harrison, Gornish, and Copeland 2015). The geographic range of a species, and the movement of that range, is determined by myriad interactions, most notably climate (Darwin 1859; MacArthur 1972; I.-C. Chen et al. 2011; Freeman et al. 2018; Mamantov et al. 2021). Across a species range, local environments select for traits that allow species to persist in different climate conditions, which creates variation in fitness and facilitates local adaptation, complementing range shifts (Clausen, Keck, and Hiesey 1941; Parmesan 2006a; Des Roches et al. 2018; Anna L. Hargreaves and Eckert 2019). However, populations are not always adapted (Pearson, Lago-Leston, and Mota 2009) and the degree of adaptation, when occurring, is challenging to characterize based solely on geography (Angert, Bontrager, and Ågren 2020). In this regard, studies that assess lifetime fitness and fitness variation under different eco-evolutionary patterns (e.g., spatial, genetic, or climate variation) are in need, especially in shifting environments and under different adaptive contexts (Angert, Bontrager, and Ågren 2020; Kawecki and Ebert 2004; Bontrager et al. 2021). In this study, we address this gap by asking the following questions: How does lifetime fitness vary across the species' range? Is there evidence of climate adaptation, and if so, in what context? Does spatial, genetic, or climate variation best predict fitness across the species range?

When addressing these questions, it is vital to observe patterns of variation among populations across a species range and at the extreme edges. Peripheral populations often experience harsh

climate conditions, which can lead to the evolution of unique genotypes that are valuable for understanding species persistence, climate adaptation, and predicting future range shifts (Lesica and Allendorf 1995; Sagarin and Gaines 2002; Gibson, Marel, and Starzomski 2009; Sexton, Strauss, and Rice 2011). However, edges have been overlooked in these contexts, perhaps due to a long-standing paradigm in range-limit studies: among-population genetic variation and fitness decline towards range margins and central-range variation is more significant (Eckert, Samis, and Loughheed 2008; A. L. Hargreaves, Bailey, and Laird 2015). Nevertheless, edge populations can be large and more variable than previously thought (Sagarin and Gaines 2002), whereas central areas can possess moderate to low adaptive signals (Angert, Bontrager, and Ågren 2020) (Pennington et al. 2021). Thus, species that are declining due to climate change are as likely to collapse into peripheries of their range as they are to collapse into the center (Channell and Lomolino 2000).

Climate effects on species vary across elevational and latitudinal gradients (Frenne et al. 2011; Mamantov et al. 2021). In this vein, montane systems experiencing harsh climates are an ideal natural laboratory to test which contexts of adaptation influence fitness patterns and which factors predict fitness and fitness variation during climate adaptation across a species range (Anderson 2016; Hampe and Petit 2005). In a montane climate-warming scenario, rear-edge (warm and low-elevation) populations must adjust or contract upslope to beat the heat, and with no place left to migrate, leading-edge (cool and high-elevation) populations are under pressure to adapt or risk shifting to a point of extinction as minimum temperatures quickly rise (Mamantov et al. 2021; Hampe and Petit 2005; Anderson and Wadgymar 2020). Ultimately, as temperatures increase and precipitation decreases in rear-edge populations, one expects reduced fitness and the elimination of genetic variation due to extreme climate stress. However, it has been unclear what is expected at high-elevation edges because they are understudied. Generally, climate change scenarios expect leading edges to have low genetic variation driven by post-glacial founder effects (Hampe and Petit 2005; Hewitt 1999).

Our study uses common gardens during a drought year to understand the role of gene flow and selection within an endemic species range of the cutleaf monkeyflower (*Mimulus laciniatus* A. Gray, Phrymaceae) in the California Sierra Nevada (Fig. 1). Clausen, Keck, and Hiesey (1948) first developed a common garden model in the Sierra Nevada in their classic study of ecotypic adaptation. They found that plant fitness was affected by interactions between population origin and garden location along an elevational gradient. Our approach, using an endemic plant with leading and rear edges confined to the western slope of the Sierra Nevada, further allows us to test theoretical models of range-edge population differentiation and adaptation and provides insights that should inform basic and applied evolutionary ecology in the context of climate change. Fitness data among populations and representative environments were used to build correlative models of fitness across the range. We tested three different adaptive contexts originally outlined by Kawecki and Ebert (2004): 1) ‘home vs. away’ (HA) where locally adapted populations are expected to outperform other populations at their home site; 2) ‘sympatric vs. allopatric’ (SA) where genotype conditions are quantified and are expected to fit within specified conditions, in this case, climate; and 3) ‘local vs. foreign’ (LF) where it is expected that local populations will outperform populations grown in the same site but originating from other habitats. The factors that predict variation in these contexts are an important consideration for both conservation and restoration efforts, particularly for areas likely to play a role in future climate adaptation (Angert, Bontrager, and Ågren 2020; Gibson, Marel, and Starzomski 2009).

1.3. Methods

1.3.1. Study species and background

Mimulus laciniatus is an annual plant with a restricted, well-defined range on the western slopes of the Sierra Nevada. Populations are mostly distributed among granite rock outcrops, mainly growing from moss (*Bryum* spp.) or spikemoss (*Selaginella* spp.) patches on ephemeral, slow-draining, seeps. Due to its distinct range and natural elevation-climate gradient (Fig. 1) it has been the subject of several studies on species range limits and adaptation to the unique, challenging environments in which it is found (Sexton, Strauss, and Rice 2011; Sexton and Dickman 2016; Dickman et al. 2019; Sexton et al. 2016; Ferris and Willis 2018). This herbaceous plant is primarily self-fertilizing, but exhibits occasional outcrossing (Sexton et al. 2016; Ferris and Willis 2018). Seeds of *M. laciniatus* were collected from 23 populations that span the elevation-climate gradient of the species range from the warm, low elevation populations of the foothill woodlands (~900 m) to the cool, high elevation populations of the alpine zones (~3270 m) (Fig. 1 and Table 3). At each population, at least 60 maternal seed families were collected to maximize the representation of microhabitat variation within each population (see Sexton et al. 2016 for sampling details). Maternal family lines from field-collected seeds were grown within a common environment allowing for the healthy growth of plants from all populations (growth chambers with 14-hr, 1000 $\mu\text{mol}/\text{m}^2/\text{s}$ light day; 23°C/4°C max/min). Seeds were sown into experimental planting trays in a randomized block design and trays were set into natural field conditions (as in Sexton et al. 2011) before the onset of winter precipitation, for a total of 4710 plants in the experiment. Using this second generation of plants reduced field-derived maternal effects and provided a more accurate assessment of interpopulation lifetime fitness across the range of climates experienced within the species range. We measured overall lifetime fitness for individuals using flower number and total fruit mass as fitness proxies (Sexton, Strauss, and Rice 2011; Sexton and Dickman 2016; Dickman et al. 2019).

1.3.2. Climate garden experiment

We established three common gardens from among the 23 populations sampled: a low-elevation, rear-edge (1000 m); a central, mid-range (1670 m); and a high-elevation, leading-edge (3095 m) (Fig. 1). Experimental common gardens were established in Fresno County, California, on National Forest land during a single growing season between October 1, 2008 and September 30, 2009. To characterize this year, we compared average temperature and precipitation from the United States Geologic Survey Basin Characterization Model (270 m resolution) (Flint and Flint 2014) to historical data obtained from a 30-year annual average (1981–2010) (see Dickman et al. 2019 for details). The average temperature minimum and maximum for the all sites in 2008–2009 was relatively normal (1.5–15.9 °C) compared to 30-year historical averages for the same sites (2.2–13.9 °C). The average annual precipitation was much lower (5.36 cm) than the historical average (78.95 cm), characterizing the 2008–2009 growing season as a drought year.

1.3.3. Statistical analysis

We used a REML model (Shaw 1987) to test for differences in lifetime fitness among populations, gardens, and their interaction. Individuals that did not emerge or survive to produce flowers were assigned a value of zero. Block was included as a random effect nested within garden. Due to high variation in mortality among gardens and populations, data did not meet assumptions of parametric analysis and were rank average transformed (Conover and Iman 1981).

We used Tukey HSD post hoc tests and planned orthogonal contrasts to answer the following questions for specific local adaptation contexts: 1) HA: We compared the fitness of each local

population grown in its home garden with that same population grown in other gardens; 2) SA: We tested whether populations originating from one climate edge (sympatric) outperformed populations from the opposite climate edge (allopatric) within their respective climate garden; 3) LF: We tested whether populations originating from each garden (local populations) performed better locally than other “foreign” populations by comparing the fitness of each local population in its home garden with all other populations grown within that garden. These analyses were conducted in JMP, version 14.2.0 (SAS Institute Inc., Cary, NC, 1989–2019).

We also compared the fitness of local populations grown within their home garden to the mean of all other populations grown within that garden using planned orthogonal contrasts. To test for differences in survival among populations, gardens, and their interaction, we used logistic regression with linear mixed effect models using Eigen and S4 values with the lme4 package, version 1.1-18-1 (Bates, Bolker, and Walker 2015) in R, version 3.6.3 2020 (R Core Team) (R Core Team 2020).

Finally, we used multiple regression analysis to test whether spatial, genetic, climate or a combination of these types of variation predicts lifetime fitness within each climate garden across a species range. Lifetime fitness data, including zeros for plants that did not emerge or survive to flower, were rank-transformed within each garden and population least-square means were derived using REML with block as a random factor. The lifetime fitness of family means, nested within populations, was also derived to increase statistical power; however, these results were qualitatively the same as population means, so we report population mean responses only. Spatial distances between populations were estimated from geographical distances. Genetic distances were estimated from 11 co-dominant markers isolated from DNA in leaf tissue (see Sexton et al. 2016 for details), and climate distances were estimated from BIOCLIM variables from 1950–2000 (see Sexton et al. 2016 for details). Linear and nonlinear (quadratic) regressions were generated for all analyses, but since no significant non-linear responses were observed, we report only linear models.

1.4. Results

1.4.1. How does lifetime fitness vary across the range?

Out of 4710 plants in the experiment, 1703 survived to produce flowers. Plant lifetime fitness, measured as the total number of flowers, and the variation of lifetime fitness between populations, fluctuated greatly across gardens and among populations. There were significant garden, population, and garden by population interaction effects (Fig. 2 and Table 1), indicating adaptive genetic variation across the species range. Lifetime fitness and fitness variance were much greater at the high garden than low or central gardens (Fig. 3 and Table 1).

1.4.2. Is there evidence of climate adaptation, and if so, in what sense or population context?

Overall, adaptive signals consistent with local or climate adaptation were detected only at the high garden. Regarding HA tests, in which the lifetime fitness of populations local to each garden was contrasted in home gardens versus away gardens, the low garden population actually had greater fitness away than at home. The central garden population exhibited no difference in lifetime fitness at its home garden versus away. The high garden population exhibited significantly increased lifetime fitness at its home garden than away (Fig. 4).

Regarding SA tests, we found evidence for climate adaptation between cold and warm climate edges. When contrasting lifetime fitness of the three high-elevation, cool-edge populations versus the three low-elevation, warm-edge populations, cool-edge populations had significantly greater lifetime fitness in the high garden, but the reverse was not initially true for cool-edge populations in the low garden (Fig. 4 and Table 2). However, one warm-edge population (see A-LOW in Table 3) had low average fitness (Fig. 4) everywhere, including in its home garden (Fig. 4). When this population was removed from the above analyses, the remaining two warm-edge populations had greater fitness in the low garden than the cool-edge populations.

Regarding LF tests, local populations were never the best performing populations in their original gardens. Instead, local populations always had lower lifetime fitness than populations from gardens with similar climates (Fig. 3 and 4). For analyses using planned contrasts comparing mean local lifetime fitness versus mean foreign lifetime fitness (i.e., mean lifetime fitness of all other populations combined), there was no significant difference between the low garden population and all other populations in the low garden, nor for the central garden population. However, high garden population plants had greater mean lifetime fitness in their own garden compared to all other populations (Table 2).

1.4.3. Does spatial, genetic, or climate variation predict fitness across the species range?

Lifetime fitness was predicted only by the climate distance of populations, and only for the high garden (Table 4), where fitness and fitness variation were the highest among all populations (Fig. 2). Spatial and genetic distances to gardens did not significantly predict mean lifetime fitness (Table 4).

1.5. Discussion

Due to post-glacial founder effects during recolonization, climate change scenarios predict increased adaptation and genetic variation at the rear-edge and reduced fitness variation at the leading-edge for plant species (Hampe and Petit 2005; Hewitt 1999). Our findings contrast with these range edge scenarios under climate warming in a drought year. Local climate adaptation was most strongly expressed at the high elevation, leading-edge where the average fitness of all populations across the species range was greatest. Low elevation, rear-edge populations also appear to be climate-adapted, but we also found evidence they are experiencing reduced fitness in their warmer, drier climates (Sexton, Strauss, and Rice 2011). These findings were most apparent in the SA adaptation test context; however, garden distance regressions also revealed climate adaptation. Climate distance from garden was the only significant predictor of fitness, and only at the high garden, suggesting that expression of climate adaptation is becoming eroded in lower elevations. In this vein, leading-edge areas may be a future refuge for the species as it responds to climate change.

1.5.1. Rear- and leading-edge adaptive responses

Both the rear and leading edges of the *M. laciniatus* range have abundant genetic variation and demonstrated adaptation to their respective climate conditions. This is likely driven by elevation-based adaptive phenological differences reported in this species in prior studies in which time to flower increases with elevation (Sexton, Strauss, and Rice 2011; Dickman et al. 2019). We found increases in plant growth and among-population fitness variance at the leading-edge. Local adaptation and high genetic variation are not often expected at the leading-edge (A. L. Hargreaves, Bailey, and Laird 2015; Hampe and Petit 2005). Plants originating from low

elevations of the species range experienced a fitness boost when grown in the high garden. In particular, as temperatures rise, gene flow from warmer, rear-edge populations contributes to adaptive genetic variation at cooler, leading-edges, as shown in the annual wildflower *Clarkia pulchella* (Bontrager and Angert 2018). For now, range limits appear to be stable for *M. laciniatus* (Sexton and Dickman 2016). Moreover, we found surprisingly low fitness in the central garden for all populations and no signal of adaptation. This suggests that central populations and rear-edge populations are under similar climatic stress (Reich et al. 2018). If climate stress increases, this could signal the beginning of an upward range contraction for a large proportion of the species range (Anderson and Wadgymar 2020).

1.5.2. Local adaptation and fitness variation at the edges of the species range

Mimulus laciniatus populations are rapidly responding to recent climate change (Dickman et al. 2019). Our study confirmed this finding and provided insights about local adaptation by assessing three adaptive contexts. First, only the leading-edge home population satisfied the HA criterion of adaptation, reinforcing the view that high-elevation environments are more hospitable while still allowing expression of adaptive differentiation. Second, in the SA test comparing cold- versus warm-climate populations, although leading-edge populations were clear winners in the high-elevation climate garden, rear-edge populations outperformed leading-edge populations in the low-elevation climate garden, producing a skewed but adaptive fitness reaction norm (Fig. 4). Finally, the LF test context was least effective in detecting adaptation: local populations never did best at their home garden. This result, although generally unexpected (Leimu and Fischer 2008), may reflect adaptive lags to climate change (Wilczek et al. 2014), especially during this drought year. Additionally, many populations across the range were assessed in each garden in this study, which may have introduced some foreign genotypes that perform better than local ones. This demonstrates the importance of adaptive differentiation among populations and the potential benefits of interpopulation gene flow.

Strong patterns of local adaptation only materialized in the high garden, a more benign environment due to its longer growing season and cool, wet climate that allowed greater genetic expression of variation to occur. In this regard, genetic variation may be inflated in more favorable conditions and depressed in unfavorable conditions (Fischer et al. 2021). Furthermore, recent climate warming may have ameliorative effects on the expression of adaptive genetic variance in leading-edge regions, such as reduced freeze stress. This differs from studies that find low quantitative genetic variation at the leading-edge of species ranges, although many such studies examine latitudinal patterns (Frenne et al. 2011; Sniegula et al. 2016). The central garden population did not exhibit local adaptation by any context. Sexton et al. (2016) found that central areas of the *M. laciniatus* range have smaller population densities, but not necessarily lower fitness variance (this study) nor lower genetic variation as measured by neutral genetic markers (Sexton et al. 2016).

1.5.3. Climate predicts fitness variation only at the leading-edge

Climate environment of origin was the only distance metric that predicted fitness, and only at the high garden where fitness and fitness variance was highest among populations. The top three performers in each garden were from populations with similar climates, consistent with other studies that have demonstrated climate as a predictor of fitness variation (Montesinos-Navarro et al. 2011; Housset et al. 2018). Distances among populations, including genetic and environmental, have also been used to predict fitness variation (Bontrager and Angert 2018) and, in many cases, fitness declines with distance from parental sites (Von Wettberg, Huber, and Schmitt 2005). In *Arabidopsis thaliana*, fitness was best predicted by populations having warmer

climates than the climate gardens in which they were grown in, suggesting adaptive lags to recent climate warming (Wilczek et al. 2014). We observed a similar effect in our central garden in which populations from lower elevations had greater fitness (Fig. 3).

There are four major implications from our fitness-climate distance findings. First, selection is likely a driver of isolation by environment patterns detected in plant studies (I. J. Wang 2013; Jiang et al. 2019), corroborating previous findings from this species' range (Sexton et al. 2016). Second, the detection of a climate-distance signal on fitness is dependent on the garden or environment in which genotypes are tested; in our study, this signal was only detected in the high garden in which the highest fitness and fitness variance was expressed. Third, since the adaptive signal was most strongly expressed at the high garden, climate change and, specifically, drought may be inhibiting adaptive signals at lower elevations, suggesting results would vary depending on where common gardens are located across the range (Angert, Bontrager, and Ågren 2020) and the year they are conducted. Finally, given the combined findings of fitness-climate distance and that local populations were never most fit, interpopulation variation and gene flow is critical for maximizing climate niche breadth and adaptive capacity under climate change (Bontrager and Angert 2019; Valladares et al. 2014). Nevertheless, this genetic variation may become muted or lost as climate change stress increases (Dickman et al. 2019).

1.6. Conclusions and implications for climate change responses

Our results signal the potential for future range shifts or contractions in response to global warming conditions, and that the leading-edge regions may already be important refugia for drought-impacted populations as they are predicted to be in the future (Gibson, Marel, and Starzomski 2009). Testing many populations across a species range will provide a more accurate depiction of how different regions respond to continued warming and which regions offer adaptive sources of gene flow. In this regard, rear-edge and leading-edge populations should be viewed as important sources of genetic variation useful for prescriptive and rescue gene flow (Rehm et al. 2015). By observing how fitness varies under different adaptive contexts, time scales, and species ranges, we will better understand and predict adaptation in future climate change events. Finally, unique genotypes with high adaptive plasticity that may be valuable for future climate responses were found across the range, and sometimes in small, peripheral populations. As climate change and drought persists, such populations will continue to be important adaptive resources (Rehm et al. 2015).

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Figures

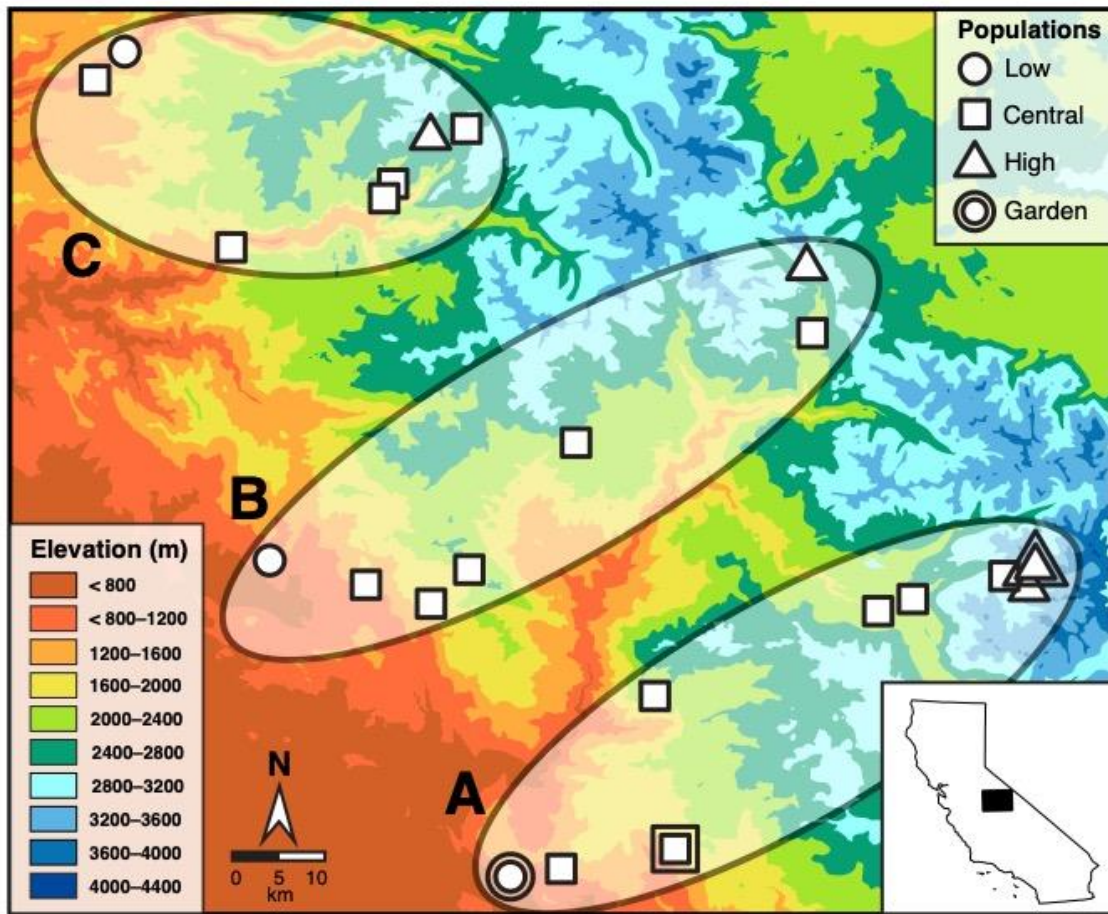


Figure 1. Climate garden experimental design across *M. laciniatus* species range. The common garden experimental design of the 23 populations across the *M. laciniatus* species range (California map cutout). The topography represents both elevation and relative temperature, where lower elevations are warmer (red) and higher elevations are cooler (blue). The three transects (A, B, and C) are represented by the shaded ellipses. Populations are marked as low edge (circles), central (squares), or high edge (triangles). The three garden populations are denoted with an extra border.

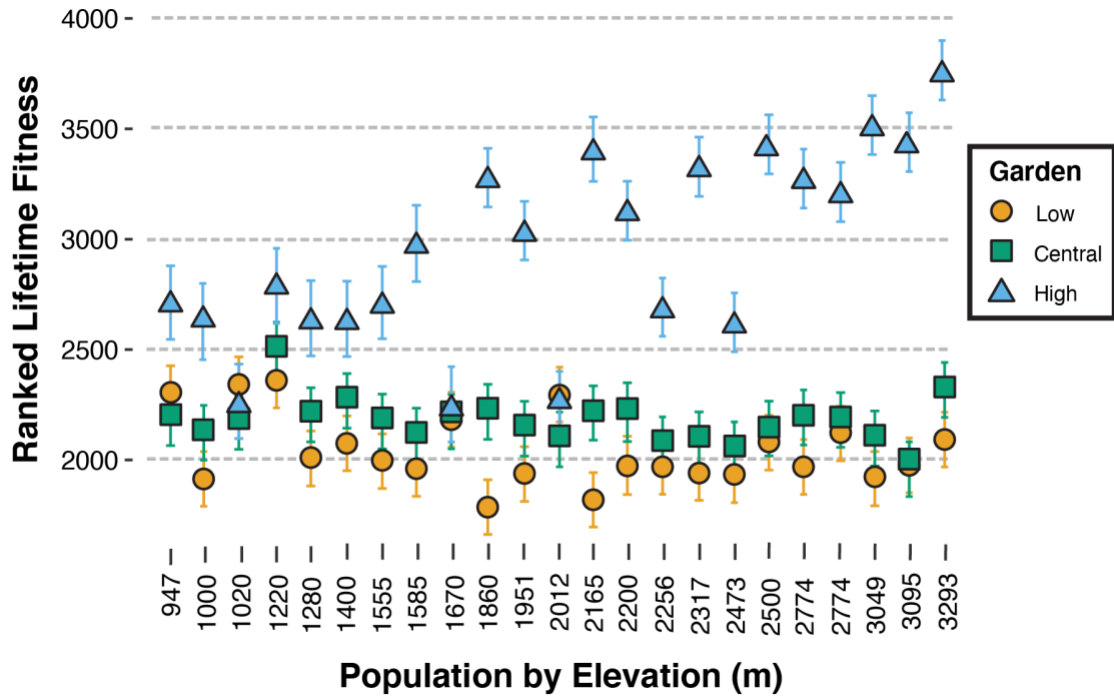


Figure 2. Overall garden trends depicted using the ranked lifetime fitness for each of the 23 populations in the three gardens ordered by elevation. Populations grown in the low garden are denoted in orange circles, populations grown in the central garden in green squares, and populations in the high garden in blue triangles. See Table 3 for details on the populations. The population with the highest fitness is Mount Hilgard (A-HIGH elevation 3293 m).

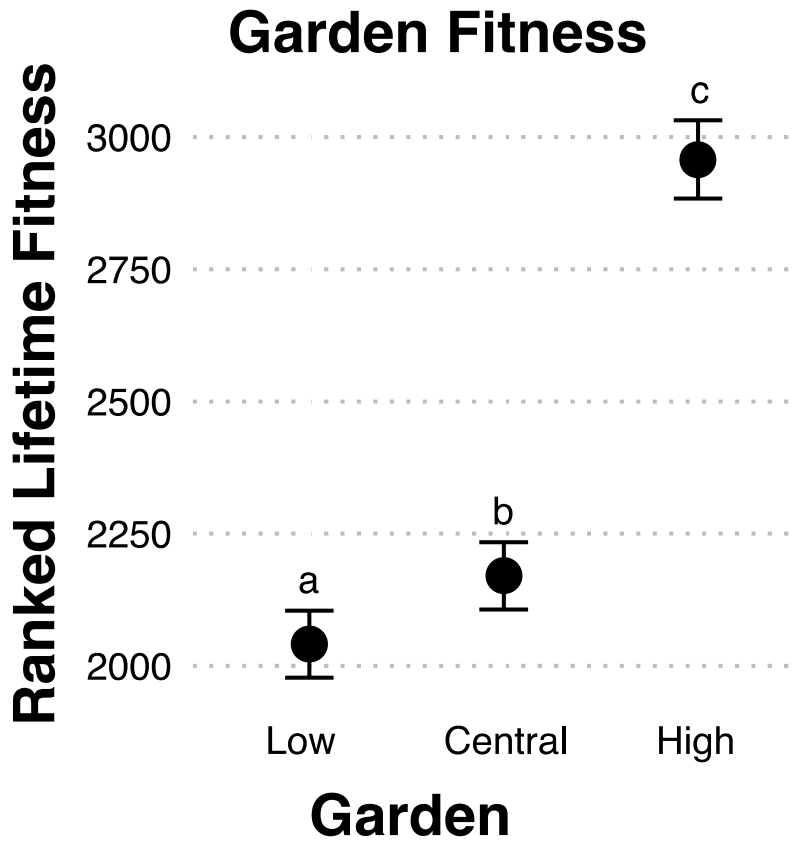


Figure 3. Garden ranked lifetime fitness from logistic regression models (REML). Each garden is significantly different from every other garden (represented by Tukey HSD values abc). Total plants in each garden are as follows: low garden n=1716, central garden n=1710, and high garden n=1281. The model set up is as follows: flowers ~ garden + pop + garden/pop + block(garden).

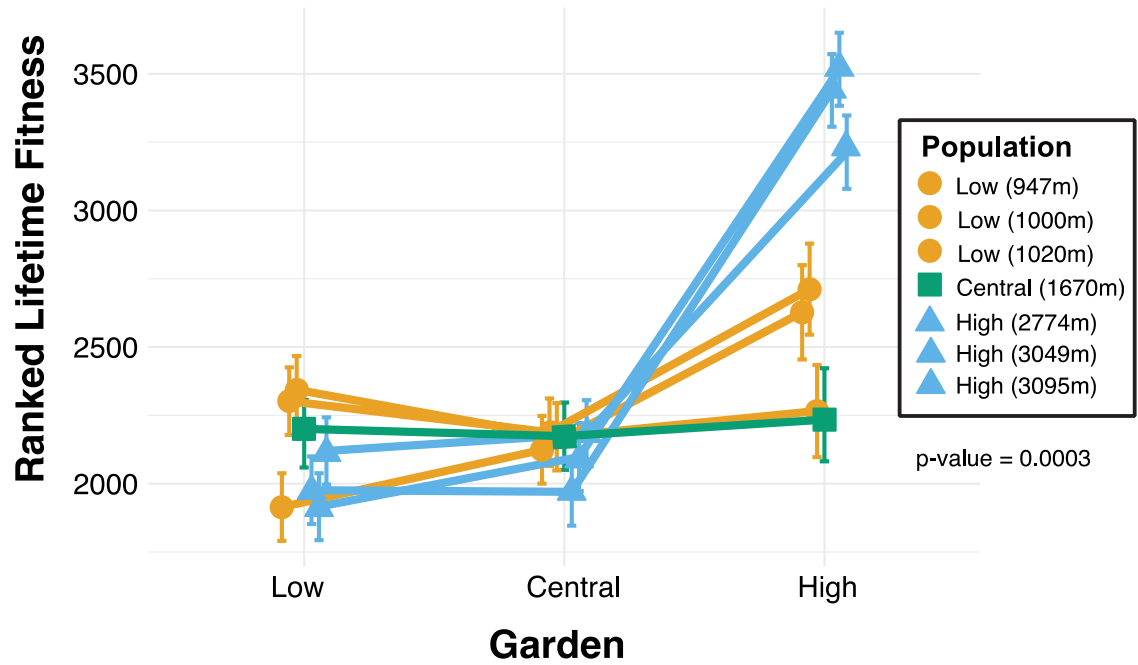


Figure 4. Adaptive trade-offs of selected population norms from the ranked lifetime fitness data of low, central, and high populations in each of the three gardens. Populations are denoted by their elevation (see Table 3 for more details).

Tables

Table 1. REML analysis of lifetime fitness variance, measured by total number of flowers, in each garden along with population and the interaction of population within garden. Block was included as a random effect and nested within garden.

Source	DF	DFDen	F Ratio	Prob > F
Garden	2	205.3	49.382	< 0.0001
Population	22	4444	4.202	< 0.0001
Population*garden	44	4443	5.481	< 0.0001

Table 2. Orthogonal contrasts between three adaptive contexts (HA, SA, and LF) in each of the three gardens (low, central, and high).

Garden	Home vs. Away			
	NumDF	DenDF	F Ratio	Prob > F
<i>Low garden</i>	1	2410	7.992	0.0047
<i>Central garden</i>	1	2369	0.0729	0.7872
<i>High garden</i>	1	1860	84.873	<0.0001

	Sympatric vs. Allopatric			
	NumDF	DenDF	F Ratio	Prob > F
<i>Low garden</i>	1	4444	8.0358	0.0046
<i>Central garden</i>	1	4441	2.6541	0.1034
<i>High garden</i>	1	4510	56.815	<0.0001

	Local vs. Foreign			
	NumDF	DenDF	F Ratio	Prob > F
<i>Low garden</i>	1	4437	1.4225	0.2331
<i>Central garden</i>	1	4437	0.0012	0.9725
<i>High garden</i>	1	4451	18.385	<0.0001

Table 3. Population and transect summary

Position	Transect	Population (Code)	Elevation (m)	Latitude	Longitude	County
Low	A1- LOW	Highway 168 (HWY)	1000	37.03977	-119.40857	Fresno
Central	A2	Peterson Road (PET)	1220	37.04580	-119.35442	Fresno
Central	A3	Grand Bluff (GB)	1670	37.07225	-119.23030	Fresno
Central	A4	Mono Hot Springs (MH)	2012	37.32672	-119.01393	Fresno
Central	A5	Bear Creek Trail (BT)	2256	37.33572	-118.98323	Fresno
Central	A6	Black Point (BP)	2473	37.23844	-119.25990	Fresno
Central	A7	Bear Creek (BC)	2774	37.35800	-118.88063	Fresno
High	A8 A9-	Mount Hilgard Garden (HE)	3095	37.35627	-118.86088	Fresno
High	HIGH	Mount Hilgard (HG)	3293	37.36328	-118.85703	Fresno
Low	B1-LOW	Yosemite Forks I	947	37.38136	-119.66533	Madera
Central	B2	McLeod Flat (MC)	1280	37.35265	-119.56288	Madera
Central	B3	Sand Creek (SC)	1585	37.33163	-119.49264	Madera
Central	B4	Little Shuteye (LS)	1951	37.37436	-119.45190	Madera
Central	B5	Jackass Meadow (JM)	2200	37.50694	-119.33867	Madera
Central	B6 B7-	Devils Postpile (DP)	2317	37.62173	-119.08628	Madera
High	HIGH	Mammoth Edge (ME)	3049	37.69661	-119.09190	Madera
Low	C1-LOW	Poopenaut Valley (HH)	1020	37.921592	-119.819067	Tuolumne
Central	C2	Hetch Hetchy Sign (HS)	1400	37.89389	-119.84903	Tuolumne
Central	C3	Turtle Back Dome (WA)	1555	37.7121	-119.706633	Mariposa
Central	C4	Snow Creek Trail (ST)	1860	37.76630	-119.54213	Mariposa
Central	C5	Snow Creek (SNOW)	2165	37.779555	-119.533982	Mariposa
Central	C6 C7-	Tenaya Lake (TN)	2500	37.8363	-119.455617	Mariposa
High	HIGH	May Lake (ML)	2774	37.8402	-119.49213	Mariposa

Table 4. Multiple regression analysis using spatial, genetic, and climate distances across each of the three gardens.

Low garden				
<i>Term</i>	<i>Estimate</i>	<i>Std Error</i>	<i>t Ratio</i>	<i>Prob> t </i>
<i>Intercept</i>	2089.7506	116.6692	17.91	<.0001
<i>Spatial distance</i>	-69.77021	153.0262	-0.46	0.6536
<i>Genetic distance</i>	-1.45413	3.217869	-0.45	0.6565
<i>Climate distance</i>	19.117267	26.72616	0.72	0.4831
Central garden				
<i>Estimate</i>	<i>Std Error</i>	<i>t Ratio</i>	<i>Prob> t </i>	
<i>Intercept</i>	2147.5421	82.64643	25.98	<.0001
<i>Spatial distance</i>	19.144335	80.63104	0.24	0.8149
<i>Genetic distance</i>	2.0376765	2.008561	1.01	0.3231
<i>Climate distance</i>	-21.02413	17.82674	-1.18	0.2528
High garden				
<i>Estimate</i>	<i>Std Error</i>	<i>t Ratio</i>	<i>Prob> t </i>	
<i>Intercept</i>	3209.1043	184.1932	17.42	<.0001
<i>Spatial distance</i>	399.78334	281.4766	1.42	0.1717
<i>Genetic distance</i>	7.4181331	5.282357	1.4	0.1764
<i>Climate distance</i>	-239.9585	55.02313	-4.36	0.0003

CHAPTER 2

Rules of plant species ranges: applications for conservation strategies.

2.1. Abstract

Earth is changing rapidly and so are many plant species' ranges. In this review, we synthesize eco-evolutionary patterns found in plant range studies and how their study can inform our understanding of such processes as they respond to climate change. We discuss whether general biogeographic “rules” are reliable and how they can be used to develop adaptive conservation strategies of native plant species across their ranges. Rules considered include 1) factors that set species range limits and promote range shifts; 2) the impacts of biotic interactions on species range limits; 3) patterns of abundance and adaptive properties across species ranges; 4) patterns of gene flow and its implications for genetic rescue, and 5) the relationship between conservation risk and range size. We conclude by outlining areas of research to better our understanding of the adaptive capacity of plants under environmental change and the properties that govern species ranges. We end by summarizing and evaluating a set of species range rules to inform future conservation and management decisions. We advise conservationists to extend their work to specifically consider peripheral and novel populations and also species with small ranges. Finally, we call for a global effort to identify, synthesize, and analyze prevailing patterns or rules in ecology.

2.2. Introduction

Since the early studies of biogeography (von Humboldt and Bonpland 1807), scientists have put forward a variety of ecological hypotheses, some of which have become entrenched or taken as granted enough to be considered “rules” (von Humboldt and Bonpland 1807; Connallon and Sgrò 2018; Liu et al., 2020). In 2016, the National Science Foundation (NSF) released a call to action urging researchers to discover and study “rules of life” in order to better develop a predictive understanding of how properties of living systems (i.e., environment, phenotype, etc.) interact (NSF 2016). A central issue is predicting how species will respond to climate change. Accelerated biodiversity loss (Urban 2015) and disruptions to global patterns of community assembly (Trisos et al., 2020) are already underway. Now, we struggle to swiftly conserve biological diversity by understanding how species respond to rapid change at the geographic scale, and whether there are broad geographic patterns or phenomena that may lead to enhanced conservation and ecosystem management. While many species are likely to become endangered or go extinct, targeted conservation measures can save many species from this fate. Every species has a story to tell, and its geographic range can provide important insights as to how it can be conserved, managed, and restored.

A core component of ecology is to recognize and understand patterns in nature (MacArthur 1972). Whether or not ecological or evolutionary patterns can serve as reliable rules is debatable since few strict laws exist in ecology, but many general ones may (Lawton 1999; Temperton et al., 2013; Dickey et al., 2021). Broadly, we consider rules to be effective, predictive hypotheses, but like any good rule, they will be broken due to the idiosyncrasies among species and the vast variation life represents. Moreover, untested rules could be useful if verified. Knowing whether species ranges provide predictive ecological information, such as in patterns of abundance,

distribution, and interactions, or evolutionary rules, such as in patterns of selection, drift, and gene flow, would allow more informed management decisions at large geographic scales (Pelletier et al. 2018).

In this article, we address various paradigms within five eco-evolutionary realms of species ranges—some long-held—that have either been empirically tested or have been hypothesized as important for species conservation and biogeography. We acknowledge that there are many more potential biogeographical “rules” relevant to species ranges that exist. We also acknowledge that these patterns are not independent of each other and overlap. We highlight new and emerging findings throughout, including needed areas of future research within each section. Although this topic applies to all forms of life, we focus our examples and conservation prescriptions primarily on plants, given their important primary roles as producers across global ecosystems. All ecosystems depend on their plant communities to influence a suite of essential ecological processes including resource use efficiency and biomass production and recycling (Cardinale et al. 2011). Thus, managing for healthy, resilient plant communities is of primary concern for ecosystem conservation and restoration. We end the paper summarizing our general findings for each rule and its associated conservation implications (Table 1).

2.3. Are plant species range limits real?

2.3.1. The essence of plant species ranges in a changing climate

Plants respond to stress and rapid change through several mechanisms. As sessile organisms in terrestrial ecosystems, plants much employ a local scale of adaptation and plasticity (Alpert and Simms 2002; Palacio-López et al., 2015). However, there is also a large geographic scale at which plant populations vary in their attributes, environments, modes of communication among populations (i.e., gene flow), and interaction with abiotic and biotic factors (Darwin 1859; Griggs 1914; MacArthur 1972; Brown 1984). This scale is commonly known as the species range (Gaston 2003). Species ranges are assumed to be a projection of niche availability in geographic space (Sexton et al., 2009). In other words, the assumption is that *range limits are niche limits*, beyond which populations tend to decline along with their available niche attributes. Whether range limits actually are niche limits is a complex and nuanced question. Support for this potential rule has been found through the use of transplant experiments and species distribution models (Hargreaves et al., 2014; Lee-Yaw et al., 2016; Connallon and Sgrò 2018; Bayly and Angert 2019; Ackerly et al., 2020). Both approaches have strengths and weaknesses, and they will vary among study systems under climate change (Araújo and Peterson 2012; Ehrlén and Morris 2015; Greiser et al., 2020). Nevertheless, with respect to species ranges, climate edges may not always correlate with geographic edges due to climate heterogeneity and geographic scale effects on climate properties (Oldfather et al., 2020). Thus, range limits can be real at particular scales, but are also constantly in motion due to climate shifts over time (Sexton et al., 2009; Halbritter et al., 2018).

2.3.2. Plant species ranges on the move

In response to global warming, plant species are shifting, contracting, and expanding into new territories beyond their range edge, poleward, and into refugia characterized by temperate conditions (Hampe and Petit 2005; J. Lenoir et al. 2008; Freeman et al. 2018; Lafontaine et al. 2018; Meng et al. 2019; Miller et al. 2020; Reed et al. 2021; Mamantov et al. 2021). Ecosystems with extreme climates (e.g., alpine, desert, and wetlands) are changing rapidly and are critical

study ecosystems to project future range shifts and identify vulnerable populations. A general rule is that, in most cases, plant species range limits are in motion and some are moving quickly.

In alpine systems, cold-edge populations are under pressure to shift and expand, if possible, in elevation and latitude while warm edges are contracting uphill to avoid rising temperatures (Hampe and Petit 2005; Gottfried et al. 2012; Kopp and Cleland 2014; Mathiasen and Premoli 2016; Reed et al. 2021). The response of alpine populations varies depending on the surrounding climatic regime and the paleontological origin of the taxon. High-elevation populations that already have contracted ranges from previously adapting to glaciation periods are at-risk of contracting to a point of extinction (Dullinger et al. 2012). In a recent meta-analysis of alpine range shift studies, Mamantov et al. (2021) found that low-elevation species are moving upslope much farther than high-elevation species, and that 28% of species shifted downslope in response to climate change. Downslope range shifts, though not as common as upslope movement, have been confirmed in a number of plant species range studies (Parmesan and Yohe 2003; Lenoir et al., 2010; Chen et al., 2011).

In deserts, plants are moving in both directions and some are not moving at all (Abella et al., 2019). Plants that rely on a chilling period for budding are shifting upslope and contracting their ranges toward cooler temperatures, as predicted for Southwestern white pine, *Pinus strobiformis* (Pinaceae) (Shirk et al., 2018). Precipitation reductions in deserts are also driving ranges upslope and constraining high-elevation populations (Guida et al., 2014). Abella et al. (2019) found that an unexpected 41% of desert plant studies demonstrated downslope range movements in response to both biotic and abiotic interactions. Nevertheless, some desert shrub plants like it hot and their ranges are incredibly resilient to climate changes (Tielbörger and Salguero-Gómez 2014).

Finally, in wetlands, mangroves, *Avicennia* (Acanthaceae), are a prime example of quickly expanding species ranges. As sea levels rise, mangroves follow the water along coastlines at a global scale, replacing existing habitats (e.g., salt marsh ecosystems) as they expand (Saintilan et al., 2014).

2.3.3. Future directions

Species range limits are real, even if indefinite depending on the scale of examination, are shifting in response to climate change (Reed et al., 2021). They continue to offer a compelling spatial context for conservation research (Serra-Diaz and Franklin 2019). Through the use of transplant experiments and robust species distribution models, key drivers and patterns across species ranges can be uncovered, (Franklin et al., 2017). A general rule is that range limits generally correspond with niche limits, but dispersal limitation does cause discordance between niche limits and range limits (Stanton-Geddes et al., 2012; Lee-Yaw et al., 2016; Sexton and Dickman 2016; Cross and Eckert 2020). Range-wide effects of climate, primarily temperature and precipitation, should be studied in combination with species interactions in different systems and across large and small geographical scales in order to develop accurate predictions for conservation and management strategies. Research on how to best facilitate new colonization at the leading edges of species ranges and to conserve rear edges of species ranges is needed (Kottler et al., 2021).

2.4. Do biotic interactions influence patterns across species ranges?

2.4.1. Evidence for the importance of biological interactions on species ranges

Biotic interactions are an integral component of a species niche (Peay 2016; Phillips et al., 2020) and are essential to consider in range limit contexts (Freeman et al., 2018; Hargreaves et al., 2014) and species distributions (Hille Ris Lambers et al., 2013). For millennia, global climate change has threatened the integrity of these important interactions (Blois et al., 2013), altering their dynamics (Hamann et al., 2021), disrupting their co-evolution (Parmesan 2006b), and influencing their role in facilitating climate-induced range shifts (Hille Ris Lambers et al., 2013). Darwin (1859) was the first to report patterns of biotic interactions across species ranges and concluded that biotic interactions tend to set range limits in abiotically benign areas, often referenced as the warmer edge of species distributions where competition may be intense. Since then, this rule has received extensive support (MacArthur 1972; Brown et al., 1996; Gaston 2003; Normand et al., 2009; Paquette and Hargreaves 2021) and is the leading hypothesis of the majority of research on patterns of biotic interactions across species ranges (Barton 1993; Bullock et al., 2000; Scheidel and Bruehlheide 2001; Holt and Barfield 2009; Louthan et al., 2015). Nevertheless, it is not a general rule for setting plant distributions.

Contrary to Darwin's theory, both positive (i.e., pollination, facilitation, and mutualism) and negative (i.e., competition, predation, herbivory, and parasitism) interactions influence plant species range limits, in warm and cold climates (Afkhami et al., 2014; Louthan et al., 2015; Benning et al., 2019; Benning and Moeller 2021a). More importantly, range limit patterns can be explained with a combination of interactions (Ettinger and Hille Ris Lambers 2017); however, most studies focus on one or a limited set of interactions often due to logistical constraints. For example, when testing fitness in *Clarkia xantiana* ssp. *xantiana* (Onagraceae) beyond its range, fitness decreased due to the lack of positive interactions (e.g., pollinators) and presence of negative interactions (e.g., herbivores) [Benning and Moeller 2019]. In this example, when pollen was supplemented and herbivores removed, fitness beyond the range tripled, demonstrating the importance of these interactions for range shifts and expansions. Furthermore, microbial and human interactions, and their impacts on plant distributions, have garnered comparatively little attention and are in need of more research. Microbes are an integral part of a plant's suitable habitat (Peay 2016) and contribute both to defining suitable limits as well as hindering expansion (Benning and Moeller 2019). Given their clear ecological importance, it is essential to include an array of biotic interactions when predicting species range patterns, especially in climate change models (Van der Putten et al., 2010). Nevertheless, this is challenging since interactions networks can be vastly complex.

2.4.2. Both positive and negative interactions matter

The prevailing paradigm is that negative interactions, particularly competition, drive warm-edge range limits (Sexton et al., 2009; Schemske et al., 2009; Louthan et al., 2015). Although this is often true, there are examples where other negative interactions, such as seed predation, influence cold-edge expansion (Brown and Vellend 2014; Hargreaves et al., 2019). Herbivory is usually categorized as a negative interaction, and sets limits with susceptible plants, such as those with delayed phenology and decreased fitness (Louda 1982; Lau et al. 2008; Benning et al. 2019). In California serpentine environments, Lau and colleagues (2008) found that increased herbivory, influenced by edaphic factors, reduced survival and persistence in the native *Collinsia sparsiflora* (Plantaginaceae), limiting the species range (Lau et al. 2008). Humans are also negatively influencing landscape alterations through habitat fragmentation and deforestation, which directly reduce species ranges (Chase et al., 2020), constraining a plant's capacity to track and expand

into their habitable niche (Chardon et al., 2019). Fragmentation can decrease range size and increase spatial isolation of plant populations, which may place them at risk of genetic erosion through drift, inbreeding depression, reduced gene flow and the extinction of local genotypes (Wright 1950; Young et al., 1996; Jump and Peñuelas 2006; García-Fernández et al., 2012). Depending on which species is under consideration, negative biotic interactions can limit or facilitate expansion of a plant species range; herbivory of one plant species may actually be beneficial to another plant. For instance, in warming tundra, herbivory protected native populations from competition (Eskelinen et al., 2017), allowing range expansion of the tundra community (Kaarlejärvi et al., 2017). Given the potential for negative interactions to limit sensitive plant species ranges, species distribution models should factor for a variety of biotic interactions (de Mesquita et al. 2016; Miele et al. 2021).

More recently, positive interactions have emerged as relevant and important for consideration in climate-range research. Mutualisms are abundant in stressful conditions (Callaway et al., 2002), affect plant fitness (Lau and Lennon 2012), mitigate climate stress on species distributions (Bulleri et al., 2016), and influence local adaptation (Pickles et al., 2015). Facultative mutualisms can facilitate expansion of species ranges into stressful habitats (Afkhami et al., 2014; Millar and Bennett 2016; Benning and Moeller 2021b) in addition to novel environments (Crotty and Bertness 2015; de Mesquita et al. 2020). An example of a key mutualism that encompasses the plant realized niche are pollinators (Phillips et al., 2020). In general, pollinator species distributions are strongly linked to their visiting plant geographic ranges (Duffy and Johnson 2017). In a four-year study of *Clarkia* x. ssp. x., pollinator availability declined with distance from the center of the plant range, contributing to the constraint of the species range limit (Moeller et al. 2012). Climate change continues to reduce the quantity and quality of pollination services globally (Burkle et al., 2013) leading to increased limitation to plant species ranges (Chalcoff et al., 2012; Moeller et al., 2012).

Microbes are becoming better known and understood as important mutualists of plants. In the endangered *Hypericum cumulicola* (Hypericaceae), soil microbes boosted population growth, persistence, and allowed the plant to expand into previously uninhabitable environments (David et al., 2019). Similarly, soil microbes in the Rocky Mountains interacted with the alpine bunchgrass, *Deschampsia cespitosa* (Poaceae), to allow the plant to grow in new, unvegetated areas beyond the range, suggesting the significance of microbes in climate-induced range expansions (de Mesquita et al. 2020). In the absence of mutualistic soil microbes beyond the species range edge, host plants experienced reduced fitness, limiting this expansion capacity (Benning and Moeller 2021a). Climate change can alter plant-microbe interactions in a variety of ways including changing microbial species ranges, community composition, functionality, and eventual fitness and occurrence of host plant species (Rudgers et al., 2020). Another important, and more obvious, mutualist

A general rule that can be gleaned from these examples is that mutualisms can determine range boundaries and range expansions at any range limit, warm or cold. In light of their importance, more research on mutualisms across species ranges is needed and mutualisms should be considered in predicting and protecting species range limits.

2.4.3. Future directions

Biotic interactions are more important to range-wide distribution patterns than previously appreciated. Mutualisms matter when considering plant distributions in a changing climate, especially when introducing a plant to a new habitat or predicting future range shifts (Hille Ris

Lambers et al., 2013; Freeman et al., 2018; Benning and Moeller 2021a). Understanding microbial community structure across species ranges will provide a better view of mutualist-mediated niche dynamics, especially as it relates to expansion in response to environmental pressures from climate (Rolshausen et al., 2020). Climate-conscious restoration efforts should aim to characterize microbial communities and identify those that confer ecosystem resistance and resilience to climate disruption (Koziol et al. 2018; Rudgers et al. 2020). Assisted migration efforts are an important conservation strategy and can help plants occupy novel habitable regions (Hällfors et al., 2017), but traditionally ignore biotic interactions and how they might influence transplanted populations (Bucharova 2017).

There is no doubt that future range limit research must include both negative and positive biotic interactions when modeling species distributions and predicting range shifts under climate change (Araújo and Luoto 2007; Kissling et al. 2012; de Mesquita et al. 2016; Miele et al. 2021). Questions that explore the interplay and evolution of abiotic and biotic interactions across species realized and fundamental niches, and under various conditions should be prioritized. Furthermore, models should include thorough biological data (Giannini et al., 2013) and test the impact of both abiotic and biotic interactions on species distributions (de Mesquita et al. 2016). A recent model has been suggested by Miele and colleagues (2021) which combines species interaction metanetwork data with environmental data and species occurrences to disentangle the effects of abiotic and biotic interaction on species distributions (see ELGRIN model, Miele et al., 2021). Overall, species interactions are largely under-researched, especially across large biogeographic scales or in remote or unique habitats. In general, more data need to be collected to incorporate important interactions across plant species ranges (Wisz et al., 2013).

2.5. Do different regions of a species range hold predictable adaptive or resilient properties?

2.5.1. The abundant center hypothesis is not a general rule

Whether specific regions of species ranges (e.g., peripheral, central, warmer, older, etc.) differ in ecological and evolutionary properties is an essential question for guiding management of plant populations under global change. As discussed earlier, range limits are niche limits and the dynamics between and within these different regions of plant species' ranges have several important paradigms to consider in order to address this question. For example, Lesica and Allendorf (1995) proposed that peripheral regions of species ranges should harbor genetically unique and isolated genotypes that are useful for conservation purposes. During range contraction events, peripheral populations are just as likely as central populations to be where the range contracts towards (Channell and Lomolino 2000), an important consideration for populations on the move.

The center of a species range is home to classic paradigms, such as the abundant center hypothesis (ACH) which posits that species will be most abundant at the center of their ranges and decrease in both size and density towards range margins (Brown 1984); nevertheless, this does not appear to be a general rule (Sagarin and Gaines 2002; Sexton et al., 2009; Dallas et al., 2017; Pironon et al., 2017). Alternatively, the niche-distance-abundance (NDA) hypothesis proposes that species will be most abundant at the center of their niche, the multidimensional space of environmental factors that a species can occupy (Dallas and Hastings 2018; Osorio-Olvera et al., 2019). This hypothesis has mixed support, (Dallas et al., 2017; Weber et al., 2017; Dallas and Hastings 2018; Jiménez-Valverde et al., 2021). A recent study of the endemic Iberian Peninsula snapdragon, *Antirrhinum lopesianum* (Plantaginaceae) found a negative relationship

with abundance and distance from the species' niche centroid (Hernández-Lambraño et al., 2020). Similarly, an analysis of European vascular plants found evidence of a negative niche distance-abundance relationship, but the relationship was weak and highly variable (Sporbert et al., 2020).

A growing body of research suggests that the history of a population is more indicative of its patterns of abundance and genetic variation than contemporary measures (Abeli et al., 2014; Koski et al., 2019; Cruz-Nicolás et al., 2020). Even so, there are still many examples where range position, niche position, and abundance do not correlate (Sagarin and Gaines 2002; Eckert et al., 2008; Sexton et al., 2009; Pironon et al., 2017; Sexton et al., 2016; Dallas et al., 2017; Kennedy et al., 2020). In some cases, plant population density actually increases towards geographic limits (e.g., Sexton et al., 2016). In this vein, it is important for researchers to continue to question the relationship between abundance, niche, and range across various taxa in climate change contexts.

2.5.2. Genetic variation determines adaptive potential

In order to conserve threatened species, it is essential to identify populations that are most vulnerable and those that have the potential to adapt to changing climate conditions. Adaptive potential is determined by genetic variation, which allows a population to respond to changes in the environment with genetic change in trait values that increases fitness (Pennington et al., 2021). Quantitative genetic variation (QGV) can lead to phenotypic variation and is a measure of the evolutionary potential and ability for species to adapt to rapid environmental change (Rice and Emery 2003; Conner and Hartl 2004). In general, QGV is higher in larger populations (Hoffmann et al., 2017) and neutral genetic variation has been shown to decline in many species towards the peripheries of species ranges (Eckert et al., 2008; Pironon et al., 2017) and niches (Lira-Noriega and Manthey 2014). This decline appears consistent with the ACH but could also indicate an *edge effect* phenomenon driven by increased drift, reduced gene flow, and increased selection towards range limits (Pennington et al., 2021). Nevertheless, neutral genetic variation and QGV are often uncorrelated (McKay and Latta 2002). Given that the ACH is not a general rule, it thus follows that QGV, and therefore adaptability, may not be highest in central regions of species ranges (Pennington et al., 2021).

Adaptive potential does follow a general rule—populations with larger effective population sizes (N_e) tend to be higher in QGV (Hoffmann et al., 2017), and so are important for conservation. This rule does have caveats. Small effective populations, especially those in adverse conditions (e.g., stressful soils), may harbor unique variation that is also important for conservation (Ellstrand and Elam 1993). Further, older populations and populations that may have been glacial refugia may also retain important variation (Hampe and Petit 2005) but may not have large numbers of individuals in their populations (N). The evidence would seem to suggest that larger, older, and unique populations are more likely to be important sources of QGV, and these may occur anywhere within species ranges.

2.5.3. Local adaptation follows adaptive potential

Local adaptation occurs throughout species ranges and is largely driven by climate (Anderson and Song 2020), suggesting that adaptive potential can be great throughout species ranges. Local adaptation has been observed in myriad species and results in differential responses to climate change across species ranges (Hargreaves et al., 2014, 2019; Peterson et al., 2019; Torres-Martínez et al., 2019; Anderson and Wadgymar 2020; Patsiou et al., 2020). Peripheral populations may be critical when considering climate-driven fitness variation and conservation (Lesica and Allendorf 1995; Channell and Lomolino 2000; Macdonald et al., 2017; Papuga et al., 2018) because they are often locally adapted to more extreme habitats and are home to

phenotypes that are not expressed in other areas of the range (Moeller et al., 2011; Papuga et al., 2018; Hargreaves and Eckert 2019; Angert et al., 2020; Morente-López et al., 2021). For example, in *Mimulus laciniatus* (Phrymaceae), an endemic Sierra Nevada annual herb, warm-edge and cool-edge populations both show signatures of local adaptation with the fitness variation leaning uphill towards higher elevations, suggesting that cooler portions of species ranges may become modern refugia (Shay et al., *in prep*). As climate change alters local adaptation (Anderson and Wadgymar 2020), patterns of variation and abundance may change. Resurrection studies are a useful approach to capture historical patterns and contrast them with contemporary patterns, to quantify how populations are responding to dramatic change. Recent resurrection studies have captured varying phenological change in response to climate change (Franks et al., 2018; Dickman et al., 2019; Vitipil and Sheth 2020; Wooliver et al., 2020; Anstett et al., 2021; Kooyers et al., 2021). Future studies may reveal further important patterns of population change in response to climate change.

2.5.4. Future directions

Patterns of adaptive potential and local adaptation, especially at the range edges, remain an important focus for understanding how populations respond to rapidly changing climate. Nevertheless, peripheral populations are understudied and, as a result, underprotected (Caissy et al., 2020). Local adaptation is widespread and varies across species ranges and peripheral populations are equally important to consider in conservation contexts as central populations. SDMs that incorporate local adaptation, such as Δ TraitSDMs (Garzón et al., 2019), should be considered when predicting species climate adaptation across geographic scales. Small populations in unique environments and older populations may harbor important, but underexplored, genetic variation. The abundant center-hypothesis is not a rule and, instead, a niche-abundance relationship deserves further study. A deeper exploration of the relationship between niche, range, and abundance patterns across plant species ranges will provide better predictions of important populations for conservation. Finally, resurrection studies are a great tool to test adaptive potential across species ranges and should be used to assist in conservation and management of plant populations. In general, these questions need to be explored in more systems as these patterns vary widely by species (Angert et al., 2020; Reed et al., 2021). We thus strongly encourage researchers to create eco-evolutionary projects that focus on native plant taxa that have not been studied or have been traditionally understudied.

2.6. Are there predictable effects of gene flow on adaptation across species ranges?

2.6.1. Gene flow across species ranges

Gene flow is widely recognized for both its enhancement and inhibition of adaptation, and it is one of the best evolutionary tools for managing species range responses to climate change (Aitken and Whitlock 2013; Sexton et al., 2014; Smith et al., 2014; Bontrager and Angert 2019; Kottler et al., 2021). Thus, understanding predominant patterns or rules of gene flow across species ranges is a key tool for a conservation biologist. Prescribing gene flow is also a game of chance, of course (Bell et al., 2019), but it can be done with good planning and strategy (Sgrò et al., 2011). Patterns of gene flow that are prevalent in the literature include environmental, geographical or spatial, and temporal (Sexton et al., 2014; Peters and Weis 2019). At species range limits, gene flow can enhance genetic variation to expand a niche (Holt and Gomulkiewicz 1997), or gene flow may potentially limit or collapse a range (Kirkpatrick and Barton 1997). The lack of gene flow is also theorized to set range limits in marginal populations that have small population sizes and high rates of inbreeding depression (Antonovics 1976; Hoffmann and Blows

1994; Morente-López et al., 2021). When interactions of different gene flow patterns are considered (e.g., environmental and geographical), eco-evolutionary patterns and consequences of these patterns are more clearly understood across species ranges (Sexton et al., 2014; Bontrager and Angert 2019; Nadeau and Urban 2019).

2.6.2. Isolation by distance is prevalent in plants

Dispersal and dispersal limitation are key features determining plant ecology, evolution, and distributions. Selection or habitat adaptation notwithstanding, dispersal limitation leads to decreased gene flow and increased drift, resulting in increased genetic isolation with geographic distance (Dobzhansky 1937; Wright 1943). Thus, genetic isolation by distance (IBD) has been the most prevalent pattern of gene flow observed across plant species ranges (Moyle 2006; Eckert et al., 2008; Orsini et al., 2013; Sexton et al., 2014; Torres-Martínez et al., 2019; Twyford et al., 2020). An obvious caveat of observed IBD patterns is that they ignore the importance of environmental variation and the effects of local adaptation on gene flow, which can be autocorrelated with spatial distances. High dispersal ability usually promotes high genetic variation in plants (Hamrick and Godt 1996; Lander et al., 2021); however, this variation can affect populations differently. In one plant family alone, Fagaceae, there are examples of little to no effect of gene flow from long-distance dispersal (Moracho et al., 2016) and high adaptive differentiation from short-distance or limited dispersal (Deacon and Cavender-Bares 2015; Gauzere et al., 2020). Importantly, the effects of IBD (i.e., dispersal ability and increased genetic isolation with distance) on plant populations can be exacerbated by habitat fragmentation resulting from human-driven habitat destruction (e.g., agriculture, urbanization, or harvesting of natural resources). Habitat fragmentation can disrupt gene flow among contiguous populations and erode genetic diversity by decreasing the effective population size and increasing the spatial isolation of populations, resulting in genetically depauperate populations subject to increased genetic drift, inbreeding depression, reduced gene flow and immigration rates (Young et al., 1996; Couvet 2002; Aguilar et al., 2019). Where IBD is detected in plants under conservation consideration, even at very small spatial scales, (Gauzere et al., 2020), genetic variation necessary to respond to rapid environmental change may be more limited and may require prescriptive or rescue gene flow (see below) from distant sources (Sexton et al., 2014).

2.6.3. Isolation by environment is also common in plants

Another gene flow pattern is the movement of alleles between populations from similar habitats or environments, creating a pattern known as isolation by environment (IBE) or “ecological isolation” (Dobzhansky 1937; I. J. Wang 2013). IBE is now known to be prevalent across species ranges and is important for climate-focused research and conservation of populations under climate change (Sexton et al., 2014; Wang and Bradburd 2014; Morente-López et al., 2021). IBE scenarios are driven by environmental heterogeneity across species ranges and are caused by natural selection or non-random mating among similar environments (Hirao and Kudo 2004; Temunović et al., 2012). A recent example of IBE across a plant species range occurs in Asian temperate deserts with a broad-leaved evergreen shrub, *Ammopiptanthus mongolicus* (Fabaceae). In this example, landscape heterogeneity in precipitation clearly promotes IBE (Jiang et al., 2019). Gene flow under IBE is often correlated with geographic distance (I. J. Wang and Bradburd 2014). Shafer and Wolf (2013) found that when IBD is accounted for, environmental variation explained roughly 5% of the total genetic differentiation among plant populations. Currently, however, most studies find that plant genetic variation is explained by a combination of IBE and IBD (Sexton et al., 2014; Moran et al., 2017; Bontrager and Angert 2019; Nadeau and Urban 2019; Da Silva et al., 2021). For example, long distance seed dispersal prevented snowmelt-driven isolation in *Salix herbacea* (Salicaceae) (Cortés et al., 2014). Future studies of

gene flow patterns will need to combine and parse the effects of environment and distance, which are often autocorrelated, and to sample a wide range of environmental variables (biotic and abiotic) across species ranges to isolate drivers of IBE. Where IBE is detected in plants under conservation consideration, genetic variation necessary to respond to rapid environmental change may require prescriptive or rescue gene flow from different (i.e., warmer) environments (Sexton et al., 2014).

Climate change has, in most cases, led to an earlier shift in plant flowering phenology (Menzel et al., 2006; Wolkovich et al., 2012; Dai et al., 2014; Leinonen et al., 2020), which influences both plant distribution patterns (Parmesan and Yohe 2003; Chuine 2010; Song et al., 2021) and gene flow events (Schuster, Alles, and Mitton 1989; Wadgymar et al., 2015). Isolation by phenology (IBP) is a form of IBE and occurs when phenology differences (e.g., flowering time) divide populations into different mating pools (Peters and Weis 2019). Climate warming is leading to more uniformity in phenology, reducing IBP (Franks and Weis 2009; Chen et al., 2018; Vitasse et al., 2018). Unfortunately, genetically-based evolution of phenology may happen too slowly to rescue populations from rapid climate change (Vtipil and Sheth 2020). In general, the overall effect of climate-change imposed IBE may be to dampen or swamp genetic differentiation due to contraction of flowering windows. Future work is needed to examine the impact of climate shifts on phenology across large geographic gradients and in assisted migration conservation efforts.

2.6.4. *The myth of gene swamping in the creation of range limits*

Maladaptive gene flow as a mechanism for stalling or degrading adaptation is known as gene swamping and has been invoked as a mechanism for creating range limits (Haldane and Ford 1956; Kirkpatrick and Barton 1997). This long-standing paradigm assumes that gene swamping reduces fitness and limits local adaptation at the range edge by flooding the region with genes adapted to different conditions, suppressing locally beneficial genes (Antonovics 1976; García-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Kawecki 2008; Lopez et al., 2008). Such reduction in fitness from mating genetically divergent populations (i.e., outbreeding depression) has been observed in several plant species (Fenster and Galloway 2000; Montalvo and Ellstrand 2001; Oakley et al., 2015). For example, Montalvo and Ellstrand (2000) documented outbreeding depression in deerweed as a result of crossing *Lotus scoparius* var. *scoparius* and *L. s.* var. *Brevialatus* (Fabaceae) and recommend caution when mixing seeds for restoration. Even so, gene swamping does not appear to be a reliable rule for explaining range limit evolution. A recent review found little evidence to support this assumption for two reasons (Kottler et al., 2021). First, gene flow is not universally asymmetrical from the centers of ranges to their peripheries, likely due to the fact that the abundant center hypothesis is not a rule, an assumption that gene swamping relies on. Second, in the very few empirical cases where gene flow has been experimentally introduced to plant populations at the edge of a species range, the results have so far been overwhelmingly positive on edge populations (Kottler et al., 2021). This is likely due to the fact that edge populations may suffer from limited effective population sizes (drift), isolation, and strong selection (Hoffmann and Blows 1994; Eckert et al., 2008; Kottler et al., 2021; Pennington et al., 2021).

2.6.5. *The potential of genetic rescue in conservation*

An alternate hypothesis to gene swamping maintaining range limits is genetic rescue, where genetic variation from outside populations is beneficial to populations suffering from inbreeding depression (Tallmon et al., 2004; Hedrick et al., 2011). Gene flow can benefit peripheral populations through the introduction of environmental specific alleles that improving fitness (Sexton et al., 2011; Bontrager and Angert 2019). When crossing *Mimulus laciniatus* between

and within warm-limit and central-limit populations, Sexton et al. (2011) found that fitness increased at the warm-limit. Similarly, Bontrager and Angert (2018) were investigating patterns of genetic diversity across the *Clarkia pulchella* (Onagraceae) species range in the Pacific Northwest when they were surprised to find fitness boosts in peripheral populations from gene flow, even though they were expecting gene swamping.

Small populations are particularly threatened by habitat fragmentation (Haddad et al., 2015) and restoring gene flow through genetic rescue is a viable option for protecting those species' ranges (Bell et al., 2019). Genetic rescue is underappreciated and a useful tool for conservation of endangered species (Whiteley et al., 2015). However, while discussed often in the literature, genetic rescue is rarely used as a conservation strategy (Frankham et al., 2017; Robinson et al., 2020) and, as previously mentioned, the fitness advantages of gene flow can be mixed due to outbreeding depression. The use of genetic rescue as a conservation and management tool is in its infancy and many eco-evolutionary aspects still remain to be understood (Bell et al., 2019). Yet, this strategy shows incredible promise, especially in the current era of genomics (S. W. Fitzpatrick and Funk 2021), and deserves immediate attention in range-wide contexts.

2.6.6. Future directions

Gene flow is a strong evolutionary force shaping adaptation across species ranges, and range limits can be heavily influenced by gene flow events in plant systems. IBD, driven by dispersal limitation and drift, and IBE, driven by selection and non-random mating, are prevalent patterns of gene flow across plant species ranges. When IBD is combined with other types of gene flow (e.g., IBE), it can synergize genetic isolation (Peters and Weis 2019). IBP, as a form of IBE, is likely to be a common phenomenon in plants, however, it is still poorly understood across species ranges and for its ramifications under climate change. Gene swamping as a creator of range limits is not a rule, as gene flow often has beneficial effects on local adaptation in marginal populations. To better understand beneficial and harmful effects of gene flow in plant conservation contexts, more research is needed across plants at different life stages, using understudied taxa, and at a wide variety of spatial and ecological scales (Table 1). Key areas of focus should include controlled cases of gene flow, measuring the effects of different types of gene flow across ranges, studying gene flow in rapid adaptation, and the measuring the effects of gene flow and transplantation across and amongst populations (Rehfeldt et al., 1999; Montalvo and Ellstrand 2001; Sexton et al., 2011; Bontrager and Angert 2018). Lastly, restoring gene flow through genetic rescue is a proven technique for combating habitat fragmentation and needs more focused research (Bell et al., 2019). We encourage plant conservation and restoration managers to use adaptive management with respect to gene flow and mixing populations (Sgrò et al., 2011).

2.7. Does range size predict vulnerability under global change?

2.7.1. Range size matters

The question why some plant species are widespread with large ranges, and others are rare or have restricted ranges, has intrigued botanists for ages. For instance, the niche breadth-range size hypothesis (Brown 1984; Slatyer, et al., 2013), predicts that a species' range size is a manifestation ultimately of its niche breadth and thus represents its ability to persist in more or fewer environments. Besides potentially having reduced niche breadth, small-ranged species may also have fewer individuals and thus lower effective population sizes. As a result, species with small ranges may be at greater risk under global change. We refer to this phenomenon as the *range size vulnerability hypothesis*.

Explanations for restricted distributions range from a lack of genetic variation, to species being newly evolved taxa, to species being very old and consisting of remnants of a past range (Stebbins 1942; 1980; Leão et al., 2020). Recent research has supported the case that plant species generally begin small, “budding” from parental species, often sympatrically within the parent species range, and then expanding over time through niche evolution and/or dispersing more widely over time (Grossenbacher et al., 2014; Anacker and Strauss 2014). Recent literature mainly sustains this view (Gastauer et al., 2015; Heydel et al., 2017; Skeels and Cardillo 2018), but there is important variation, nuance, and exception, and a variety of forms of speciation and specialization in plants (Boucher et al., 2016; Rajakaruna 2018; Salariato and Zuloaga 2021). For example, a species may evolve through adaptation to a niche that is very widespread (e.g., ruderal plants), and so it has the potential to fill this niche quickly and will appear, geologically, as if it expanded its niche rapidly and exploded. Alternatively, clade radiations may fill unused habitats, creating sudden bursts of diversification, followed by gradual broadening of ecological niche and range sizes (Tanentzap et al., 2015; Folk et al., 2019). More diverse plant lineages may typically be comprised of species with smaller ranges (Leão et al., 2020). From these incipient speciation events, what ultimately determines plant species range size can be determined by myriad factors. Sheth et al. (2020) performed a meta-analysis and review on this topic and found that niche breadth, species’ age, niche availability (i.e., how common a niche is), and range position (i.e., range characteristics such as latitudinal breadth) were consistently strong factors associated with range size, but concluded that much more research is needed to confirm these effects on plant range sizes plus other potentially important effects such as mating system, ploidy, and dispersal ability. Grossenbacher et al. (2015) found strong support that more highly selfing plants have larger range sizes, and Grant and Kalisz (2020) recently confirmed that selfing plants indeed generally possess greater niche breadth than more outcrossing plant species. Moreover, polyploid plants with higher numbers of chromosomes tend to differentiate their niches faster (Baniaga et al., 2020). Finally, although logistically challenging, very few studies exist testing whether rare plant species are limited by genetic variation, but research thus far suggests that they are (Sheth et al., 2020).

2.7.2. *No range should be left behind in conservation*

Reviewing recent literature for plants, there are clear cases finding strong support for rarity predicting increased vulnerability or conservation risk under climate change. For example, Zettlemoyer et al. (2019) found that rare, more specialized plants are more likely to go extinct in a study in Michigan, USA. Aspinwall et al. (2019) demonstrated experimentally that *Eucalyptus* (Myrtaceae) trees with smaller range sizes were more susceptible to experimental heat waves. Many studies have found positive associations between niche breadth and species range sizes based on species distributions models (see Slatyer et al., 2013). Such correlative species distribution models (cSDMs) show potentially causal relationships between range size and species performance. Nevertheless, an important caveat is that spatial autocorrelation between the number of possible environments sampled and larger geographic extents can mask or overemphasize causal relationships (Moore et al., 2018; Journé et al., 2020). Another caveat is that such observational studies base patterns on the observed, or realized niche, rather than the fundamental niche, which is of primary interest for understanding environmental tolerances (Sexton et al., 2017; Liu et al., 2020), but see above discussions on biotic interactions and the realized niche. Nevertheless, experimental data can confirm true relationships between vulnerability and range size, when they exist. Historical considerations may also be quite strong. For example, Rapoport’s Rule states that species at higher latitudes should have larger ranges due to the greater stress and variability of those environments (Brown et al., 1996). Thus, more

tropical species may be driven or boxed into smaller ranges than their higher-latitude relatives due to evolutionary history. Huang et al. (2021) recently presented evidence supporting this hypothesis in plants: greater climate variability has a large potential effect on the evolution of large range sizes.

Cases and considerations in contrast to range size vulnerability hypothesis, or with mixed findings, clearly exist also (Lacher and Schwartz 2016; Hirst et al., 2017; Cai et al., 2021). Microhabitats, local, or sub-surface factors can buffer plants under climate change stress (Franklin et al., 2013; Gremer et al., 2015; Denney et al., 2020), and so small-ranged species that occupy highly heterogeneous landscapes may be able to weather rapid global change through more accessible escape environments. Rarity does not limit genetic variation or preclude subpopulation structure in the geographically restricted desert forb, *Astragalus lentiginosus* var. *piscinensis* (Fabaceae), which has small populations and a limited geographic range (Harrison et al., 2019). Additionally, specialized plants, such as soil endemic plants (“edaphism”) (Mota et al., 2017), may be limited more by competition outside of these environments than the climates they experience. Indeed, adaptation and diversification in rare, stressful environments can cause cradles or hotspots of diversity of taxa with smaller range sizes (Buire et al., 2021). Moreover, microhabitat variation may buffer populations via “portfolio effects,” but such effects may not be enough to save rare species from extirpation under rapid climate change (Abbott et al., 2017). In this vein, a species’ realized niche may be vastly smaller than that fundamental or potential niche. In such cases, a plant with a very small range may be able to weather a great variety of climates experienced outside of its current realized niche. Finally, a complex and nuanced reality likely exists for many species regarding this question. For example, Hirst et al. (2017) found only mixed results in support of the niche-breadth range size hypothesis in Australian alpine daisies; rarer daisy species showed evidence of increased tolerance at the cost of lower growth rates, but their seeds were also resilient to a wider range of germination environments. Thus, species may have increased vulnerability in a critical stage only (e.g., germination requirements) and such limiting stages may take a fair amount of experimentation to confirm.

2.7.3. Future directions

Generally, the range size vulnerability hypothesis appears to hold as a potential rule: smaller ranges tend to be more vulnerable to global change, but exceptions and patterns can vary greatly by taxon. For example, Tanentzap et al. (2019) found range size to be more strongly associated with extinction risk in conifers than in palms. Thus, we recommend that special status species with smaller geographic ranges receive high conservation priority, including reserve establishment in regions having many restricted endemics plants. Rare species can also be highly important for conservation and evolutionary study for a variety of reasons (Stebbins 1979) and should be assumed to be of high value, including for ecosystem function and services (Lyons et al., 2005). Nevertheless, larger-ranged species are no less important as conservation targets and can be vulnerable from falling through the cracks of political boundaries (Bisbing et al., 2021; Vázquez-García et al., 2021). For such species, we recommend greater focus on connectivity, dispersal habitat corridors, and multi-stakeholder and intergovernmental conservation plans. In this vein, local adaptation is likely to be a mechanism by which widespread species maintain their distributions (see above) and thus population conservation of populations in unique environments is critical.

Regarding future research, several avenues can be explored to uncover the conservation risk associated with range size (Table 1). More experimental assessments of plant performance at different life stages, under variable conditions, and between different taxa with varying range

sizes are required to better assess the range size vulnerability hypothesis. While a challenging area of research, tests of plant species range size vulnerability at the population and individual level is lacking (Slatyer et al., 2013). For example, metrics such as heat shock protein response can be used to assess the vulnerability of rare versus common plant taxa to predicted climate change stress (Al-Whaibi 2011; Aspinwall et al., 2019). In order to determine if smaller ranges are, indeed, more at-risk from modern habitat alterations, extinction debt (Kuussaari et al., 2009) should be assessed in taxa varying in range size (Jamin et al., 2020; Makishima et al., 2021). Questions concerning the relationship between range size and particular plant groups or life histories should be investigated. For example, as stated earlier, highly selfing species are expected to have larger geographic ranges and greater niche breadth, but this was not found for *Epipactis* (Orchidaceae) species in Europe (Evans and Jacquemyn 2020).

2.8. Conclusions

Our world is in a constant state of flux, exacerbated by rapid climate change, and researchers, managers, and stakeholders would benefit from adopting goals that attend to the impact of these changes and develop methods that can accommodate uncertainty (Rollinson et al., 2021). We provide an initial list of plant species range rules, identify gaps in the research, layout assumptions to consider, and tasks to complete in order to enhance our understanding of how ranges are governed and how they will change (Table 1). Generally, we envision this set of rules as a guideline for upcoming areas of study and efforts to conserve native plant populations. We ask that these rules of plant species ranges be fundamentally tested, under a variety of conditions, and, if necessary, ruled out, in order for us to advance our understanding of how plants are adapting to climate change, where to effectively target conservation efforts, and how to more holistically consider species ranges in our species distribution model predictions. We also acknowledge that there may be additional rules of species ranges not considered in this review, and we encourage the field to shine on them, especially as they relate to conservation. Finally, we call upon the scientific community at large to develop intelligent systems to detect and evaluate patterns and potential rules across disciplines in order to inform effective conservation and ecosystem management. For example, clustering is a form of unsupervised machine learning that can analyze and cluster large datasets based on pattern recognition (Caron et al., 2018). We foresee clustering techniques being employed to evaluate trends in the literature in the very near future. In the case of ecological or biogeographical patterns, rules can be judged or weighted by important factors, such as phylogenetic, geographical, or environmental parameters, etc. We encourage ecologists to put forward the rules they wish computers of the future to tally. We trust that human ecologists will always be the final judges.

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Tables

Ranges in Motion		
<i>Rule</i>	<i>Suggested Research and/or Conservation Implications or Strategy</i>	<i>References</i>
Range limits are real, are equal niche limits, and in motion.	Plan to help populations expand at leading edges and conserve important warm-adapted genetic variation at warm limits. To study range limits and their movement, use reciprocal transplant experiments and/or species distribution models (SDMs).	Sexton et al., 2009; Stanton-Geddes et al., 2012; Hargreaves et al., 2014; Lee-Yaw et al., 2016; Franklin et al., 2017; Halbritter et al., 2018; Lafontaine et al., 2018; Cross and Eckert 2020
Climate, often temperature, drives species ranges poleward or upward in elevation.	Use temperature as proxy for climate in climate-range research, and in combination with species interactions, in different systems, and at large scales.	Hampe and Petit 2005; Kopp and Cleland 2014; Freeman et al., 2018; Meng et al., 2019; Reed et al., 2021
Biotic Interactions		
<i>Rule</i>	<i>Suggested Research and/or Conservation Implications or Strategy</i>	<i>References</i>
Both positive and negative interactions set range limits and drive range shifts.	Assume there are myriad interactions influencing range limits and incorporate as many as possible into SDMs. For example, see ELGRIN model (Miele et al. 2021).	Hille Ris Lambers et al., 2013; Afkhami et al., 2014; Lothan et al., 2015; Bueno de Mesquita et al., 2016; Benning and Moeller 2019
Facultative mutualists (e.g., microbes and pollinators) facilitate range expansions into stressful habitats.	Assisted migration should consider mutualists in order for a plant species of interest to successfully establish or be restored to a habitat. Research to maximize and manipulate mutualist diversity is greatly needed.	Hille Ris Lambers et al., 2013; Afkhami et al., 2014; Millar and Bennett 2016; Koziol et al. 2018; Phillips et al., 2020; Rolshausen et al., 2020
Population Properties		
<i>Rule</i>	<i>Suggested Research and/or Conservation Implications or Strategy</i>	<i>References</i>
The abundant center hypothesis is not a rule, large populations are likely to be found across geographic ranges for many species.	While it is unlikely that population size and abundance decrease from geographic centers to edges, it is possible that size and abundance follow niche limitations. Conserving optimum habitat is recommended.	Lesica & Allendorf 1995; Channell and Lomolino 2000; Sagarin and Gaines 2002; Sexton et al., 2009; Moeller et al., 2011; Dallas et al., 2017; Pironon et al., 2017; Papuga et al., 2018; Hargreaves and Eckert 2019; Angert et al., 2020; Morente-López et al., 2021
Large populations have higher quantitative genetic variation, however, small, older, and/or unique populations need more consideration.	Assume these populations harbor important genetic variants or are locally adapted. Conservationists should aim to protect these populations.	Ellstrand and Elam 1993; Hampe and Petit 2005; Hoffman et al., 2017
Local adaptation is widespread and varies greatly across species ranges.	Research should investigate adaptation across a wide range of species across their entire range. Managers should assume local adaptation is happening, but	Hargreaves et al., 2014; Peterson et al., 2019; Torres-Martinez et al., 2019; Anderson and Song 2020; Hargreaves et al., 2020; Patsiou et al., 2020;

	is being affected or dampened by rapid climate change.	Anderson and Wadgymar 2020; Shay et al., <i>in prep</i>
Gene Flow		
<i>Rule</i>	<i>Suggested Research and/or Conservation Implications or Strategy</i>	<i>References</i>
Isolation by distance is the most commonly observed pattern of gene flow across plant species ranges.	Determine dispersal capacity and limitations for plant populations under conservation consideration. Assume genetic variation is related to dispersal, regardless of the size of the range.	Moyle 2006; Eckert et al., 2008; Sexton et al., 2014; Osrini et al 2013; Torres-Martinez et al., 2019; Twyford et al., 2020
Isolation by environment is very common in plant species ranges.	Assume isolation by environment is in play across a heterogenous environments and landscapes. If IBE is detected in plants under conservation consideration, genetic rescue from warmer environments may be warranted.	Hirao and Kudo 2004 Temunovic et al., 2012; Sexton et al., 2014; Wang and Bradburd 2014; Morente-López et al., 2021
Isolation by phenology is a type of IBE and impacts plant species distributions.	Studies should explore the impact of climate shifts on phenology-based gene flow, especially at large geographic scales and as a result of assisted migration efforts.	Chuine et al., 2010; Song et al., 2021
The idea that gene swamping creates range limits is not a rule, however, genetic rescue serves as a promising use of gene flow for conservation.	Genetic rescue is worth considering as a conservation and managements tool, especially at species range limits to avoid contractions or extirpations.	Sgrò et al., 2011; Bell et al., 2019; Kottler et al., 2021
Range Size		
<i>Rule</i>	<i>Suggested Research and/or Conservation Implications or Strategy</i>	<i>References</i>
Range size is determined by several factors, including niche-breadth, species' age, niche availability, and range position.	When classifying a range as small, and therefore of great consideration for conservation, these factors should be considered, but there may be others. More research that assesses plant performance across various conditions, taxa, and range sizes are needed.	Slayter et al., 2013; Baniaga et al., 2020; Grant and Kalisz 2020; Leão et al., 2020; Sheth et al., 2020
Small ranges may be more vulnerable; however, no range should be left behind.	Special status species with small ranges should receive higher conservation priority and larger ranges should be included in multi-stakeholder discussions and policy.	Lyons et al., 2005; Tanentzap et al., 2020; Bisbing et al., 2021; Vazquez-Garcia et al., 2021

Table 1. Rules of species ranges, suggested research for conservation efforts, and supporting references.

CHAPTER 3

Spatial, temporal, and environmental variation structures native monkeyflower endophytes

3.1. Abstract

As climates continue to warm and become drier, drought events threaten plant species worldwide. All plants associate with a community of microbes that make up their microbiome and those that reside within the plant are known as endophytes. Yet, we are limited in our understanding of the species that comprise these communities, how they form, and what environmental factors influence their composition and distribution. Here we report the first investigation of the endophytic community of the cutleaf monkeyflower, *Mimulus laciniatus*, with access to its native soil. As an endemic monkeyflower on the western slopes of the Sierra Nevada, *M. laciniatus* is adapted to a variety of environmental conditions, this system offers insights on the effects of abiotic stress of endophyte communities. We examined the bacterial and fungal endophyte communities through Illumina amplicon sequencing in a drought experimental context using soil collected from the plant host habitat. Community richness and diversity estimates revealed that the *M. laciniatus* microbiome is affected by plant compartment, temporal variation, and drought. Root communities were more diverse than shoot communities and time between harvests effected root communities to significantly shift. We saw a reduced level of species richness in drought conditions and an enrichment of specific taxa adapted to harsh conditions or engaged in plant growth promotion. We conclude with an initial assay of the root endophyte community, general functional implications, and ecological associations with their host.

3.2. Introduction

Drought stress from rising global temperatures is having major ramifications on species and their community compositions (Mann and Gleick 2015). As sessile organisms, plants are particularly susceptible to water scarcity (Zhao and Running 2010) and are forced to adapt and tolerate stresses (Exposito-Alonso et al. 2018; Dickman et al. 2019; J. T. Anderson and Song 2020) or shift their species range (Shirk et al. 2018); however, they are not alone in these challenges (Rodriguez and Redman 2008; Lau and Lennon 2012; Afkhami et al. 2014). Increasing evidence suggests that microbes extend the plant host phenotype and can help their plant hosts cope with stress from a changing environment (Marulanda et al. 2009; Compant et al. 2010; Rolli et al. 2015; Armada et al. 2018; González-Teuber et al. 2018). When a plant experiences stressful conditions, it can select distinct microbial taxa with specific beneficial functions (Lemanceau et al. 2017; Xu et al. 2018). For example, during drought events, plant root systems can attract and select drought-resistant microbes endowed with plant growth promoting traits (Bulgarelli et al. 2012; Marasco et al. 2012). These endophytes form diverse communities of eukaryotes, bacteria, archaea, and viruses and serve important ecological roles controlling pathogens, promoting plant health, and ameliorating stress from drought (Arnold et al. 2003; Hardoim et al., 2008; Sherameti et al. 2008; Rodriguez et al. 2009; Reinhold-Hurek and Hurek 2011; U'Ren and Arnold 2016). Most notably, plant-microbe associations are important facilitators for plant range expansion, or alternatively, constraining such expansion if microbes cannot match host dispersal (Afkhami et al. 2014; Benning and Moeller 2021). Although these associations clearly exist, are influenced by environmental variation, and are important for plant climate response, we have a limited

understanding of how ecological processes impact these communities, especially in wild plant communities experiencing drought stress.

In the field of ecology, a fundamental area of research is aimed at understanding patterns of community composition and diversity of species in space and time (Vellend 2010). By exploring endophyte community structure and assembly, we can gain more insight into the significance of these communities and the role they play in their ecosystems (Bulgarelli et al. 2012; Trivedi et al. 2020). Bacterial and fungal community compositions are most often determined by plant compartment, environmental factors, and host genotype (Edwards et al. 2018; Hamonts et al. 2018; Morella et al. 2020; Trivedi et al. 2020). A few studies on plant cultivars have demonstrated the influence of spatial (Coleman-Derr et al. 2016), temporal (Ek-Ramos et al. 2013), and climatic (Simmons et al. 2020) variation on endophyte community composition. For example, a comprehensive study of both cultivated and native *Agave* species demonstrated the important role of plant compartment in microbiome composition across geographic scales (Coleman-Derr et al. 2016). Thus, it is clear that space, time, and climate are strong selective forces on both cultivated and native microbial communities; nevertheless, the extent to which drought affects native systems adapted to drought is less clear. By directly testing the effects of drought on a native plant microbiome, we can gain insights into how plant microbiomes in highly variable ecosystems respond to climate change. The aim of this project is to examine changes in endophyte communities across plant compartment, time, and drought conditions. We sampled above- and below-ground endophytes from the tissue of an endemic wild plant grown in three treatments (pre-drought, control, and drought) with access to its native soil and seed microbial community.

To understand how these microbial communities are assembled in nature, it is important to study these questions using native plant seeds and soil. Our study used seeds from a central population in the species range of an endemic, annual, herbaceous plant of the California Sierra Nevada: the cutleaf monkeyflower, *Mimulus laciniatus*. We ask whether a fluctuating environmental condition, such as drought, will affect the native community of endophytes of *M. laciniatus* from soil and seed communities. This plant is an ideal system for this question for its ease of collection, manipulation in the lab, and its unique ecology. Ultimately, this system provides a perspective in a drought-stressed context, giving its potential to inform the complexity of interactions from drought-affected regions (Dickman et al. 2019). To our knowledge, this is the first study to report the endophytic microbiome of a monkeyflower under environmental variation.

3.3. Methods

3.3.1. Study system

Mimulus laciniatus has a well-defined species range across an elevation gradient on the western slopes of the Sierra Nevada, with cool, high-elevation populations, warm, low-elevation populations, and a relatively neutral range center (Fig. 1). Due to its distinct range and natural elevation-climate gradient, it has been the subject of several studies concerning adaptation to the unique and challenging environments in which it is found (Sexton et al. 2011; Ferris and Willis 2018; Dickman et al. 2019). Recent studies have confirmed that *M. laciniatus* is experiencing differential environmental stress (e.g., rising temperatures at the warm-edge and low precipitation along both the warm and cool edges), though these conditions are growing more stressful with climate change (Sexton et al. 2016; Shay et al. *in prep*). Typically, populations are distributed

among granite rock outcrops, growing from moss (e.g., *Bryum* spp.) or spikemoss (e.g., *Selaginella* spp.) patches on ephemeral, slow-draining, seeps that acquire water seasonally from snowmelt runoff. As an annual plant, *M. laciniatus* plant lifetime fitness is relatively easily measured as total number of flowers (Sexton and Dickman 2016).

3.3.2. Experimental design and sampling

Our experiment is a randomized block design of three treatments (pre-drought, control, and drought) with field-collected *M. laciniatus* seeds. In 2017, a dozen spikemoss patches, each containing hundreds of *M. laciniatus* seeds, were randomly collected from a central-range population of *M. laciniatus* in the Sierra Nevada (1585m). Spikemoss patches were homogenized and sieved (2 mm) into sterile bins, creating a substrate mix with seeds. The substrate mix was then potted in 3 36-cell potting plug trays, totaling 108 cells (2" x 2.25" x 3.25") and placed into tray inserts for bottom watering. Nutrients were added using Miracle-Gro formula to accommodate for the nutrient-poor substrate mix detected in a prior growth trial. Trays were cold stratified at 4 °C for 2 weeks to induce seed germination. Trays were then transferred to controlled growth chambers (PGC Flex, Conviron, Winnipeg, Canada) and grown on 14-hour day cycles ranging from 23 °C to 10 °C for day and night conditions, respectively. Plants were bottom-watered with sterile MilliQ™ (MilliporeSigma, Burlington, MA, USA) every 1–2 days for 4 weeks. Many seedlings germinated throughout this period, and each cell was thinned to only allow 1 plant per cell. Trays were rotated occasionally to minimize chamber position bias. A total of 42 plants survived to flowering.

Upon flowering, each cell was randomly assigned to a treatment group: 13 plants for the pre-drought, 14 for the control, and 15 for the drought treatment. Sampling occurred at these two timepoints to account for temporal community shifts or community shifts associated with plant life stage. After 4 weeks, the pre-drought treatment was harvested, and the remaining plants were reorganized into trays representing the control and drought treatments. The control tray continued with the same watering regimen, whereas the drought tray received one additional watering and was not watered again. After 2 weeks, all remaining plants were harvested and separated, using a fresh sterile razor, at the point where the root tissue meets the shoot tissue, representing 2 compartments. The roots and shoots were surface-sterilized by sonication for 5 minutes (Lundberg et al. 2012), and stored in -80° C. To prepare for extraction, root and shoot tissues were frozen with liquid nitrogen and homogenized into a powder through mechanical grinding.

DNA was extracted from root and shoot samples using the MoBio PowerSoil DNA kit (MoBio, Carlsbad, CA, USA). Samples were sent to the Department of Energy (DOE) Joint Genome Institute (JGI) for PCR amplification and sequencing following the recommended JGI protocols (Rivers 2016). PCR amplification was done using the JGI sample amplification QC (version 1.5) protocol, which calls for a mixture containing 12 µl of nuclease-free Molecular Biology Grade Water, 1 µl of Bovine Serum Albumin (BSA, 10mg/ml), 10 µl of 5 PRIME HotMasterMix (VWR Catalog # 10847–706 OR 10847–708), 0.5 µl of forward primer (10 µM), 0.5 µl of reverse primer (10 µM), and 1 µl of template DNA (10ng/µl), totaling 25 µl (Daum 2017). The fungal internal transcribed spacer region (ITS2) was amplified using the forward primer ITS9F (GAACGCAGCRAAIIGYGA) and the reverse primer ITS4R (TCCTCCGCTTATTGATATGC) (White et al. 1990; Ihrmark et al. 2012), and the bacterial ribosomal RNA (16S, V4–V5) was amplified using the forward primer 515F-Y (GTGYCAGCMGCCGCGGTAA) and the reverse primer 926R (CCGYCAATTYMTTTRAGTTT) (Parada et al. 2016). Peptide nucleic acids (PNA) were used to block host amplification of plastid sequences (Fitzpatrick, Lu-Irving, et al. 2018). Thermocycling conditions consisted of a denaturation at 94 °C for 3 min, followed by 30

cycles of 45 s of denaturation at 94 °C, 1 min of annealing and extension at 50 °C, and 90 sec at 72 °C for the dissociation stage, finishing with 72 °C for 10 min and held at 4 °C. Presence of PCR products was checked on an agarose gel. DNA concentrations were measured using a Qubit dsDNA HS kit (Life Technologies Inc., Gaithersburg, MD, USA) and tested for quality using a NanoDrop 2000 (Thermo Scientific, Waltham, MA). Sequences were run on an Illumina MiSeq PE300 sequencing platform (Illumina, Inc., CA, USA) in 2x300 run mode at the JGI (Walnut Creek, CA, USA). Raw sequence data were processed using a JGI-specific pipeline (Tremblay et al. 2015).

3.3.3. Statistical methods

Data were analyzed in QIIME2, version 2020.11 (Bolyen et al. 2019). Raw Illumina data were imported using the Casava 1.8 paired end demultiplexed fastq format. DADA2 (Callahan et al. 2016) was used to remove sequence errors from amplicon reads. Sequences were aligned using MAFFT (Kato and Standley 2013) and piped into FastTree (Price et al. 2009) to generate phylogenetic trees from the masked alignment. Feature tables were filtered to remove mitochondrial and chloroplast sequences as well as unassigned taxa at the phylum level for both ITS and 16S. Taxonomic assignment was completed using sklearn (Pedregosa et al. 2011). Classifiers were trained on the QIIME released UNITE, version 8.2 (<https://unite.ut.ee/repository.php>), (Köljalg et al. 2013; Abarenkov et al. 2020) reference database with a 97% sequence similarity threshold for fungi and on the Greengenes 99% full-length sequences (DeSantis et al. 2006; McDonald et al. 2012) for bacteria. All UNITE classification follows the current classification from Index Fungorum (<http://www.indexfungorum.org/>). Community richness (alpha diversity) was quantified using Faith's Phylogenetic Diversity (Faith 1992) and community dissimilarity (beta diversity) was quantified using unweighted UniFrac distance (Lozupone and Knight 2005; Lozupone et al. 2011). Principle Coordinate Analyses (PCoA) were performed and visualized using EMPeror (Vázquez-Baeza et al. 2013). Fungal sequence variants were matched to fungal groups using FUNGuild (Nguyen et al. 2016), and functional profiles were predicted for bacteria using PICRUSt2 (Douglas et al. 2020). Bacterial gene function between plant compartment was inferred by matching ASVs to known genome sequences from the Greengenes database. For 16S sequence data, we used BURRITO to visualize taxa-informed gene function (McNally et al. 2018). We assessed community similarity by conducting a non-parametric multivariate analysis (ANOSIM) (Clarke 1993) and identified factors that influence variation of community structure between plant compartment and treatment using PERMANOVA (Adonis) (M. J. Anderson 2001) in the beta-group-significance pipeline implemented in QIIME2 with unweighted Unifrac distance matrices. We assessed associations between species and groups through the 'indicspecies' package, version 1.7.5 (Cáceres and Legendre 2009; De Cáceres and Jansen 2015), in R (R Core Team 2020). From the indicator species analysis, we report taxa that are significantly associated with groups at the species level when available, however, some taxa were significant in specific treatments, but were not resolved to species level (e.g., *Firmicutes* in drought root).

3.4. Results

We analyzed the endophyte communities associated with *M. laciniatus* from a drought experiment. We confirmed that the drought-treated plants were stressed with a Welch two sample t-test (p-value = 0.008) between the treatment group and life stage. Stressed plants sped up their life cycle and senesced earlier than the controlled plants. The 42 *M. laciniatus* plants harvested

were analyzed through Illumina sequencing on the MiSeq platform. We obtained 12,325,100 and 21,265,480 total high-quality reads, which, after quality control, filtering, and determination of amplicon sequence variants (ASVs) resulted in 1,547 and 5,135 sequences of fungal and bacterial ASVs respectively. We detected 15 distinct bacterial phyla and 8 fungal phyla; bacterial and fungal communities were substantially dominated by Proteobacteria and Ascomycota, respectively. After filtering for unassigned taxa, within bacterial taxa, Proteobacteria dominated >60% of the root community and >90% of the shoot community. Within fungal taxa, Ascomycota dominated >80% of the community in both drought and control groups, but less so in control plants where Glomeromycota and Basidiomycota were present at higher percentages than in other groups (Fig. 2).

3.4.1. Above- and below-ground diversity of *Mimulus laciniatus*

In general, taxa were more phylogenetically diverse in the below-ground root compartment. In all groups, except bacterial shoot communities, bacterial and fungal alpha diversity were marginally lower in drought treatments than in control or pre-drought assemblies (Fig. 3). Root communities were significantly more diverse than shoot communities in both fungi and bacteria (Fig. 3). Within plant compartment, the control and drought fungal communities were the most phylogenetically distinct from each other, whereas all other groups were more phylogenetically consistent (Fig. 3).

3.4.2. Factors influencing microbial root communities in *Mimulus laciniatus*

Analysis of beta diversity from unweighted Unifrac distance matrices revealed that plant compartment was a stronger factor than treatment in shaping the microbial communities (Fig. 4). Within compartments, treatments affected root communities but not shoot communities. Within plant compartment, treatment within root was the factor that explained 14% of the variation in fungal communities and 19% in bacterial communities (Table 1). In bacterial root communities, the pre-drought community was most dissimilar to the control treatment community, whereas the drought and control and drought and pre-drought were more similar to each other (Table 2). In fungal root communities, the control was somewhat dissimilar to both the pre-drought and drought treatments, while the drought and pre-drought treatments were more similar (Table 2). Communities did not significantly shift between treatments in the above-ground tissue (Fig. 4).

3.4.3. Relevant taxa from treatment groups

Using such and such analysis, we found no species that were significantly correlated to treatments within shoot or within fungal communities, only in bacterial root treatments. Of those significant bacterial species in roots, there were six major classes belonging to Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, Firmicutes, Bacteroides, and Acidobacteria (Fig. 5). Some taxa were significantly correlated to every treatment in roots, such as *Acidopila rosea* with, a known soil microbe (Catão et al. 2014). Other taxa specialized to specific treatments, such as the stress and starvation activated Acidobacteria *Candidatus Koribacter versatilis* (Challacombe et al. 2011), which was indicated as a significant species only in the drought root treatments (Fig. 5). Although, our fungal indicator species analysis did not result in significant species within treatment, we did find phyla that were enriched in treatments (Fig. 2). Based on the taxa bar plots, Firmicutes and Ascomycota were enriched in drought treatments, Glomeromycota in the controlled root, and Basidiomycota in the controlled shoot treatments.

3.4.4. Taxonomy-informed gene and ecological function

For bacteria, the taxonomy-linked functional analysis resulted in four functional groups (cellular processes, environmental information processing, genetic information processing, and

metabolism) with the remainder representing unclassified genes (Fig. 6). Every function was strongly linked to Proteobacteria. The most abundant gene function was metabolism, followed by genetic information processing. Environmental processing was strongly linked to Acidobacteria in addition to Proteobacteria and represented the third most abundant gene function in the dataset. Fungal ecological functions were characterized from the entire dataset, not specifically for any group (Fig. 7). For fungal ecological associations, most taxa were tied to saprotrophic (found 151 times) guilds, followed by plant pathogens (found 128 times), and symbiotrophs (found 124 times).

3.5. Discussion

3.5.1. The effect of spatial, temporal, and climate variation

The microbial communities of *M. laciniatus* were shaped spatially by plant compartment, temporally across two different harvest time points, and environmentally with the water availability. Drought was not a leading driver of variation; however, drought-stressed communities harbored less phylogenetic diversity than other treatments, which points to selective pressures from drought on microbial communities (Fig. 3). In our study, the shift between the two timepoints saw an enrichment in Firmicutes bacteria, particularly in the drought group, suggesting these microbes may have colonized the roots via soil during the few weeks the plant was stressed from drought. Firmicutes may also be drought tolerant, which allowed for their abundances to be enriched in drought. The drought treatment enriched specific taxa related to growth promotion, stress response, and drought tolerance in *M. laciniatus* (see Firmicutes and Ascomycota in drought treatments (Fig. 2, Fig. 5) (Fitzpatrick, Copeland, et al. 2018). A similar finding was recently reported in sorghum root microbiomes, where drought enriched for specific taxa and delayed the development of the root microbiome (Xu et al. 2018). In fungal communities, pre-drought and control communities were more dissimilar than the drought and pre-drought, suggesting that the presence of water allowed fungi to flourish. Although fungal communities are known to be significantly affected by experimental drought (Lagueux et al. 2021), we found no strong relationship. Alternatively, fungi might be unaffected by drought entirely, as seen in a study on carbon turnover where drought had little to no effect on fungal communities in plant roots (Fuchslueger et al. 2016). Moreover, these microbial communities could be generally drought tolerant akin to the monkeyflowers they occupy.

Both fungal and bacterial endophyte communities were influenced by time between harvests. Root bacterial communities significantly shifted between the pre-drought treatment and the control group (Table 2). This aligns with previous studies of cultivated plants that have reported shifts in plant microbiomes over time, as in the case of cotton (Ek-Ramos et al. 2013) and wheat (Comby et al. 2016). The role of time is multifaceted in how it influenced endophyte community structure. Time allowed microbes to associate with each other and determine assembly based on their position and relationship with the host. For example, in wild lima bean (*Phaseolus lunatus*), the order of endophyte arrival was influenced by fungi that were resistant to pathogenic bacteria, preventing them from entering right away (Adame-Álvarez, Mendiola-Soto, and Heil 2014). Competition, particularly around the roots between rhizosphere microbes could affect which microbes enter the plant over time. Host sampling and selection of microbes into the endosphere could also shift over time based on the plant's life stage and needs. The element of time should continue to be included as a factor of endophyte community assembly and future research should aim to understand its specific role and level of influence in different systems and contexts.

3.5.2. The root microbiome of *Mimulus laciniatus*

This study was the first to our knowledge to characterize whole plant endophyte communities associated with a monkeyflower species under an environmental context. We confirmed our expectations and found strong differences between above- and below-ground communities, a general finding in plant microbiome research (Coleman-Derr et al. 2016). Root, but not shoot, communities shifted in response to the effects of temporal and drought treatments. This suggests that bacterial root communities may be important for *M. laciniatus* as it responds to environmental stress (Trivedi et al. 2020). Although a novel finding for a monkeyflower, high species diversity in root communities has been found in an array of plant microbiome studies and under different conditions (Edwards et al. 2018; Xu et al. 2018; Simmons et al. 2020; Zeng et al. 2021). The taxonomy-informed gene function analysis showed an association between communities and metabolism and environmental processing, another indicator that these microbes are involved in sensing and responding to abiotic stress responses. Shoot communities were less diverse than root communities and did not vary significantly with treatments (Fig. 3). With little effects on shoot communities, we only discuss the root microbiome.

Bacterial endophyte taxa were dominated by Proteobacteria, a major phylum of diverse symbiotic bacteria (Stackebrandt 2001; Liu et al. 2017). We confirmed the presence of the core bacterial classes typically found in all plant root endophytic communities including Betaproteobacteria, Gammaproteobacteria, Alphaproteobacteria, Firmicutes, Bacteroides, and Acidobacteria (Edwards et al. 2015; Liu et al. 2017; Kalam et al. 2020). Our indicator species analysis revealed several significant taxa that strongly associated with treatments within the root (Fig. 5). In the drought treatment, bacterial taxa associated with microbial starvation and stress were enriched, as in the case of *Candidatus Koribacter versatilis* (Fig. 4). We also identified several root taxa involved in plant growth promotion and biocontrol, such as the commonly found *Burkholderia gladioli* (Gunjal and Kapadnis 2013), methylotrophs like *Methylovorus glucosotrophus* (Kumar et al. 2017), and polysaccharide-producing bacteria like *Mucilaginibacter kameinonensis* (Urai et al. 2008). In general, the characterized bacterial taxa have a diverse array of important roles for plant health. Although it is not exactly clear what the specific function of these communities are, we know these bacterial taxa are linked to genes involved in environmental sensing (Fig. 5) and are ecologically characterized in plant growth and nutrient cycling, suggesting that these bacteria may provide beneficial functions to their plant host.

Fungal endophyte taxa were largely identified as Ascomycota, which are known to play a variety of important roles in arid ecosystems, including soil stability, carbon and nitrogen cycling, and symbiosis (Challacombe et al. 2019). Within Ascomycota, we found typical endophytic fungal groups, including members of the Pezizomycotina subdivision (Rodriguez et al. 2009); particularly, Dothideomycetes, Eurotiomycetes, Leotiomycetes, and Sordariomycetes (Wang et al. 2006; Tedersoo et al. 2013; Chen et al. 2015; U'Ren and Arnold 2016), some of which are strongly associated with bryophytes (Davey et al. 2014) and occur in a variety of ecosystems (Zimmerman and Vitousek 2012). Since *M. laciniatus* arises from and interacts closely with bryophytes, this finding may suggest some horizontal transmission from the moss microbiome to the herb. A large portion of taxa were unassigned (34%) at the phylum level or higher, a common issue in fungal datasets (Köljal et al. 2013; Abdelrazek et al. 2020). BLAST (Altschul et al. 1990) results were also inconclusive with these taxa. This is likely due to the limitations and gaps of undescribed taxa within publicly available datasets, the low resolution of ITS (Tedersoo et al. 2020), and the need for a larger diversity of sampled study systems.

Though our indicator species analysis did not reveal specific fungal taxa within treatment groups, there were several ecologically important taxa from the dataset that are well-known, rock-inhabiting fungi that occur in arid ecosystems. For example, *Chaetothyriales* (Eurotiomycetes) (Barr 1987) are typically found in extreme, adverse conditions on hot rock surfaces, similar to the granite rock outcrops where *M. laciniatus* live, and are characterized as opportunistic in harsh conditions (Teixeira et al. 2017). *Capnodiales* (Dothideomycetes) (Woron 1925) were also prevalent in several groups and are known as a rock-inhabiting group that are tolerant to drought and heat (Schoch et al. 2009). When we characterized their ecological interactions, the vast majority of Ascomycota were in the saprotrophic guild (Fig. 7), which may be a result of opportunistic endophytes shifting ecological strategies during plant senescence (Promputtha et al. 2007). Surprisingly, a few species of coprophilous (dung-loving) fungi in the *Conichaeta* genus were associated with root tissues, suggesting horizontal transmission from animal scat or possibly herbivore vectors dispersing endophytes through root mastication, which is known to occur in arid regions similar to where these plants occur (Herrera et al. 2011). Basidiomycota are not expected to be isolated from endophytic communities; however, recent studies are identifying various basidiomycetes that engage in the endosphere for part of their life. For example, the orchid mycorrhizal basidiomycete, *Serendipita vermifera*, was isolated in this study and has recently been recognized as a beneficial root endophyte that combats competitor microbes by spending part of its life inside the plant and part of its life outside the plant forming a protective barrier in the rhizosphere (Sarkar et al. 2019). Several plant pathogens were identified, namely *Fusarium*, however, it is not clear how these known toxigenic fungi are interacting with *M. laciniatus* since fungi tend to behave differently with wild plants than with the more well-studied and susceptible cultivated plants (Lofgren et al. 2018).

It is important to recognize the limitations of conducting this experiment in a controlled growth chamber as well as the role of microbiome assembly. The plants in this study were grown in a controlled environment without access to propagules transmitted by pollinators, or air/wind, and deprived of what phyllosphere community is maintained in the field. This likely caused lower diversity in the above-ground endophyte communities (U'Ren et al. 2012; Maignien et al. 2014). Nevertheless, above-ground endophyte communities are usually less diverse than those isolated from below-ground tissue (Martins et al. 2016). Some of the endophyte community may be assembled from the seed communities through vertical transmission, although we could not directly test for this (Walsh et al. 2021). Both bacterial and fungal vertically transmitted endophytes are known to affect plant growth and biocontrol (Truyens et al. 2015; Shahzad et al. 2018). Vertical transmission of fungal endophytes is especially common in forbs (Hodgson et al. 2014). It is possible that a portion of taxa were vertically transmitted; this would further explain why shoot communities remained relatively unchanged in both bacterial and fungal assemblies across treatments.

3.6. Conclusions

In the wild, we have a limited understanding of endophyte diversity and function and what we do know from cultivated systems does not necessarily translate into knowledge about natural plant-microbe interactions. For instance, a toxic plant pathogen to a crop may be a mutualistic growth promoter in the wild, but these associations will continue to remain a mystery until we gather more environmental information from a variety of systems. From our initial survey, we were able to capture ecologically important taxa involved in plant growth promotion, environmental stress

response, and rock-inhibitors known to occur in harsh, arid conditions. These communities may be specific to the challenging environment from which they came, suggesting that they are adapted to these conditions and may ameliorate stressful conditions to their plant hosts. Alternatively, these habitat-adapted microbes may be exceptional opportunists that are taking advantage of a stressed plant for its carbon and resources (Saikkonen et al. 2004). Although this specific interaction is not clear, what is clear is that these communities are heavily influenced by their spatial, temporal, and resource environment and deserve more exploration to untangle their role in wild plants.

Thinking ahead, further understanding of how the structure and function of endophyte communities shift in response to time, drought, and temperature, especially from field-collected plants across species ranges experiencing climate stress, is a crucial avenue for identifying significant plant-microbe interactions that are not only implicated in plant climate adaption, but also in determining host species range shifts as climate conditions worsen (Van der Putten et al. 2010). Several ASVs sequenced in this study were either unassigned or unidentified and are most likely novel taxa. This serves as a reminder of the critical importance of identifying taxa from a breadth of hosts experiencing various environmental conditions, as well as the magnitude of unknown endophytic diversity in nature. Future research should continue to explore variation in communities across large heterogeneous host species geographic ranges and in plants experiencing stresses, such as those from climate change. Here, we report the first view of the microbiome for a native herbaceous plant of the Sierra Nevada, yet there remains a world of plants, across diverse ecosystems in need of deep microbial exploration.

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Figures

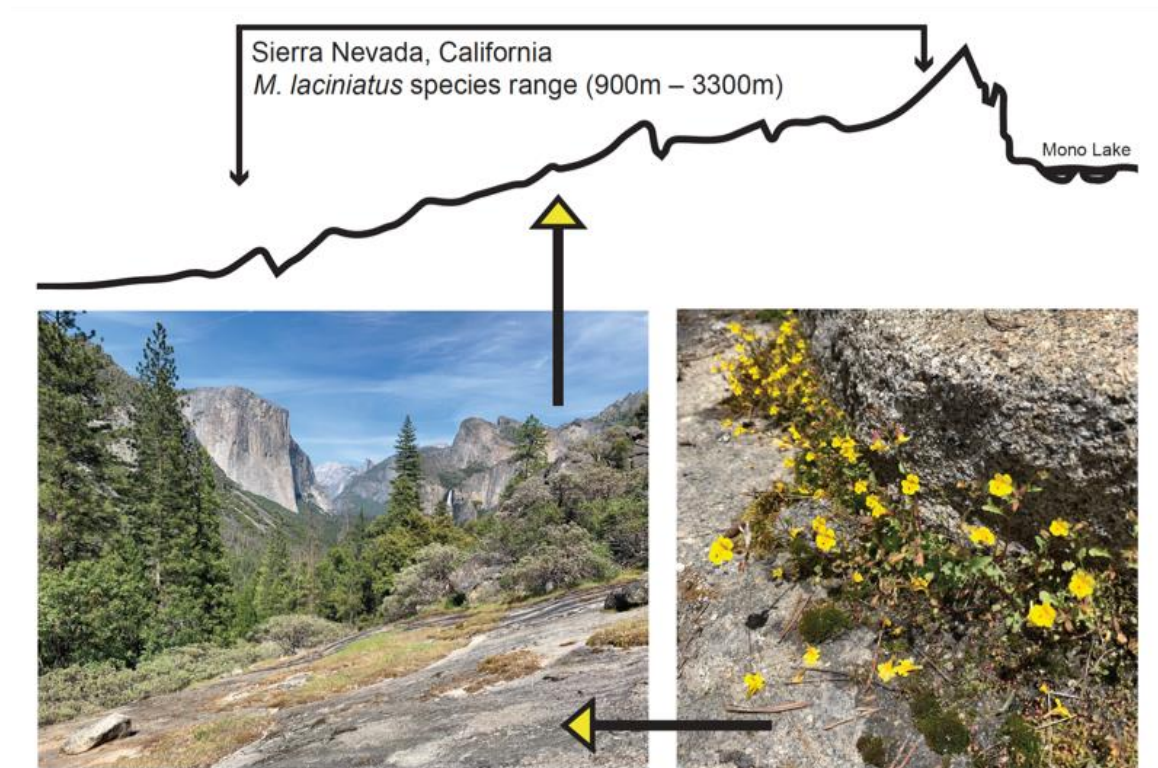


Figure 1. Seeds were collected from a central population in the *M. laciniatus* species range across the western slopes of the Sierra Nevada. The annual, herbaceous monkeyflower is shown growing from moss settled at the base of a granite rock. Moss patches can be found scattered across granite rock bluffs (seen here).

Mimulus laciniatus Endophyte Community Phyla

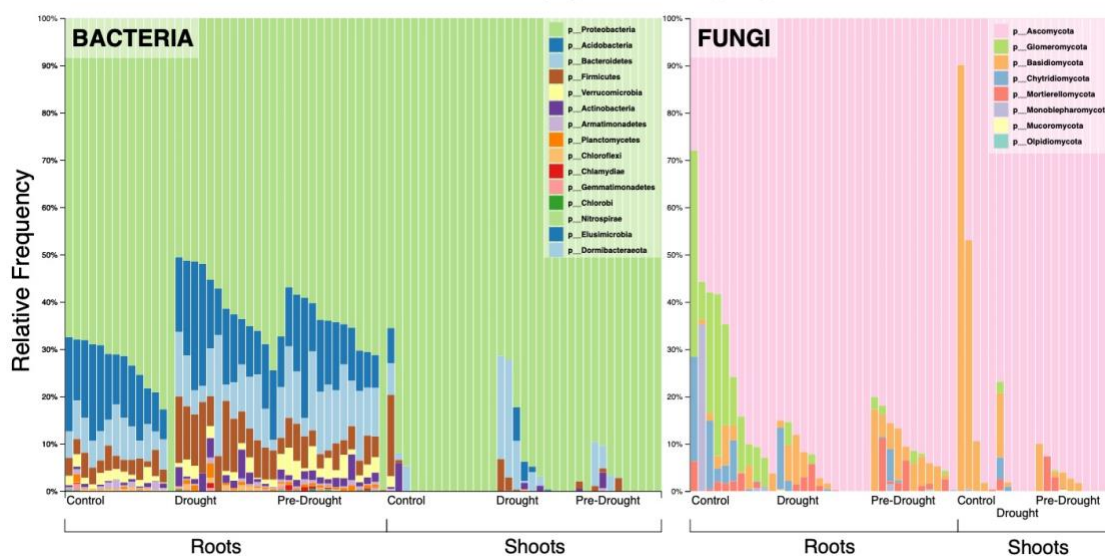


Figure 2. Taxa bar plot of identified bacterial and fungal phyla. Filtered taxonomic plots produced in QIIME2 with relative frequency of taxa in each treatment group within plant compartment. Taxa are ordered first by plant compartment, followed by treatment, and finally by frequency of *Proteobacteria* and *Ascomycota* in ascending order.

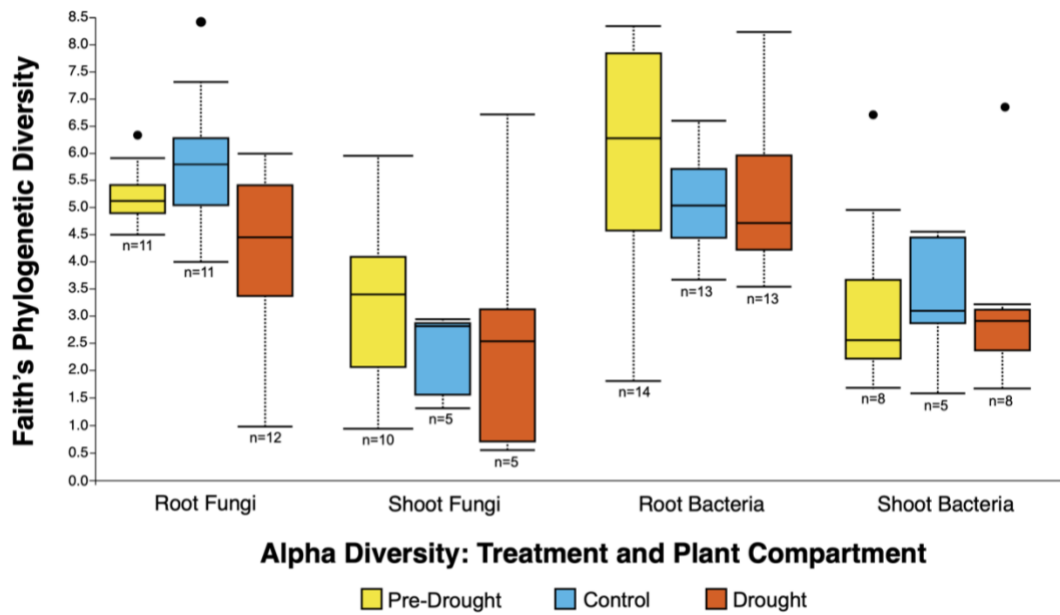


Figure 3. Alpha diversity plot of treatment groups within plant compartment. Estimated phylogenetic diversity (Faith's PD) in fungal and bacterial communities associated with each treatment group (pre-drought, control, and drought). Kruskal-Wallis p-value is 0.0002, meaning there are at least two groups that are different from each other. Details on differences between groups can be found in Table 1. The dots represent outliers.

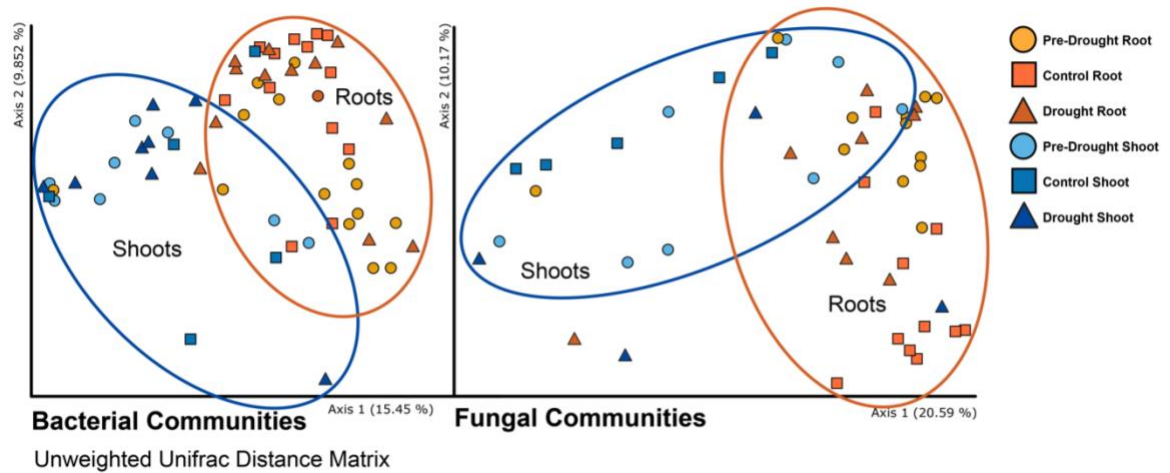


Figure 4. Beta diversity PcoA plot of treatment groups within plant compartment. PcoA plots were made with unweighted Unifrac distance matrices. The space between points represents the dissimilarity between communities of microbes from an individual plant (points). The closer the points, the more similar the communities. Variation on axis 1 is explained by plant compartment while variation on axis 2 in bacteria is explained by time in bacteria and water in fungi. Statistical support is provided in Table 1.

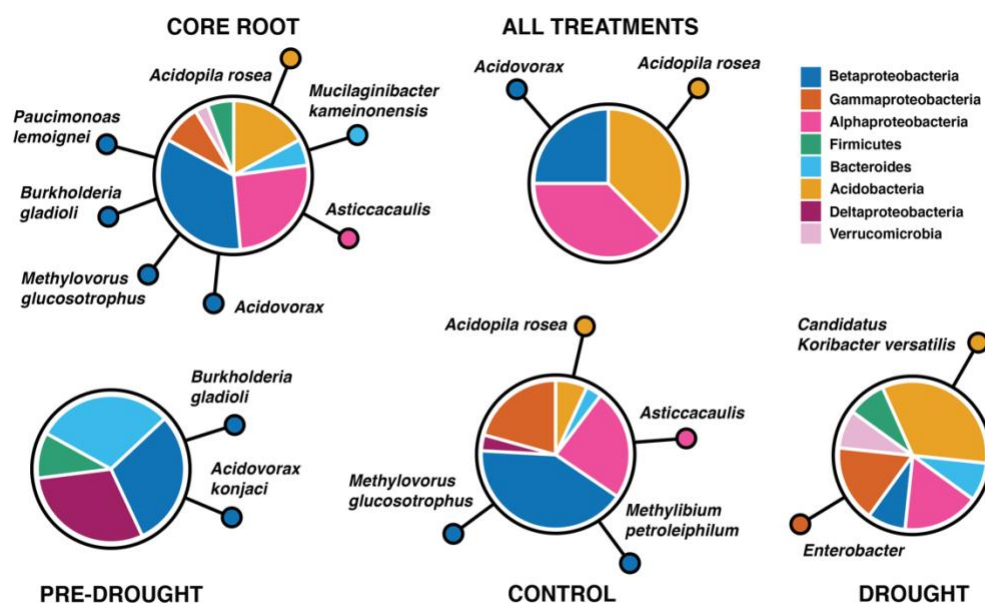


Figure 5. Results of indicator species analysis of significant bacterial taxa to the species level ($p < 0.05$). Associated bacterial taxa (at phyla level) for treatment groups. Proteobacteria are split into their subsequent classes (e.g., Beta, Gamma, Alpha, and Delta). Bacterial phyla and classes are indicated in colors. Pie charts represent the number of species indicated as significant from each phylum. Pop-out circles show all significant taxa resolved to the species level. Taxa plots are separated by treatment, taxa in all three treatments, and taxa significant in the root.

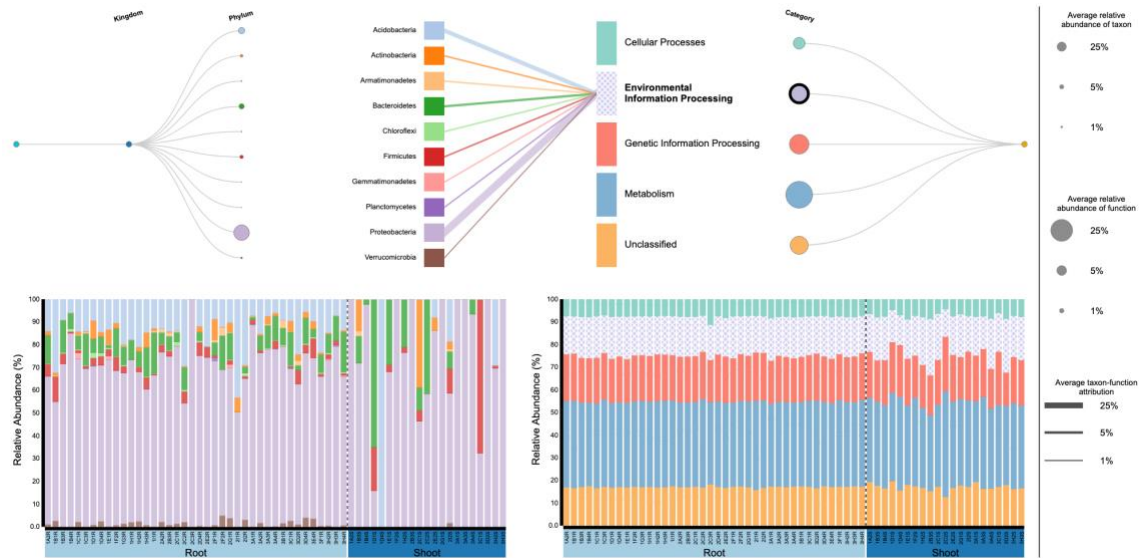


Figure 6. BURRITO analysis for 16S gene function. Representative taxa (top left) are grouped by phyla based their average relative abundance (size of circles). Selected gene functional groups (top right) are based on the average relative abundance of the function in the dataset (size of circles). Stacked bar plots represent the relative abundance of taxa (bottom left) and functions (bottom right) in each sample. Taxon-function associations are linked (environmental information processing is highlighted here) and the width of the bars represents the average association of the gene function to the highlighted taxa.

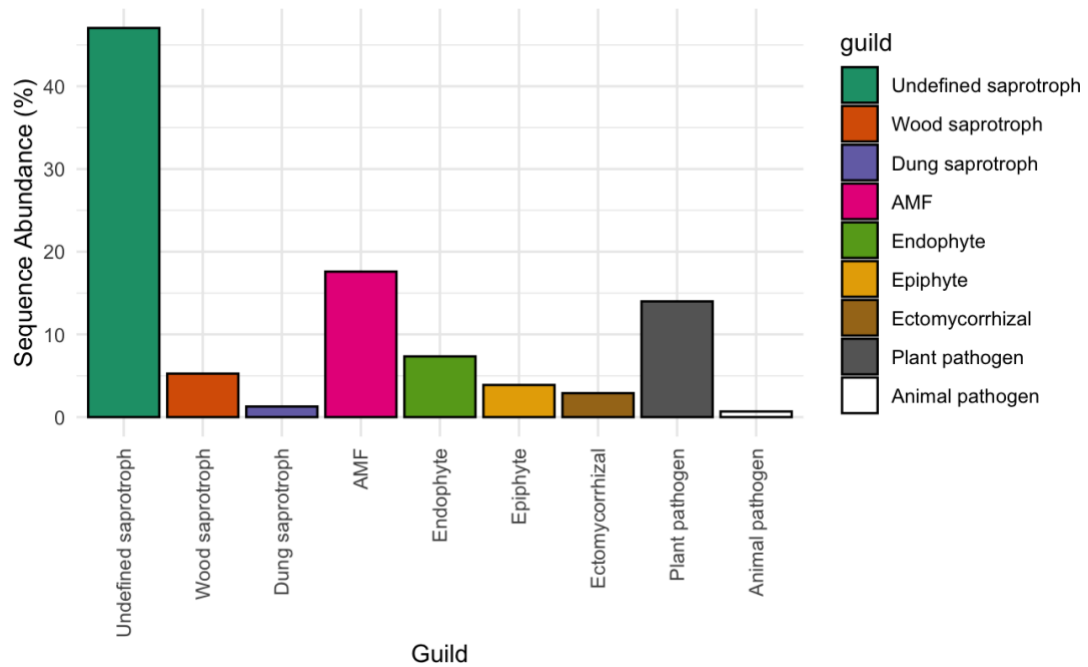


Figure 7. Fungal ecological guilds produced by FUNGuild using relative frequency of sequences assigned to each guild. The first three represent saprotrophs fungi, the next three symbiotrophic fungi, and the last two are pathogenic fungi. The majority are in the saprotrophic guild and largely unidentified saprotrophs.

Tables

Table 1. Factors that influence community structure using PERMANOVA (Adonis).
Significance of R-squared values are denoted as follows: $p \leq 0.001$ ***, $p \leq 0.01$ **, $p \leq 0.05$ *,
 $p > 0.05$ ns.

	Fungi	Bacteria
Compartment	R² = 0.085 ***	R² = 0.102 ***
Treatment	R ² = 0.052 *	R ² = 0.072 *
Treatment within Root	R² = 0.140 ***	R² = 0.194 ***
Treatment within Shoot	R ² = 0.143 ns	R ² = 0.130 ns
Treatment*Compartment	R ² = 0.041 ns	R ² = 0.045 *

Table 2. Changes within community structure. ANOSIM pairwise comparisons of treatment groups within *M. laciniatus* root communities. Distances were calculated using unweighted Unifrac matrices. An R value closer to “1.0” represents groups that are more dissimilar to each other and values closer to “0” imply more even distribution between groups.

FUNGI						
<i>Group 1</i>	<i>Group 2</i>	<i>Sample size</i>	<i>Permutations</i>	<i>R</i>	<i>p-value</i>	<i>q-value</i>
Control	Drought	22	999	0.2654	0.001	0.003
Control	Pre-Drought	22	999	0.4069	0.002	0.003
Drought	Pre-Drought	22	999	0.1540	0.008	0.008
BACTERIA						
<i>Group 1</i>	<i>Group 2</i>	<i>Sample size</i>	<i>Permutations</i>	<i>R</i>	<i>p-value</i>	<i>q-value</i>
Control	Drought	26	999	0.3319	0.001	0.001
Control	Pre-Drought	26	999	0.7827	0.001	0.001
Drought	Pre-Drought	26	999	0.2315	0.001	0.001

CHAPTER 4

Tackling real-world environmental paper pollution: A problem-based microbiology lesson about carbon assimilation. *

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4.1. Abstract

Governmental and educational organizations advocate for the adoption of inquiry-based, student-centered educational strategies in undergraduate STEM curricula. These strategies are known to benefit students by increasing performance, enhancing mastery of class content, and augmenting affect, particularly in underrepresented racial/ethnic minority students. Among these strategies, case study and project-based learning allow students to master course content while collectively tackling relevant, real-world societal problems. In particular, environmental pollution with paper-based products provide a current problem by which microbiology students learn about the role of microorganisms in paper waste management as well as the microbiological and biochemical processes involved in protein secretion, nutrient uptake, and energy metabolism. Delivered in a flipped, hybrid class in a Technology-Enabled Active Learning (TEAL) laboratory, this lesson taught students about exoenzyme secretion, biopolymer hydrolysis, intracellular transport of sugars, and sugar catabolic reactions. Students demonstrated increased comprehension of exoenzyme function and secretion, as well as how cells uptake the products of exoenzyme hydrolysis. However, students had challenges in placing the transported exoenzyme products within metabolic processes. Our results show increased perceived learning from the students as well as an understanding of the societal implications of these microbiological concepts. Our lesson deviated from knowledge silos in which students learn information in discrete topics. While departing from employing traditional, compartmentalized learning approaches, this student-centered guided lesson frames the systemic nature of the microbiological and biochemical processes underlying the decomposition of organic matter in a real-world context.

4.2. Introduction

Institutions and faculty are revolutionizing their science, technology, engineering, and mathematics (STEM) educational programs to effectively engage and train tomorrow's scientists (AAAS, 2009). Students need more than a traditional biology education to tackle the most pressing issues facing science and society; they need to learn how to address real-world problems. By presenting a problem in an experiential learning environment, students can build their own conceptual framework through an active learning process that encourages them to socially engage, share ideas, and participate in their own inquiry-based learning (D. Allen & Tanner, 2005; Chorazy & Klinedinst, 2019). Problem-based learning fosters deep, accurate understanding of a subject and contributes to developing process skills such as research, teamwork, and verbal communication (D. E. Allen et al., 2011; Orsmond & Zvaunya, 2015). Problem-based learning increases awareness and connects students to local challenges, which is instrumental in actively engaging them in their education, especially for underrepresented student populations (Nelson-Hurwitz & Buchthal, 2019).

When students are passionate about a subject, they engage deeply in their learning (Harackiewicz et al., 2016), and one issue that students are passionate about is the environment (Desa et al., 2011). A growing global crisis is waste pollution, and contrary to many perspectives, paper is a major contributing factor to global waste. The average American consumes 700 pounds of paper each year and paper represents 25% of waste in landfills and 33% of municipal waste (Desilver, 2016). Now more than ever, it is important to foster students who can critically assess these issues by understanding how natural biodegradation cycles operate to ameliorate stress on our global ecosystem (Marope, 2016). The use of deliberate pedagogy can increase awareness of paper waste pollution and emphasize how microbial degradation provides an alternative to paper waste management (Nelson-Hurwitz & Buchthal, 2019).

In this paper, we test the use of evidence-based teaching practices in combination with problem-based learning in a flipped classroom to ask if these have a positive impact on students' understanding of complex biological material in the context of a real-world problem. Specifically, we wanted to ascertain if the activity would increase student's mastery of the concepts of bacterial protein secretion, nutrient transport, and carbon cycle. Furthermore, we wanted to evaluate if the activity would influence the student's confidence about their knowledge of these topics. Here we present an effective lesson that utilizes paper waste degradation as a platform to teach students about the carbon cycle. Our work shows that this lesson increases student's awareness of paper waste pollution and management, content knowledge about microbial paper degradation, and mastery of the process of bacterial protein secretion, nutrient transport and metabolism.

4.3. Pedagogical Framework and Principles

4.3.1 The Technology-Enabled Active Learning (TEAL) Laboratory Environment

The General Microbiology course at the University of California, Merced (UC Merced) is a hybrid class taught using flipped pedagogy, and it is delivered in a TEAL laboratory environment. The TEAL lab is a classroom space designed to offer students the opportunity to enhance their cognitive and behavioral engagement through small group discussions, peer evaluation, and shared experiential learning. Students interact more with each other, share resources, and experience a more equitable learning environment in the TEAL lab (Beichner et al., 2007). These labs facilitate the implementation of active learning strategies to best utilize the classroom space (Cotner et al., 2013).

The General Microbiology TEAL lab houses 90 students in ten working tables (Supplementary Figure 1). These are arranged to allow equal view of two large class projector screens located in the front and back of the room. Each table is equipped with docks to power laptops, a document camera to display paper-based work, a whiteboard, an HDMI monitor and a control panel. The instructor control lectern is centered in the room and has the ability to orchestrate the technology offered in the space. In this way, class content can stream from the lectern, or from any of the ten working tables to the entire class (Office of Information Technology, UC Merced, 2020).

4.3.2. Active Learning and Flipped Hybrid Classrooms

Active learning is a student-centered pedagogy where students interact with their learning as opposed to passively listening to a lecture. It is widely accepted for its efficacy in increasing students' performance, especially in STEM courses (Freeman et al., 2014). In order to use the space and time with the students most effectively, we developed a flipped classroom model

designed to center the class around the students through facilitated experiential learning. Students watch class content videos at home and attend their scheduled lecture hour prepared for activities with a heightened sense of engagement (Gilboy et al., 2015). The hybrid flipped classroom provides students with a more intimate experience with their instructors, where they can benefit from team-based learning, demonstrate their knowledge, and receive immediate feedback.

To amplify the effects of an active team-based learning environment, our lesson structure follows a recommended instructional protocol by Michaelsen and Sweet (2011). Students begin class with readiness assurance practices, where they 1) watch video lectures before class; 2) respond individually to a 10-minute lecture comprehension quiz at the start of lecture (Supplementary Material 2); 3) review the quiz as a table to confirm answers; and 4) discuss the quiz results as a class to identify and diffuse misconceptions and establish consensus. The readiness assurance is followed by a 55-minute flipped lecture, which consists of a brief review of material followed by an activity based on the 5E model.

4.3.3. The 5E Model: Engaging Large Classes

To address the needs of a large active-learning class, it is necessary to implement a well-documented form of learning that focuses on the desired learning outcomes and considers the cognitive engagement of the students. The learning-cycle method known as the “5E model” (Bybee, 1997) is a common method for organizing large biology-based lessons infused with active learning (D. Allen & Tanner, 2005). The 5E model, comprised of five stages (engage, explore, explain, elaborate, and evaluate), is known for its efficiency and positive effects on students’ broad-scale academic achievement, attitudes towards lessons, and science process skills (Cakır, 2017).

In order to bring the 5E framework into the context of our microbiology lesson, we integrated a constructivist approach to help students assemble their own knowledge and build a cooperative teamwork dynamic (AAAS, 2009; Arik & Yilmaz, 2020; Glasersfeld, 1987). We used backwards design to create class materials, first defining the learning outcomes and then charting steps to reach the desired level of understanding (Wiggins & McTighe, 1998). We developed activities, discussions and questions that would help students accomplish those outcomes. We integrated a team-based learning experience, proven to foster authentic perspectives to complex problems beyond the scale of individual learning (Michaelsen & Sweet, 2011). This established team-based approach, together with active-learning, helps underrepresented students succeed in STEM courses (Freeman et al., 2014; Snyder et al., 2016). This is particularly important at UC Merced, a Hispanic Serving Institution with over 70% first generation students.

4.4. Methods

4.4.1. Research Setting

4.4.1.1. The University of California, Merced

UC Merced is the first research university built in the 21st century in the United States and serves the predominantly underserved communities of California’s San Joaquin Valley. UC Merced holds a diverse student population, with 87% of students from traditionally underrepresented groups. The university has over 8,800 students and is designated as both a Hispanic Serving Institution (HSI) and an Asian American/Native American/Pacific Islander Serving Institution (AANAPISI).

4.4.1.2. Participants

Our student population included 89 students in the Fall 2019 course and 90 students in the Spring 2020 course ($n = 179$). The predominant racial and ethnic make-up of the combined cohorts consisted of 34.1% Asian and 33.5% Hispanic/Latinx, reflecting the University's HSI and AANAPISI designations. The remaining student population in the course was comprised of 2.8% American Indian/Alaska Native, 5% Black, 3.4% Native Hawaiian/Pacific Islander and 15.6% White. The cohort is primarily comprised of female students (65.4%). The mean age is 21 years of age. The primary difference between the cohorts is class level; the Fall 2019 class was mostly Senior students (93%) while the Spring 2020 cohort had 31% Juniors and 69% Seniors. Students GPA did not differ significantly between the two cohorts ($t(177) = -1.22$, $p = 0.22$), being 2.89 for Fall 2019 and 2.96 for the Spring 2020 (Table 1).

4.4.1.3. Format of General Microbiology at UC Merced

General Microbiology (BIO120) is an upper division course taken primarily by biology majors, and it is a graduation requirement for students in the Microbiology and Immunology emphasis track. The course requirements include General Biology, Molecular Biology, and Cellular Biology. The most relevant content to General Microbiology that students learn in these pre-requisite courses includes the overall organization and structure of cells, principles of metabolism and nutrient cycles, as well as the function of enzymes.

General Microbiology meets twice a week for 75 minutes in the TEAL lab, limiting the class size to 90 learners. The teaching team includes the instructor of record (García-Ojeda), one co-instructor (Shay), and one undergraduate learning assistant (Solis). This course was transformed into a flipped, team-based learning class in Fall 2015 and converted into a hybrid course in Fall 2019. The hybrid model used in this class consist of in-person flipped lectures with online discussion sections (Supplementary Material 5). The academic year 2019–2020 was the first full year to incorporate all learning and teaching techniques discussed in this manuscript.

4.4.2. Learning Outcomes

We chose the global issue of environmental pollution by paper-based products to teach students about the role of microorganisms in paper waste management. At this point in the course, students have been introduced to the history and fundamentals of microbiology, the structures and organization of microbial cells, the evolution and diversity of microbes, microbial motility, microbial growth, nutrient transport, and the nitrogen cycle. This section of the class focuses on carbon acquisition as well as how microbes secrete exoenzymes to hydrolyze macromolecules. Specifically, students learn about the process of bacterial protein secretion, and how different exoenzymes break down lipids, nucleic acids, proteins, and carbohydrates. Students are then asked to connect the process of active nutrient transport via symporters, ABC transporters, or phosphoenolpyruvate-carbohydrate phosphotransferase system (PTS). Lastly, students demonstrate how the transported molecules are further modified by converting enzymes and then metabolized.

4.4.2.1. General Microbiology Course Learning Outcomes

The General Microbiology Course Learning Outcomes were designed to integrate previous learning and develop advanced scientific skills such as research and critical thinking. The following course learning outcomes are related to this activity:

1. Recognize microbiological concepts and terms used in the primary scientific literature and to communicate with other microbiologists and scientists.
2. Discern the molecular, metabolic, structural, and ecological differences between microbial cells and be able to explain how these differences allow microbes to a) live in almost any environment on earth, b) sense, react, and interact with their environment as well as with other organisms, and c) serve as tools for science, medicine, and industry.
3. Synthesize knowledge gained in previous courses and apply it to novel microbiological questions.

4.4.2.2. *Carbon Acquisition Lesson Learning Outcomes*

This lesson has the following learning outcomes:

1. Given the biotic and abiotic sources of carbon and carbon-containing compounds, illustrate the biological flow of carbon, starting from an initial, complex carbon-containing molecule to a final product (CO₂ or fermentation product).
2. Illustrate the enzymatic reactions that hydrolyze carbohydrates, nucleic acids, lipids, and proteins, and identify the various exoenzymes involved in this process.
3. Identify how different microbes secrete important proteins.

For this lesson, we focused on the first two outcomes by asking students to work with a complex carbon-containing product: paper. Students work through the process of carbon flow, from the initial biomolecule carbon source in paper (cellulose) to the final metabolic product (CO₂ or a fermentation product) within Gram-negative bacterial cells. Students build an understanding of the overall carbon cycle and how biomolecules are hydrolyzed by exoenzymes secreted by bacteria. Exoenzyme secretion reinforces learning outcome 3, where students learn about the Sec and Tat translocators as well as the Type V secretion system and related proteins. Students also connect this lesson to previously taught material concerning the transport of monomeric sugar molecules across the cell membrane via PTS transporters as well as the catabolic reactions needed to extract energy under aerobic or anaerobic conditions (glycolysis, Entner-Doudoroff Pathway and Krebs cycle). Lastly, this lesson reinforces previously learned concepts of bacterial cell wall structure, proteins, cell membrane and their functions.

4.4.3. **Pedagogical Format**

4.4.3.1. *Combining Strategies: Carbon Assimilation Group Exercise Using the 5E Model*

The novelty of this pedagogical approach is the combination of strategies which seamlessly merge our highly active and collaborative learning initiatives. For this particular 75-minute flipped lesson, students prepare by watching online videos about the carbon cycle, protein secretion systems, exoenzymes, and converting enzymes (for slides of this lecture, see Supplementary Material 1.1). They are also encouraged to listen to a podcast titled “How much of our stuff actually gets recycled?” (Brand, 2018).

A detailed summary of the lesson’s timeframe can be found in Supplemental Table 1. For the *engage* phase of the 5E model, students spend the first 8 minutes of class taking a readiness assurance quiz (Supplementary Material 2). The quiz is followed by a short discussion of the lesson’s learning outcomes (2 minutes) and a 5-minute in-class lecture where students are introduced to current recycling challenges resulting from the adoption of *National Sword*, a 2018 recycling policy instituted by China, as well as a similar policy by India (Staub, 2020), that banned the import of most recycled materials and set strict contamination limits on recyclables (Allan, 2018). These nations used to purchase the majority of recycled paper from the USA

(Hamel, 2018), which now piles up in landfills. To maintain their engagement and bring home the effects of this policy, the class is asked “*What type of paper products would be rejected under China’s National Sword policy?*” After a 5-minute discussion, students reply that most paper products contaminated with food, such as pizza boxes, soiled newspaper and paper towels would be rejected. They further conclude that these paper products would end up in a landfill (for slides used during this flipped lesson, see Supplementary Material 1.2).

This introduction is followed by a short 5-minute lecture on how cardboard and paper products originate from the processing of plants and trees. A figure from Bayer and colleagues (2007) is used to illustrate the fate of plant material processed by the paper industry, which generates paper products for human consumption. Once used, these products end up in municipal solid waste facilities where some are recycled, composted, or incinerated. The large majority end up in landfills becoming environmental pollution (Bayer et al., 2007).

Before they reflect on the material, students spend a few minutes answering a metacognitive survey question “*Before today, to what extent did you understand the role played by microbes in the biodegradation of paper waste products?*” via clickers (Supplementary Materials 1.2). Following this, students are asked to predict what happens to paper in a landfill, selecting one of the following options in a clicker question: “*It remains in the landfill, as paper is not degradable,*” “*It will decompose by microbial activity involving respiration,*” “*It will decompose by microbial activity involving fermentation,*” and “*Something else will happen...*”. Together, these questions provide a baseline assessment of the students’ understanding of the role played by microorganisms in the biodegradation of paper waste products (Figure 1C, Supplementary Figure 2A).

For the *explore* phase, students spend 10 minutes researching and reflecting on different aspects of the molecular composition of paper, the microbial communities that degrade paper, the process of exoenzyme secretion, cellulose hydrolysis, and glucose transport and discuss their findings with their table mates. Each student group is divided into 2 subgroups, and each subgroup is tasked with providing the answer to 3 questions (Supplementary Materials 1.2). One subgroup researches the answers to following questions: “*What is the molecular composition of cardboard and paper?*”, “*Which microorganisms would degrade cardboard and paper?*”, “*Are these biochemical processes happening aerobically or anaerobically?*”, and “*Which exoenzymes would these organisms use?*”. The second subgroup researches the questions: “*How would these exoenzymes be secreted?*”, “*How would the products of the exoenzymatic reactions be transported into the cytoplasm of the bacteria?*”, and “*Once in the cytoplasm, what biochemical processes would be used in catabolic reactions?*”. For the *explain* phase, the instructors help students synthesize their new knowledge and clarify misconceptions during a 10-minute class discussion where students from different tables discuss the answers to these questions as a whole class.

In the second part of the activity, the *elaborate* phase, students spend 15 minutes applying their knowledge by drawing the entire paper-degradation process in their table groups. The drawing must include the main components of exoenzyme secretion, the enzymatic hydrolysis of cellulose happening outside the cell, the transport of glucose into the cell, and finishing with glycolysis (Figure 2). After discussing their drawings, students are again asked to predict what happens to paper in a landfill, and their responses are recorded using clickers (Figure 1C, Supplemental Figure 2B). Finally, the class spends the last few minutes discussing their predictions and editing their figures to present their final drawing online.

4.4.3.2. Data Acquisition, Analysis, and Statistics

Students are *evaluated* at multiple points. Formative assessments include metacognitive questions before, during and after class via clickers or the learning management system (LMS), drawing their images as well as re-drawing them after in-class discussion. Summative assessments include the readiness assurance quiz and midterm exam questions related to the topic (Supplementary Materials 2 and 3).

Students in the Spring 2020 semester were given a post-class metacognitive survey via the LMS where they were asked the following metacognitive questions:

1. What was the most confusing concept in today's class?
2. Based on today's work, tell us what you think about the following statement: The power of microbes can be harnessed to reach environmentally sustainable goals.
3. Does today's work illustrate the relationship of microbiology to society? Explain.
4. Tell us how much you agree with this statement: Today's activity did NOT influence my opinion about recycling paper waste.
5. Tell us how much you agree with this statement: After today's activity, I will be able to explain how enzyme secretion and sugar transport are related to cell wall and membrane function.

Students in the Fall 2019 cohort only answered the first question. All statistical analyses were conducted in Microsoft Excel version 16.37.

4.4.3.3. Generation of a codebook

Students responses to the metacognitive question “*What was the most confusing concept in today's class?*” were analyzed for emergent themes in potential missing knowledge, misinterpretation, and misconceptions of class content, following the process described by Offerdahl & Montplaisir (2014). Briefly, two coders (García-Ojeda and Solis) independently read 20 randomly selected student responses (10 from each cohort) for emerging patterns and established an initial code. This initial code was used to code all 157 submissions (From Fall 2019 and Spring 2020 cohorts). The coders then compared and discussed the initial codes for each independent submission, exploring discrepancies in detail, adding new codes, and collapsing or eliminating other codes. This process was done twice more, using the entire sample, to verify and generate a final codebook (Supplementary Material 4). Codes that appeared at least 3 times were kept in the final codebook, consisting of 12 codes in 3 categories. After these discussions, both coders recoded the entire sample. Cohen's kappa values were calculated to determine intercoder reliability (Dewey, 1983; Glen, 2014) using the following equation

$$\kappa = \frac{p_o - p_e}{1 - p_e}$$

where p_o is the relative agreement between both coders, and p_e is the hypothetical probability that agreement was achieved by chance. Kappa values between 0.61 - 0.8 are considered substantial agreement, while values between 0.81 - 0.99 are considered near perfect agreement (Landis & Koch, 1977).

Jaccard coefficients have been used to establish the level of consistency between coders (Smith et al., 2013). We calculated the Jaccard coefficient T using the equation $T = n_c / (n_a + n_b - n_c)$, where n_c is the number of times a statement was coded the same by both coders, n_a is the number

of times a statement was coded the same by both coders plus the times it was coded by coder 1 but not coder 2, while n_b is the number of times a statement was coded the same by both coders plus the times it was coded by coder 2 but not coder 1. Jaccard coefficients closer to 1 are indicative of high consistency between coders (Niwattanakul et al., 2013).

4.4.3.4. Word Cloud Analysis

Word clouds can be used to investigate patterns in text data (DePaolo & Wilkinson, 2014). For metacognitive questions 2 and 3 (see *Data Acquisition, Analysis, and Statistics* section for Metacognitive Topic Questions), we identified and ranked key topics emergent in the students' answers by using the online software wordclouds.com (<http://wordclouds.com/>). Briefly, we transferred the text responses from the LMS to Microsoft Word and identified and deleted words and phrases that were directly taken from the questions. Such language included phrases like “the power of microbes” or “the relationship of microbiology to society.” We also expunged phrases like “I agree because”. We then uploaded these .docx files into the wordclouds.com website to create the initial word clouds and produce a term table with the term's respective weights, shown in parenthesis (#, Supplementary Table 2).

Using the term tables, we narrowed down the number of terms in the word cloud by identifying terms that had similar iterations or meanings and adding their respective weights. For example, the terms Microbes (11), microbes (61) and microbe's (2) were collapsed into the term microbes (74). Words that had meanings unrelated to the question were also eliminated, such as “yes”, “yet”, “terms”, etc. This process reduced the number of terms in the Power of Microbes word cloud from 575 to 126, while reducing the number of terms in the Microbes and Society word cloud from 722 to 248. To reduce the complexity of the word cloud images, we did not include in the illustration terms that appeared less than 4 times.

4.4.4. Adapting to the COVID-19 pandemic

The COVID-19 pandemic escalated a few weeks into the Spring 2020 semester and university campuses worldwide shut down to prevent the spread of the SARS-CoV2 virus (Richardson, 2020). Since this course is a flipped, hybrid class, transitioning to Emergency Remote Instruction (ERI) required minor adjustments. Although the activities described here were deployed in-person before transitioning to ERI, the exam evaluating the content of this activity was administered online. The Spring 2020 students had to navigate the transition period only a week before they took the exam discussed in this paper.

To deter cheating, the exam was open book, open note, timed and delivered via the LMS using Respondus Lockdown Browser® without the Respondus Monitor®. The exam questions were written to build upon foundational knowledge, which require students to use higher order thinking to answer them correctly. Therefore, the answers to these questions could not be easily found online. To clarify exam misconceptions and answer students' questions, the instructors were available via Zoom during the entire exam period.

4.5. Results

4.5.1. Formative Assessments

4.5.1.1. Role of microorganisms in paper degradation.

During their pre-class metacognitive survey, we asked students to evaluate their understanding of the role of microbes in paper biodegradation. The majority of students demonstrated improved understanding after the activity (Figure 1). Before the activity, over half of students (51%) reported “do not understand at all” or “understand a little,” while 41% reported “somewhat understand” and 8% reported “understand a lot” (Figure 1A, blue bars). After the activity, the vast majority of students reported, “understand a lot” (66%) or “completely understand” (3%) while only 28% reported “somewhat understand” and few (2%) reported “understand a little” (Figure 1A, orange bars).

Using Alluvial plots (Figure 1B), we examined the changes in reported understanding of the material by comparing their responses at baseline and after the lesson. Most students reported improvement in their understanding of the material. Some students who initially reported “do not understand at all” (Figure 1B, light blue) reported “understand a little” after the lesson, while the majority of students in this baseline response reported either “somewhat understand” or “understand a lot” after the lesson. The group of students who initially answered, “understand a little” (Figure 1B, lilac), split equally to reporting “somewhat understand” and “understand a lot” after the lesson. The students initially reporting “somewhat understand” (Figure 1B, light green) reported “understand a lot” after the lesson. From students (8%) who reported “understand a lot” at baseline (Figure 1B, rose), subsequently reported “completely understand” and “understand a lot” after the lesson. Taken together, our data indicate that students perceived gains in their understanding of the role of microbes in paper biodegradation after completing the lesson.

4.5.1.2. Mechanism of paper biodegradation

Before starting the activity, students were asked to predict the fate of paper in a landfill via a multiple-choice clicker question. Paper buried in a landfill would be degraded primarily by anaerobic mechanisms (Pommier et al., 2010). Initially, the majority of students in the Spring 2020 semester (71%) predicted that respiration played a role in paper biodegradation in landfills (Figure 1C). About 18% stated that paper would be degraded via fermentative pathways, and 11% predicted that something else would happen (Figure 1C). When asked the same question after the activity, students in the Spring 2020 cohort changed their prediction. Only 41% predicted that paper would be degraded via respiratory mechanisms, while the great majority (57%) stated that fermentation would be the primary pathway of paper degradation (Figure 1C). Fewer students (2%) were unsure about the fate of paper in landfills after the activity than before. The greatest transition in answers shifted from students predicting that respiration would play a role to predicting that fermentation would play a role (Supplementary Figure 2B (light green)). Taken together, the majority of students correctly predicted that paper would be degraded anaerobically via fermentative processes after participating in the lesson.

4.5.1.3. Active Learning Group Exercise: Drawing the enzyme secretion, macromolecule degradation and nutrient transport pathways

Students demonstrated their overall understanding of class material in a group formative assessment, where they drew the processes of enzyme secretion, cellulose degradation, glucose transport and glucose metabolism (Figure 2). This activity was designed to evaluate the extent by which groups of students engaged in model-based reasoning by using a drawing-to-learn approach (Quillin & Thomas, 2015). A group with high mastery of the material would place the Sec/Tat protein secretion systems across the cell membrane, the Type V Secretion System and the porin channels in the outer membrane, and the glucose phosphotransferase system (PTS) across the cell membrane (compare Figure 2B and Figure 2C). Their drawings will also show the phosphorylation of glucose to glucose-6-phosphate after transport via the PTS system, leading

directly to its hydrolysis via glycolysis. Moreover, they would illustrate cellulose as a polymer as well as have appropriate labels for the cellulase enzyme and other components.

4.5.1.4. Metacognitive Topic Questions

4.5.1.4.1. Students identified the topics most confusing to them.

Question 1: What was the most confusing concept in today's class?

To evaluate this question, we established and validated a 12-item codebook (Supplementary Materials 4) to explore emerging patterns in students' responses. These codes were subdivided into 3 categories: 1) Concepts, 2) Competencies, and 3) Affect. Table 2 shows the frequency (f) of these codes for each semester, as well as the Jaccard coefficient (T), and Cohen's kappa ($\kappa \pm SE$) values evaluating inter-rater reliability. The high Jaccard coefficient (≥ 0.92) and Cohen's kappa values (≥ 0.81) indicate a high level of consistency between the two coders.

The Concepts category includes codes that deal with students' questions about class content. In this category, students from both semesters primarily identified Protein Export as the most confusing concept. This code was used 19 times by Fall 2019 students and 27 times by Spring 2020 students. Biochemistry and Misconceptions were more represented in the Fall 2019 cohort compared to the Spring 2020 cohort. To a lesser degree, but with similar frequencies, both cohorts identified Exoenzyme Activity, Location, and Nutrient Transport as confusing topics.

The Competencies category includes codes that describe skills students ought to master over the course of the lesson. Under this category, Big Picture was the most common code (overall frequency of 39 times), found more frequently in the Spring 2020 cohort. Illustration was also frequently cited, (20 times) with the Fall 2019 cohort displaying this code at a higher frequency. Some students in both cohorts reported having challenges with Time to complete the activities (3 times total).

Although not prompted by the question, students from both cohorts expressed ideas about feelings of self-improvement in conceptual understanding or skills. Some students also expressed feelings of concern about their understanding or feared potential negative consequences for not understanding the material. To reflect the frequency of these statements regarding feelings, we created a category of Affect (Table 2). Improvement was the most frequently used code in this category, with both cohorts displaying similar frequencies for this code. Similarly, Concern was also equally represented to a lesser degree in both cohorts.

4.5.1.4.2. The power of microbes and the relationship of microbiology to society.

Question 2: Based on today's work, tell us what you think about the following statement: The power of microbes can be harnessed to reach environmentally sustainable goals.

Concerning the power of microbes to reach environmentally sustainable goals, the 10 most used terms (in order of weight) included waste, help, degradation, break, paper, landfill, recycle, clean, decompose and microorganisms (Figure 3A and Supplementary Table 2). The frequency of use for these terms indicates that students perceived microorganisms as a clean alternative to help the degradation (break or decompose) of waste in landfills. The following student responses were selected because they represent the breadth of perspectives and backgrounds of the students:

“...Microbes are definitely beneficial for sustainability ... One example can be like bioremediation where microorganisms aid in cleaning air, soil, and water.”

“...We could potentially use bacteria to degrade cardboard that does not fall under conditions to be recycled by parties in other countries. We would be able to continue working on sustainability efforts of decreasing soiled cardboard and paper pollution.”

“...In the lecture prep videos we were able to see the importance of degradation of oil and petroleum by bacteria using oxygenase for bioremediation.”

4.5.1.4.3. Students understand the connection to of this microbiology lesson to society.

Question 3: Does today's work illustrate the relationship of microbiology to society?

Concerning the relationship of microbiology to society, students connected the activity to four overall societal applications, 1) waste management, 2) sustainability and environmental solutions to pollution, 3) biodegradation as a tool, and 4) energy cycles essential for human life (Figure 3B and Supplementary Table 2). In particular, students reported that the activity helped them understand the real-world implications of the biology they were learning:

"... Listening to the newscast about the garbage problem with China really made it apparent to me how important it is for us in America to find a reliable, safe, and sustainable solution to the ever-increasing garbage problem. I see how microbes can be used to help break down certain types of waste and I am very curious to see if there will be newer research and findings regarding microbe usage in the waste management sector."

“The activity helped me to connect everything together especially by relating it to the current problem we have with paper not being properly recycled. The activity helped me envision how paper was broken down via cellulase enzyme then brought in by porins and PTS systems and through catabolic reactions we harvested energy. The activity made everything full circle for me.”

4.5.1.4.4. Students agree: the lesson influences their opinion about paper recycling

Question 4: Tell us how much you agree with this statement: Today's activity did NOT influence my opinion about recycling paper waste.

To evaluate if the lesson influenced the students' opinion about paper recycling, we deployed the above 5-point Likert scale question via LMS. Most students either Strongly disagreed (33%) or Somewhat disagreed (36%) that the activity did not influenced their opinion about recycling paper waste (Figure 4A). About a fifth of the students (22%) Neither agreed nor disagreed with the statement, while less than 10% of the students Somewhat agreed or Strongly agreed.

4.5.1.4.5. Students agree: the lesson increases their confidence to explain the relationship between enzyme secretion, sugar transport to cell wall and membrane structure.

Question 5: Tell us how much you agree with this statement: After today's activity, I will be able to explain how enzyme secretion and sugar transport are related to cell wall and membrane function.

We evaluated the students' confidence in their ability to explain the relationship between enzyme secretion and nutrient transport to cell wall and membrane structure. After the lesson, we asked students to rate how much they disagreed or agreed with the question above. Most students (81.8%) reported that they could explain how enzyme secretion and sugar transport are related to cell wall and membrane function. About 9% of the students were neutral about this statement, while very few of them either "strongly disagreed" (4.5%) or "somewhat disagreed" (4.5%) with the statement (Figure 4B).

4.5.2. Summative Assessments

4.5.2.1. Readiness Assurance Quiz

During the first 10 minutes of class, students took a 5-point quiz containing 5 multiple choice questions to test their comprehension of the video lectures (Supplementary Materials 1.1). Both cohorts of students performed similarly on the quiz ($t[176] = 0.44$, $p = 0.66$). The Fall 2019 class scored 3.6 ± 1.1 points (mean $\pm$ SD, 71%) and the Spring 2020 class scored 3.5 ± 0.9 points (70%). Students were successful at understanding how exoenzymes hydrolyze polymers (questions 3–5) but were less successful in describing where in the cell these processes are happening and how the materials are transported (questions 1–2).

4.5.2.2. Midterm Exam

The midterm questions were designed to assess the students' mastery of the lesson's core concepts (Crowe et al., 2008) and have evolved over the development of the course. All exams for this course are open-ended with brief essay responses. For context, after making the course hybrid in Fall 2019, the exams shifted from asking low-order questions to asking students higher-order synthesis questions about the entire process of macromolecule degradation (Supplementary Material 6). In Fall 2018, students averaged 71% on the questions relevant to this lesson, providing basic answers about exoenzyme identification and secretion systems (data not shown). However, students did not demonstrate mastery of the entire macromolecule degradation process. In Spring 2019, students were challenged to describe the degradation process of a protein without the problem-based learning exercise. Their answers averaged 31% for these questions (data not shown). Overall, students from the pre-hybrid course demonstrated a rudimentary understanding of the processes of macromolecule degradation but were challenged when connecting macromolecule degradation to a non-carbohydrate substrate. This demonstrates a breakdown of knowledge with the macromolecule degradation process, thereby inspiring the shift of the questions to the higher-level processes assessed here.

In the exam questions pertaining to this lesson, students are asked to detail the process of decomposition and metabolism of a triglyceride to their final carbon-based products (CO_2 or a fermentation product). This shift in substrate in the exam, from cellulose used in the lesson, would allow us to ascertain if students transferred the knowledge obtained in the lesson to a new scenario (Nokes-Malach & Mestre, 2013). The exam question had four parts assessing students' mastery of protein secretion, exoenzyme function, nutrient transport and metabolism. For both semesters, these exam questions were graded by the same grader, using the same key, and the same rubric (Supplementary Materials 3). These two cohorts were the first to be assessed with this question.

When comparing the entirety of the exam, both cohorts performed similarly ($t[176] = 1.08$, $p = 0.28$). The Fall 2019 cohort had a mean score of 77% and the Spring 2020 had a mean score of

74%. Since Fall 2019, students do not keep their exams after reviewing the material in discussion so there is little chance that the Spring 2020 cohort had access to exam questions ahead of time. Both cohorts demonstrated similar comprehension of the exoenzyme secretion process (Figure 5A). Students in the Spring semester cohort performed better than the Fall semester cohort in the question concerning Exoenzyme function (Figure 5B). On the other hand, Fall semester students performed better in the question relating to nutrient transport (Figure 5C). Although the Spring 2020 students performed better, both cohorts had challenges identifying the products of triglyceride degradation (glycerol and fatty acids) and placing these within the beta oxidation pathway to generate Acetyl-CoA (Figure 5D).

4.6. Discussion

4.6.1. Practical Implications and Lessons Learned

Peer-led, team-based learning is known to give students an opportunity to develop positive interdependence, scientific reasoning, critical thinking, and communication skills (Scager et al., 2020; Trempey et al., 2002). Additionally, peer-led learning provides a more inclusive and supportive learning environment, particularly to underrepresented students who come from culturally interdependent communities (Covarrubias et al., 2016). General Microbiology utilizes project-based learning in the TEAL classroom environment to facilitate the exploration of real-world scenarios (Dourmashkin et al., 2020) and help students synthesize their own understanding of material (Leupen, 2020). This lesson helped students connect first-hand with the global impact of paper pollution and the role microbes play in the biodegradation of paper waste. Students demonstrated a deep understanding and increased awareness of the societal issues surrounding paper waste management and sustainability

Illustrating the entire biodegradation process of cellulose was the most challenging part of the activity, as students are required to engage in systems thinking, bringing together the material not only from this lesson, but from two other previous lessons (cell wall structure and nutrient transport). This activity revealed misconceptions in students' understanding of the material (Figure 2A, 2C). Some students found it difficult to differentiate between the Sec and Tat protein translocation system, centered around a knowledge breakdown between which system translocated unfolded (Sec) versus folded (Tat) proteins. Furthermore, students were confused by which protein secretion system was used to transport proteins across the outer membrane (Type V Secretion System) and where these secretion systems were located in the cell envelope. This in-class evidence was confirmed through the coding of the post-class metacognitive response to "*What is the most confusing concept in today's class?*", where Protein Export was the most commonly represented code, followed by Big Picture and Illustration (Table 2). Another misconception centered on the transport of cellulose, as some students misidentified cellulose as a monomer and missed including glucose in their diagrams. Concerning nutrient metabolism, some students had challenges ascertaining the biochemical pathways utilized to digest glucose monomers as a source of energy. These topics also surfaced in the coding of the answers to the metacognitive question "*What is the most confusing concept in today's class?*", where Biochemistry, Nutrient Transport and Exoenzyme Activity codes were abundantly represented (Table 2). Combined, these misconceptions demonstrated a general challenge with visualizing structure/function relationships in the cell envelope. This informed us of the importance of emphasizing the structural components of the lesson for future students. In the next iterations of the class, we will prime students to review these topics during the preparatory stage of the carbon

cycle lesson. In this way, the aspects that are least understood to the class would be emphasized during individual lectures and incorporated into activities to solidify this knowledge.

Overall, students were effective at explaining how exoenzymes are secreted (Figure 5A), their role in macromolecule hydrolysis (Figure 5B) as well as the transport of their fatty acid products into the cell (Figure 5C) but were less effective at connecting the biochemical pathway for fatty acid metabolism to energy production. We strategically chose to ask students about triglycerides hydrolysis instead of cellulose to provide them the opportunity to demonstrate their ability to transfer the knowledge they gained from the cellulose lesson to the triglyceride exam question (Nokes-Malach & Mestre, 2013). In this regard, students showed a breakdown of knowledge in the level of completion in their answer regarding the specific pathway by which fatty acids are processed (Figure 5D). For example, some students would correctly state that fatty acids are processed into acetyl-CoA via beta oxidation but could not follow through connecting acetyl-CoA to the Krebs cycle and the electron transport chain. Some students mentioned the Krebs cycle and electron transport chain, but not the beta oxidation pathway. This breakdown may reflect the fact that these questions were the most challenging on the exam and required students to think at a higher-cognitive level than other questions. Moreover, students did not have the opportunity to practice transferring knowledge of the various macromolecule degradation processes from one substrate to another before the exam. This signaled to the instructors to consider providing more opportunities to practice this skill, particularly in the asynchronous discussions and homework. While the exam scores were low, they were an improvement from the scores of the pre-hybrid Spring 2019 cohort, which did not have the aid of the problem-based learning exercise (data not shown). This demonstrates an improvement between the pre-hybrid and hybrid cohorts when approaching higher-cognitive level questions.

Based on their formative assessment results, students showcased evidence of their learning and achievement of the lesson's learning outcomes. This is illustrated in the transition in predictions about the fate of paper in a landfill (Figure 1C, and Supplementary Figure 2). Most students came to class incorrectly believing that respiration played a role in the biodegradation of paper waste products. By the end of the activity, most students stated that fermentation plays a primary role in this process. However, a good proportion of students (41%, Figure 1C) still stated that paper would be degraded via respiratory processes. We hypothesize that the lack of clear understanding of landfill architecture as well as images used during the introduction to the activity of paper exposed to air in landfills might have influenced student's answers (Supplementary Material 1.2). In future iterations of the class, we will ensure to reinforce these concepts in the pre-flipped lecture preparation material.

Students were able to visually work through the mechanism of nutrient transport and correctly illustrate the biochemical processes accurately. Misconceptions of the structural organization of these processes were identified quickly and resolved through visual exploration and discussion (Schnotz, 2014). Our metacognition survey demonstrated that students self-identified their growth in understanding before and after the activity (Figure 4B). The students synthesized and demonstrated their understanding through the summative assessments, being able to recall the process of protein secretion and nutrient absorption but struggled to transfer knowledge from one polymer (cellulose) to another (triglycerides). Future class iterations will provide discussion forum questions and homework that will give students more opportunities to examine a variety of macromolecules and work through the entire biochemical pathways needed for their degradation.

4.6.2. Conclusions and Recommendations

Using a variety of teaching strategies and technology, this lesson departed from a traditional teaching model by giving students an opportunity to address a real-world problem. Students demonstrated their learning by building their own systems-level understanding of the microbiological and biochemical processes involved in the breakdown of paper. This lesson was facilitated by, and took advantage of, the TEAL lab learning environment and a hybrid online model, both components that may not be accessible to all universities. The TEAL lab facilitates the use of active learning, but it is neither required to use these strategies nor required to create a collaborative learning environment. There are many ways to foster collaborative learning including structured discussion, reciprocal teaching, and problem-solving (Barkley et al., 2014) that are independent of the TEAL lab. For courses that do not have access to online resources, face-to-face interactions can produce statistically similar grades from online learning as well as increased levels of student satisfaction (Summers et al., 2005).

There are some inherent challenges that come with implementing a flipped lesson with evidence-based teaching strategies of this nature. It takes a significant amount of time to prepare each component, which requires intention and alignment with the learning outcomes to be effective for student learning and engagement. Additionally, these active learning strategies may be new to instructors accustomed to traditional teaching and may require additional training to model effectively. To facilitate the amount of activity in the given time, the timing during flipped lecture needs to be managed closely to incorporate all the components of a 5E model lesson. Timing itself could be a limitation if courses are less than the 75 minutes discussed here and if instructors feel there is not enough time to cover the content with active learning (Graffam, 2007). This lesson may be difficult to implement in a large (>200 person) class and may require additional levels of organization or facilitation. Furthermore, there may be systemic resistance to incorporating active learning techniques (Bathgate et al., 2019) that faculty may need to overcome without reward (Michael, 2007).

In order to increase content retention and reduce misconceptions, students could perform a pre-lecture homework where they can review the materials discussed in the lesson's video lectures as well as from previous coursework. We found that, overall, students improved their understanding of the concepts after the 5E lesson. Where some students struggled was drawing the structures related to the biological processes. We recommend that instructors incorporate more model drawing activities to help identify misconceptions and provide students with an opportunity to promote reasoning skills (Quillin & Thomas, 2015). We also recommend offering a variety of examples for students to practice transferring their knowledge from one example to another. Lastly, we recommend and encourage faculty to center activities, when possible, on real-world scenarios that are relevant to students, especially scenarios that impact underserved student communities, to connect the concepts and their implications to student experiences (Harackiewicz et al., 2016).

4.7. Summary

Today's students suffer from the burden of climate change and global pollution and need to develop skills to think critically about these problems. Problem-based learning can engage students in real-world scenarios while simultaneously learning complex microbiological and

biochemical concepts. By using deliberate teaching strategies, outlined in this lesson, we demonstrate increased student conceptual understanding and perceived understanding of microbial carbon assimilation and its role in paper waste degradation. We coded student responses from a reflective survey and identified common misconceptions and perceived gains. Student performance was measured through a variety of assessments including a drawing activity and exam questions. We were able to determine areas where student performance could improve and address them accordingly. We recommend instructors consider using real-world scenarios when teaching complex topics to foster student engagement and interest in the topic in and beyond the classroom.

4.8. Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

4.9. Ethics Statement

The studies involving human participants were reviewed and approved by UC Merced Institutional Review Board. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

4.10. Acknowledgments

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4.11. Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmicb.2020.588918/full#supplementary-material>

4.12. References

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Figures

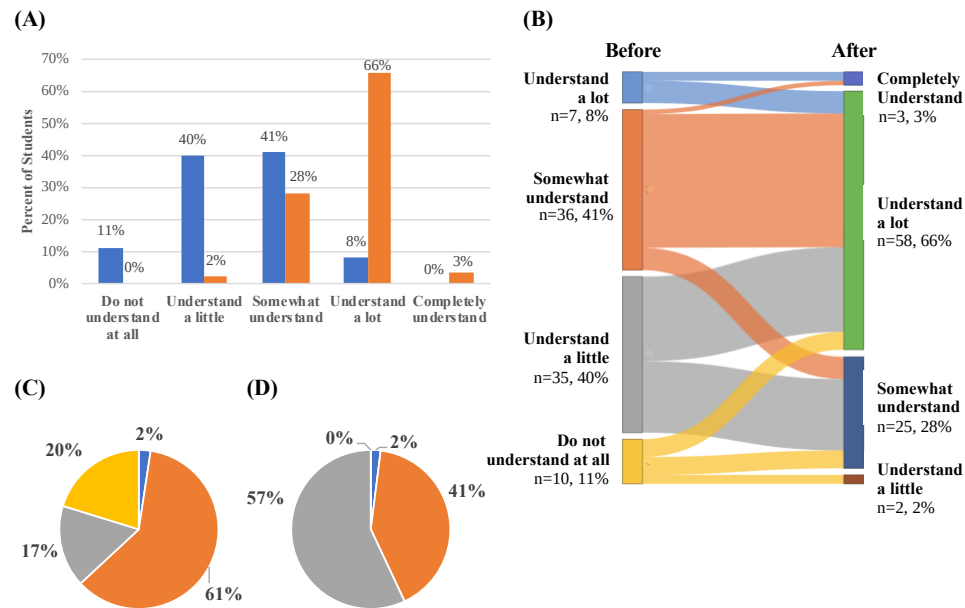


Figure 1. Comparison of student’s responses to metacognitive questions before and after the activity. (A) Students were asked the question “*To what extent did you understand the role played by microbes in the biodegradation of paper waste products?*” before and after the class activity. The graph displays the responses before the activity (blue bars) and after the activity (orange bars) for the Spring 2020 semester (n = 88). This 5-point Likert survey question was deployed via clickers before the activity or via the Learning Management System after the class. Numbers above the columns represent the percent of students selecting a response. (B) Alluvial plot mapping the change in prediction before and after the activity from students in Spring 2020. The left column indicates the predictions before the activity, while the right column indicates the predictions after the activity. Only statements included in the data are shown. (C) Pie chart showing student’s predictions of the fate of paper before and after the activity. After a brief introduction, students were asked to predict the fate of paper in a landfill via a multiple-choice clicker question. The answer choices included “*It remains in the landfill, as paper is not degradable.*” (blue), “*It will decompose by microbial activity involving respiration.*” (orange), “*It will decompose by microbial activity involving fermentation.*” (gray), and “*Something else will happen...*” (yellow). The graphs display the before (left pie) and after (right pie) responses for the Spring 2020 semesters (n = 88).

[illegible]

Figure 3. Word clouds illustrating terms in the responses to metacognitive questions. A) Microbes and environmental goals. Following the activity, students were asked the question: *Based on today's work, tell us what you think about the following statement: The power of microbes can be harnessed to reach environmentally sustainable goals.* B) The relationship between microbiology and society. Following the activity, students answer the question: *Does today's work illustrates the relationship of microbiology to society? Explain.* Both of these free-response survey questions were deployed via the Learning Management System after the class. Text from the student's responses was organized using a word cloud software that highlights, by word size, how often a term was used. The images display the responses from the Spring 2020 semester (n = 88). To simplify the image, only terms used at least 4 times are shown.

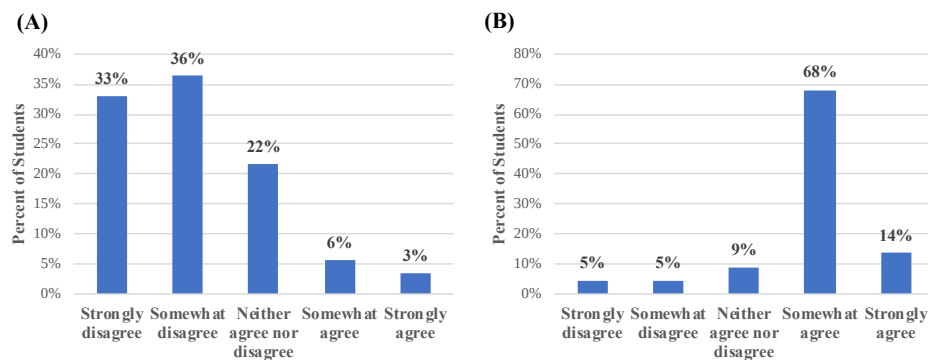


Figure 4. (A) The activity strongly influences student's opinions about recycling. Following the activity, students were asked: "Tell us how much you agree with this statement: *Today's activity did NOT influence my opinion about recycling paper waste.*" (B) The activity strongly enhances student's understanding of enzyme secretion and nutrient transport. Following the activity, students were asked: "Tell us how much you agree with this statement: *After today's activity, I will be able to explain how enzyme secretion and sugar transport are related to cell wall and membrane function.*" These 5-point Likert survey questions were deployed via the Learning Management System after the class. The graphs display the responses from the Spring 2020 semester (n = 88). Numbers above the columns represent the percent of students selecting a response.

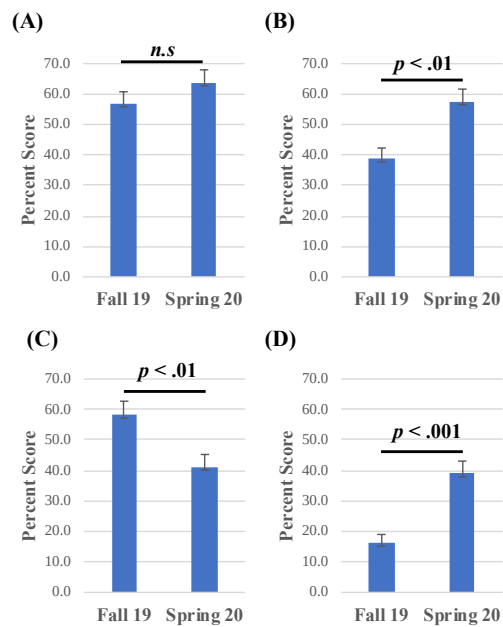


Figure 5. Comparison of exam performance in summative assessment questions for the Fall 2019 and the Spring 2020 cohorts. All graphs compare the mean percentage score and error bars are the standard error of the mean. (A) Question 5a: Protein Secretion, $t(177) = -1.20$, $p = 0.232$. (B) Question 5b: Exoenzyme Function, $t(177) = -3.24$, $p = 0.001$. (C) Question 5c: Nutrient Transport, $t(177) = 2.63$, $p = 0.009$. (D) Question 5d, Metabolism, $t(177) = -4.68$, $p < .00001$. Bar graphs display the responses for Fall 2019 ($n = 89$) and Spring 2020 semester ($n = 90$). All statistics are unpaired, two-tailed, Student's t -Tests. For details on the questions and their answers, please see the Supplementary Materials.

Tables

Table 1. Student participant demographics.

	Fall 2019		Spring 2020		Overall	
	n = 89		n = 90		n = 179	
	n	(%)	n	(%)	n	(%)
Gender						
Male	26	(29.2)	36	(40.0)	62	(34.6)
Female	63	(70.8)	54	(60.0)	117	(65.4)
Race/Ethnicity						
American Indian/Alaska Native	2	(2.2)	3	(3.3)	5	(2.8)
Asian	28	(31.5)	33	(36.7)	61	(34.1)
Black	5	(5.6)	4	(4.4)	9	(5.0)
Hispanic/Latinx	32	(36.0)	28	(31.1)	60	(33.5)
NH/PI ¹	3	(3.4)	3	(3.3)	6	(3.4)
White	14	(15.7)	14	(15.6)	28	(15.6)
Two or More Races	3	(3.4)	4	(4.4)	7	(3.9)
Declined ²	2	(2.2)	1	(1.1)	3	(1.7)
Age — mean (range)	21.3 (18-23)		21.3 (20-27)			
Class — (%)						
Junior	(7)		(31)			
Senior	(93)		(69)			
GPA ³ — mean±SD	2.89±0.38		2.96±0.42		2.92±0.41	

¹ NH/PI = Native Hawaiian/Pacific Islander. ² Students who declined to report race/ethnicity.

³ Grade Point Average.

Table 2. Codes used to evaluate responses to the question: "What was the most confusing concept in today's class?"

Category ¹	Code	T ²	κ ³	SE (κ)	f_{Fall19}	$f_{Spring20}$	f_{Total} ⁴
Concepts	Biochemistry	0.99	0.96	0.04	10	4	14
	Exoenzyme						
	Activity	0.99	0.89	0.07	5	4	9
	Location	1.00	1.00	0.00	3	2	5
	Misconception	0.99	0.95	0.04	16	6	22
	Nutrient						
	Transport	0.97	0.82	0.09	4	6	10
	Protein Export	0.98	0.95	0.03	19	27	46
	Other	0.99	0.85	0.15	2	1	3
Competencies	Big Picture	0.92	0.81	0.05	14	25	39
	Illustration	0.97	0.89	0.05	12	8	20
	Time	1.00	1.00	0.00	1	2	3
Affect	Concern	0.99	0.89	0.11	2	2	4
	Improvement	0.99	0.96	0.03	12	15	27

¹Categories: Concepts refer to codes that address students' questions about class content. Competencies refer to codes describing skills students master throughout the course. Affect refers to codes associated with student's feelings. ²Jaccard coefficient (T) and ³Cohen's kappa (κ) values were calculated with the combined Fall 2019 and Spring 2020 data ($n = 157$). ⁴Frequency (f) indicates the number of times a code, selected by both coders, appeared in student's responses. Fall 2019 ($n = 72$), Spring 2020 ($n = 85$).

CHAPTER 5

Resilient Instructional Strategies: Helping Students Cope and Thrive in Crisis *

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5.1. Abstract

Recent times of excessive stress call for a reflection and reformation of how people interact and support one another. This is particularly true in science, technology, engineering, and mathematics (STEM) discipline-based education where it is becoming increasingly important for course instructors to adopt student-centered teaching approaches that engage students, maintain rigor, and consider the students' learning experiences, including stress. What are some pedagogical strategies that instructors can draw upon to help students cope with trauma and regain a healthy state of learning in an already challenging field? To prepare instructors for the transition to remote instruction, a variety of evidence-based pedagogical and technological approaches were designed to promote resilient student-centered classrooms and facilitate student development and care in times of crisis. This perspective provides an overview of the salient research behind these strategies, highlights those that instructors found most useful, and concludes with planned next steps in the continued effort to support instructors.

5.2. Introduction

For students in STEM academic programs, the challenges associated with learning the hallmark skills of a successful scientist such as navigating scientific obstacles, persevering through the pressure to perform and publish, and coping with failure (1) are exacerbated by the unique excessive stressors from recent crises (2). The unnatural act of social distancing and increased remote interactions initiated by COVID-19 has dramatically changed living conditions, causing economic-, health-, and education-related hardships. A recent survey of 2086 college students found that remote learning has disrupted students' lifestyles, with most students experiencing anxiety (91%), disappointment or sadness (81%), and loneliness or isolation (80%) (3). Of those surveyed, nearly half (48%) have experienced financial setbacks and forced relocation (56%), and over half of students also reported difficulty a) maintaining a routine, b) getting enough physical activity, and c) staying connected with others (76%, 73% and 63%, respectively) (4). The growing socio-political divisiveness in the United States and ravaging forest fires in the West have intensified the level of trauma, which pose additional disruptions to remote teaching and learning (5). For persons excluded due to ethnicity or race (PEERs), the culmination of these challenges has added to the long-term struggle associated with the systemic consequences of exclusion and underrepresentation in STEM (6). Many low-income, first-generation students also struggle with a lack of quiet workspaces, the absence of internet and other technological tools, housing and/or food insecurity, and the added responsibilities associated with being at home and helping the family (e.g., employment, care, etc.). Stress, anxiety, and traumatic events contribute to students' *cognitive load*, making the focus on learning more challenging. *Cognitive load theory* refers to the number of resources in working memory that are used for processing, encoding, and recalling new information (7, 8). Under high intensity conditions, the human brain manages by developing a system to tolerate or minimize excessive stress and overrides cognitive sources of

energy deemed less of a priority, such as learning (9). Specifically, traumatic incidents interrupt executive functioning and self-regulation (10), making it harder for students to plan, remember and focus (11), which often results in lower performance and reduced student engagement (12–14). Given the impact of stress on learning in STEM, how can instructors help students cope and thrive in these unprecedented conditions?

5.3. Pedagogies that help students cope

Teaching requires developing a safe and flexible learning environment and in times of crisis this environment must be resilient. Resilience refers to the capacity to adapt to changing circumstances and can be obtained by implementing evidence-based teaching practices that encourage flexible approaches to learning (15). Using student-centered pedagogies, instructors can intentionally design resilient online learning environments without compromising rigor (16, 17). Such techniques include reducing cognitive load by amplifying core concepts and competencies, rethinking assessment, and trauma-informed teaching.

Cognitive load, core concepts, and competencies. A number of evidenced-based strategies can be implemented to reduce cognitive load and support student learning. First, instructors must focus on amplifying core concepts and competencies outlined in the course learning outcomes. In *Vision and Change* (2011), the American Association for the Advancement of Science emphasizes the importance of shifting focus from content “coverage” to clear and aligned learning outcomes (18), which results in improved student learning (19, 20). This is particularly important in STEM, where “the drive to ‘cover content’ presents a formidable barrier to incorporating more student-centered practices” (21). *Core concepts* are discipline-based ideas and/or principles for which students must have a deep and lasting understanding and *competencies* are the skills students must possess to be successful within the discipline beyond the classroom. *Vision and Change* proposed core concepts for STEM education and several disciplines have followed suit (22), including the American Society for Microbiology (23). Second, instructors should consider making content relevant to students’ lives. Deep and lasting learning requires that students engage with, reflect upon, and apply content to real-world problems (24–28). Additionally, using model examples helps students develop a conceptual understanding of the material and place those understandings within the larger context of the discipline (21). For example, in microbiology, symbiotic interactions, nutrient cycling, and fermentation can all be discussed using the relationship between a cow and its rumen microbiome. By utilizing this particular model, students better understand the interrelatedness of these concepts and how to apply them in other biological scenarios. Lastly, chunking content into smaller, more manageable, pieces can assist the brain with organizing and encoding new information into long-term memory (25, 29, 30). Providing learning guides, explicit expectations, and a user-friendly organization of content in the online learning management system (LMS) also provides students with a pathway to conceptual understanding (31).

Rethinking assessment. The purpose of assessment in the student-centered classroom is to gather relevant information about student performance related to course learning outcomes. Formative assessments help monitor student progress and adjust instruction accordingly. These assessments (e.g., reflections, quick writes, discussion boards, quizzes, participation, homework, etc.) often contribute a smaller percentage toward the final grade, while summative assessments (e.g., mid-terms, finals, projects, papers, performances, etc.) are used to evaluate mastery of learning outcomes and are often weighted more heavily in the course (32, 33). Instructors should

reevaluate the frequency, weight, and distribution of assessments in a resilient online course. It is critical in times of crisis to consider using frequent, low-stakes assessments that will give students ample opportunity to practice, receive feedback, and monitor their progress, thereby reducing the stress and anxiety associated with performing well on a few high-stakes assessments (34, 35).

Trauma-informed pedagogy and creating a sense of community. Student motivation, or the willingness to engage actively with course content and instruction, is influenced by a student's sense of belonging, value, and well-being in the classroom (11, 36–38). *Trauma-informed pedagogy* aims to recognize student experiences inside and outside the classroom and create an emotionally and psychologically safe environment where students feel comfortable sharing their ideas, opinions, and perspectives free of ridicule or harassment (11, 39, 40). Communication and collaboration are also central to building a safe learning community (41). Methods to facilitate this form of teaching online include giving students multiple opportunities to share their experiences with each other and collaborate on learning tasks. Instructors must a) provide expectations for in-class and online communications, and b) prepare to manage emotional responses that students may display when discussing emotionally charged topics (e.g., genetics, evolution, etc.). Instructors who intentionally create space for students to safely share their challenges and successes and offer their own personal experiences in turn will help students connect as people first and as learners second (11). This can be done asynchronously in a discussion board or synchronously by spending a few minutes before or after class having a casual conversation (11, 38). Most importantly, being authentic and establishing a strong presence will help develop trust and community in online environments. Trauma-informed practices are inherently flexible and help students to regulate their own learning through mindfulness and alternative pathways to learning (42).

5.4. Strategies and tools for resilient classrooms

The professional development materials outlined here focus on reducing cognitive load, amplifying core concepts and competencies (25, 43), implementing trauma-informed pedagogy (11, 38, 41) and engagement strategies (28), and rethinking assessment practices (44, 45). It is recommended that instructors utilize the following strategies to establish conditions within the learning environment to help students deal with additional stress and regain their sense of equilibrium.

5.4.1. Identify and amplify critical content.

In order to identify core concepts critical to meeting course outcomes, instructors should first ask: What are the essential bodies of knowledge, skills, and dispositions associated with this course? What extraneous material may be causing unneeded complexity and/or distraction for students? Second, instructors should ask: How can the course be organized such that students can more easily navigate the content and materials in the LMS? Instructors are encouraged to discuss core concepts with students and demonstrate how they can achieve these competencies through a roadmap based on the learning outcomes. For example, a core concept of microbiology may be the relationship between the structure and function of a cell and a learning outcome of the course may be to explain how to use a microscope to determine cell wall features and their function (23). Core concepts may connect to multiple units of the course. Instructors should remind students when this happens so they can understand the relationship of core concepts within course materials. One technique for creating a logical, user-friendly visual organization is chunking content into modules by units of study or weeks in the semester. With regard to content delivery,

instructors should avoid long, synchronous lectures, and instead use time spent with students to a) emphasize major concepts or principles, b) address common misconceptions in the discipline, and c) clarify expectations for upcoming assignments. Instead of lengthy or dense readings, consider selecting multimedia sources such as online videos, tutorials, or concise articles that will help convey content. Consider creating mini-lectures or “recap” videos to ease cognitive load and support student learning.

5.4.2. Increase online communication and collaboration.

Clear, open, and frequent communication is the linchpin to any successful relationship, including those in the classroom. Students desire connection in an online learning environment and perceive the instructor as the facilitator of classroom climate and student-student rapport (46). To mitigate feelings of loneliness and isolation, instructors can build classroom climate by increasing communication between students and themselves. Student-instructor interactions can be facilitated through regular announcements, which give students focus and clarify assignment expectations. Feedback through rubrics, messages, videos, or audio technologies in the LMS keeps students on track and communicates care and concern to students. Student-student interactions can be facilitated through group activities such as discussion boards, Zoom breakout rooms, shared documents, or synchronous discussions. Beyond the socio-emotional benefits, working in groups can also reduce cognitive load since processing content and completing complex tasks can be shared among several individuals. While individuals still need to integrate information and coordinate their learning, group work often results in deeper processing, elaboration, and more meaningful learning than individual work (47).

5.4.3. Engagement strategies.

Deep learning is reflected in one’s ability to apply core concepts, principles, and competences in novel situations, and is made possible by generative activities that prompt reflection and elaboration. To help students engage deeply with the content during synchronous sessions, instructors can integrate purposeful pauses such as quick writes, minute papers, or polling (48, 49). Cognitive aids, whether instructor-provided or collaboratively produced, can facilitate deeper understanding by making explicit important concepts, principles, and connections among ideas (50). Some examples of cognitive aids include timelines, flow charts, concept maps, visual images, etc. Each of these examples can be used to cover multiple concepts in a course. For example, consider the various stages of pathogen infection along with its associated virulence factors. By using a concept map, student can describe how each stage connects to a virulence factor, by using a timeline, students can explain the order of events, or by using a diagram, students can distinguish where each stage of infection occurs. Regarding engagement of the whole class, learning guides in the LMS can pave the way for achieving course learning outcomes. By breaking down complex tasks or ideas into their component skills or essential understandings, students can practice subskills individually and then in combination to master the material (27). Finally, in content-heavy STEM courses, *active learning* strategies improve student engagement and provide opportunities for focused practice and discussion of core concepts, benefitting female and PEER learning in particular (28).

5.4.4. Prioritize learning and rethink assessment.

In times of crisis, a student-centered class where students have a choice and authority over their learning creates a sense of power and responsibility in a time when many things seem beyond their control. When instructors focus on the process of learning they communicate the student onus of developing an understanding and mastery over memorization and recall. To give students some choice in their process, instructors can provide a few topic options for students to

investigate and then let them choose how they prefer to demonstrate their newfound understanding (e.g., a podcast, a poster-type presentation, a written paper). Similarly, reflections and short essay-style prompts offer a method for expressing individual thoughts and ideas about the material. Assessing learning can be as varied as the process of learning itself. The primary purpose for assessment should be to gauge student learning and collect data that can inform subsequent instruction. A thoughtfully designed rubric is a powerful assessment tool in the trauma-informed classroom. Rubrics offer clear, comprehensive, and agreed upon scoring that ensures equity and confidence in the grading and feedback process, thereby improving student-instructor communication of how and when learning is achieved. Whether using an analytical rubric to assess creativity from a writing assignment (51) or specifications grading, a flexible grading system designed to assess performance without a letter grade, point value, or attendance (52), to assess competency of laboratory skills, rubrics are key in rethinking the purpose of, and approaches to, assessment. Lastly, when designing a student-centered course focused on learning, the use of frequent low-stakes assessments (i.e., polls, quizzes, quick writes, discussion boards, etc.) is correlated with high student self-efficacy and motivation (53).

Using these strategies as a framework, pedagogical coaches from the Center for Engaged Teaching and Learning (CETL) at the University of California, Merced, partnered with instructional designers from Academic and Emerging Technologies (AET) to help instructors plan ahead for unforeseen and uncontrollable disruptions to teaching and learning by intentionally designing resilient courses for the remote environment (Table 1). Content was targeted to all instructors across the campus with particular emphasis towards instructors new to online instruction. Instructors from all levels (teaching assistants, lecturers, assistant and associated professors) and from all schools (engineering, social sciences and humanities, and natural sciences) participated. Provided examples were varied to accommodate instructors teaching labs, small discussions, or large lectures. Instructors were provided several opportunities to learn and practice teaching online. The crash course “Faculty Institute” and “TA Institute” covered the basics of planning, building, and teaching an online course with 111 faculty participants and 87 graduate teaching assistants (GTAs). The more targeted workshops focused on pedagogy and technology and had a total of 76 faculty participants and 12 GTAs. One-on-one consultations provided individualized support and troubleshooting to 49 faculty and 46 GTAs. Of those reported, a total of 236 faculty and 145 GTAs were supported through these programs. Detailed descriptions of the workshops offered are outlined in Table 2. The team also contributed to a campus-wide, bi-weekly resource newsletter for faculty and created both a faculty and graduate student course in the campus LMS, containing a plethora of additional resources, videos, tutorials, and discussion board support.

5.5. Conclusions and recommendations

As fall instruction progresses, the strengths and areas for improvement regarding the focused workshops and the Faculty Institute are becoming clear. Instructors came away from the workshops with a better understanding of the importance of reducing cognitive load, building community, and engaging students. They shared that chunking content into modules and organizing the course using a visual guide has proven to be highly effective for communicating with students, clarifying course expectations, and reducing cognitive load. For many classes, particularly smaller, discussion-based courses, the Zoom breakout rooms and interface are working well to establish community and give students opportunities to work together and build community. Instructors noted that recording lectures through video, creating introductory

welcome videos, or offering alternative resources outside of a traditional lecture, has been effective in delivering content while freeing up time to dive into material during synchronous “lecture” hours. Lastly, instructors believe strategies for engaging students online, such as polling, relatable examples, and being personable has motivated students to participate more than was experienced during the emergency remote instruction of spring 2020. Frequent polling has proven to be a successful engagement strategy in large classes (> 200) and serves as a manageable, low-stakes assessment. One faculty reported feeling pleased to see the impact of “reaching students in different ways” and will consider using many of these strategies even after returning to face-to-face instruction.

While many instructors have shared positive feedback, others are experiencing a number of challenges as they adjust to teaching in a remote setting. The vast majority found the learning curve of novel teaching practices (e.g., communicating frequently, offering frequent low-stakes assessment, creating relatable examples, etc.) and technology (e.g., organizing breakout rooms, creating online course content, recording lectures, setting up the course in the LMS, etc.) to be time-consuming. For large classes (> 100), Zoom breakout rooms are not an effective strategy and often result in management challenges (e.g., organizing/assigning breakout rooms) that distract from instructional time. Courses that require hands-on instruction, such as labs, are finding it particularly difficult to teach techniques and skills remotely without laboratory tools and equipment. The UC Merced pedagogical and technology coaches are learning the importance of reducing the cognitive load and anxiety carried by instructors who are teaching remotely for the first time and expect perfection when implementing several new pedagogies. In the future, it is important to emphasize that transitions are progressive and iterative processes. Instructors are encouraged to start implementing less-complex strategies (e.g., creating a welcome video, organizing content in the LMS, polling, etc.) and work up to more complex and time-consuming strategies (e.g., pre-recording mini-lectures for asynchronous learning, polling students, etc.) based on their level of comfort and willingness to commit to novel pedagogical approaches. In general, instructors are feeling overwhelmed, finding it difficult to restructure their courses for the remote environment in such a short amount of time.

The transformation to resilient instruction is without a doubt time-consuming and frustrating, however, the flexibility built into these online courses will provide a better learning experience for students now and in the future. The resilient instructor must recognize that teaching is already a demanding practice of care and that an elevated level of stress and trauma requires elevated care of both students and the instructor themselves. To prevent instructor burnout, instructors are reminded to simply do their best, focus on balancing self-care with the care of their students, and recognize and express that this is an emotional time for everyone (54). Strategies implemented by instructors during this time of crisis will not only bolster the integrity of STEM courses but will continue to do so as stressors from recent traumatic events dissipate. Despite being overwhelmed, students have expressed feeling supported and appreciative of the level of care going into classes. One biology faculty member shared, “students were praising me in the Zoom chat today because they feel like they are learning a lot from my class. That is entirely because I followed [CETL’s] advice.” Another faculty member expressed feelings of relief after participating in several workshops and consultations — “I felt the clouds over me lift immediately.” Throughout these challenging times, a unique transformation is in progress in how instructors are thinking about teaching and learning. Equipped with student-centered, resilient online teaching strategies, instructors can help students not only cope, but thrive during these unprecedented times.

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5.7. Supplemental materials

Supplemental materials available at <http://asmscience.org/jmbe>

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Tables

	Resilient Strategies and Salient Literature	Faculty Workshops and Courses	Learning Outcomes
1	<i>Amplify critical content and reduce cognitive load</i> (8, 11, 21, 22, 27, 43, 47)	1. Faculty and TA Institutes: Plan, Build, and Engage 2. Zoom for Instruction and Zoom Security 3. Using Canvas to Organize/Chunk Course Content into Modules 4. Creating Instructional Videos	1. Identify strategies to reduce cognitive load. 2. Identify core concepts, principles and competencies for your course design. 3. Utilize the appropriate technologies to engage and support student learning in the online environment.
2	<i>Build community and collaboration</i> (4, 17, 24, 26, 28, 38, 44, 45)	1. Faculty and TA Institutes: Plan, Build, and Engage 2. Implementing Engagement Strategies and Mitigating Resistance/Non-participation 3. Using Microsoft Teams for Instruction 4. Zoom for Instruction and Zoom Security	1. Create a “to do list” for developing a welcoming and inclusive learning community that reflects “best online practices.” 2. Identify 3 to 5 evidence-based engagement strategies to use in the fall. 3. Identify 2 evidence-based strategies to mitigate student resistance to active learning. 4. Utilize the appropriate technologies to engage and support student learning in the online environment.

3	<i>Employ engagement strategies</i> (24, 26–28, 43, 44)	1. Faculty and TA Institutes: Plan, Build, and Engage 2. Overview of Canvas Features, covering basic navigation and how to create assignments, announcements, discussion boards, and quizzes	1. Identify 3 to 5 evidence-based engagement strategies to employ in the fall. 2. Identify evidence-based strategies to mitigate student resistance to active learning. 3. Utilize the appropriate technologies to engage and support student learning in the course shell.
4	<i>Rethink assessment</i>	1. Rethinking Assessment: Integrity, Best Practices and Creating Rubrics 2. Using Canvas Gradebook and Analytics to Keep Students on Track	1. Evaluate the appropriateness of previously planned assessments with regard to high- versus low-stakes. 2. Determine potential modifications to reflect a variety of purposes and types of assessment, with more frequent low-stakes assessments. 3. Utilize features of Canvas Gradebook to monitor student engagement and support their learning.

Table 1. The four strategies for resilient remote instruction are outlined with associated supportive literature. These strategies are directly tied to the offered workshops and courses that cover each strategy discussed. Some workshops covered more than one strategy. General learning outcomes for the represented categories are outlined and cover the overall goals for instructors participating in the workshops offered.

Instructional Workshops	Description
Faculty and TA Institutes: Plan, Build, Engage (3 hours)	This three-part series covers the basics of pedagogy and technology and "best practices" for developing resilient online courses. In part one (plan), instructors learn about cognitive load and how to center a course on the "core concepts.". In part two (build), instructors learn many technology tools available to implement a course online. In part three (engage), instructors learn how to build a community and engage students online, along with pedagogical methods to mitigate resistance to engagement in an online setting.
Implementing Engagement Strategies and Mitigating Resistance (1 hour)	This workshop dives deeper into the ways to engage students online by focusing on simple strategies such as being personable, offering relatable examples, creating clear expectations and guidelines, and providing varied ways for students to interact with the instructor, the content, and each other. Examples for both synchronous and asynchronous courses, as well as small and large classes, were also discussed.
Rethinking Assessment (1 hour)	This workshop focuses on the purpose of different assessment types. Instructors discuss the benefits of frequent, low-stakes assessments in an online learning environment. Examples of both high-stakes, low-stakes, synchronous and asynchronous assessments are discussed in detail with technological tools that can facilitate each type of assessment.
Canvas LMS Sessions	
Creating Instructional Videos (1 hour)	This workshop covers how to record, edit, and upload instructional videos using Zoom, Camtasia, and the Canvas learning management system.

Overview of Canvas (1 hour)	This workshop covers a deeper exploration of all the tools and technologies in Canvas. Specifically, instructors learn basic system navigation, how to create assignments, announcements, and quizzes, and how to set up the learning environment effectively.
Using Canvas to Organize/Chunk Course Content into Modules (1 hour)	This workshop covers benefits, tips, and templates for chunking course content into modules that can be easily to navigate and access by students. Instructors are encouraged to be interactive during this workshop by opening their course sites and begin chunking content into more manageable pieces. It also covered the set-up of assignments, quizzes, discussion boards, files, pages and home page with reference to overall course organization.
Using Canvas Gradebook and Analytics (30 minutes)	This workshop covers the nuances of grading and understanding grades in Canvas. Specifically, instructors are given tools on how to keep students on track and monitor performance effectively. Examples are provided for how to build quizzes and questions that will produce quality course data.

Zoom and Teams

Zoom for Instruction (1 hour)	This workshop guides instructors through all the features, settings, and tools integrated in Zoom. Specifically, they are given the best tips for teaching classes such as enabling breakout rooms, reactions and raising hand features, Zoom whiteboard for annotations, sharing screen and audio, and polling.
Zoom for Security (1 hour)	This workshop introduces ways in which Zoom security can be compromised and discusses ways to safeguard the classroom and student's privacy. This includes how to set up the Zoom room to manage participants, create passwords, establish a waiting room culture, and controlling the chat.

Using Microsoft Teams for Instruction (1 hour)	In this workshop, instructors are guided through Microsoft Teams, and learn about the benefits of using teams, conditions where this technology will be more useful, how to set up the Teams, how to navigate and facilitate the Teams environment, and how to communicate with Team groups and Team networks.
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Detailed descriptions of all workshops and courses offered by UC Merced's CETL and AET teams as they prepared instructors to transition from emergency remote instruction to resilient remote instruction.