UNIVERSITY OF CALIFORNIA

Santa Barbara

Missing in the forest if not for the trees:
Ectomycorrhizal neighbor effects in temperate forests

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

Gabriel C. Runte

Committee in charge:

Professor Holly V. Moeller, Chair

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March 2025

The dissertation of Gabriel C. Runte is approved.

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December 2024

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What a joy it is to learn about the places I love. Throughout the challenges of this dissertation, I have always been lucky – even when I lost sight of it – to study the forests of California and beyond. Without the people who have guided and supported me along the way, I would never have made it this far and had the chance to do and see the things that made this adventure so wonderful. To you all, thank you.

Beginning with my greatest scientific advocates, my committee, I want to express my gratitude. Without this incredible group of people, I would simply be a pile of enthusiasm for forests without the first clue of how to spend it.

Chief among them is Holly, my advisor. Holly has been both patient and rigorous at each of the times I have needed her to be. She has taught me to think clearly about the questions that make up this PhD and the curiosities I continue to hold. She asks difficult questions, finds excitement where I initially find defeat, and turns drafts red with edits when she knows they can be better. Holly has been an incredible mentor to me and a close friend, and I am so grateful to have pursued my dissertation in her lab.

In the early years of my PhD, I was lucky to also overlap with Laura as she began a postdoc in our group. Laura ended up acting as a secondary advisor to me while she was in Santa Barbara. Her way of thinking nicely complements Holly's and contributed to a broader set of knowledge for me. I was so pleased that she agreed to continue as a committee member after she left to start her own lab at UC Davis.

Carla was my first advisor from my undergraduate years and has remains a crucial part of the way I interrogate the natural world. Her undying curiosity and incredible instinct for new and exciting research directions has made her an invaluable committee member.

Carla helped launch my ecological career and continues to make the work I do more robust and my drafts more impactful. Thank you, Carla.

As I found my way into grad school, Ryoko welcomed me into her lab group. She helped me wade into the depths of molecular ecology and develop the skills necessary to study the unseen microbes in the soil. Even after my Masters, she has helped me to understand and overcome challenges and raised important, yet troubling, questions about the limitations of our techniques. I will be forever appreciative of Ryoko's guidance.

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Finally, my dad. He was person who brought me into the forests early on in life and showed me the magic of the natural world. Through adventures, times on chair lifts recognizing and checking on the same trees for decades and watching them grow, hikes going too far in too few days through the Sierra, and his general enthusiasm for the beauty and healing power of the forests, he shaped everything I do now. Through his passing, I am reminded to be grateful for all of it. Dad, I wouldn't be who I am today without you.

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GC Runte, HV Moeller (*In prep*). Is the friend of my friend my enemy? The maintenance of multiple hosts and symbionts in a mutualism network.

HV Moeller, A L'Etoile-Goga, L Vincenzi, A Norlin, GS Barbaglia, **GC Runte**, JT Kaare-Rasmussen, MD Johnson (2024). Retention of blue-green cryptophyte organelles by Mesodinium rubrum and their effects on photophysiology and growth. *Journal of Eukaryotic Microbiology.* https://doi.org/10.1111/jeu.13066

GC Runte, R Oono, NA Molinari, SR Proulx, CM D'Antonio (2022). Restoring bigcone Douglas-fir post-fire in drought-stricken Southern California: Assessing the effects of site choice and outplanting strategies. *Frontiers in Forests and Global Change*. https://doi.org/10.3389/ffgc.2022.995487

J Weverka, **GC Runte**, EL Porzig, CJ Carey (2022). Exploring plant and soil microbial communities as indicators of soil organic carbon in a California rangeland. *Soil Biology and Biochemistry*. https://doi.org/10.1016/j.soilbio.2023.108952

GC Runte AH Smith, HV Moeller, LM Bogar (2021). Spheres of influence: Host tree proximity and soil chemistry shape rRNA, but not DNA, communities of symbiotic and free-living soil fungi in a mixed hardwood-conifer forest. *Frontiers in Ecology and the Environment*. https://doi.org/10.3389/fevo.2021.641732

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Honorable Mention, NSF Graduate Research Fellowships Program	**2020**
Sonoma County Mycological Society Scholarship ($1,000)	**2020**
NSF Research Experiences for Undergraduates	**2018**
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Presentations

Guest Lecture on Climate Niche Modeling, UCSB	**2024**
International Conference on Mycorrhizas	**2024**
Ecological Society of America Annual Meeting	**2023**
Terrestrial Microbiology Guest Lecture on Fungi in the Environment, UCSB	**2023**
8th Annual California Oak Symposium	**2022**
Ecological Society of America Annual Meeting	**2022**
Yosemite Symbiosis Workshop	**2022**
Conservation Seminar Series, UC Santa Barbara	**2021**
Terrestrial Microbiology Guest Lecture on Fungi in the Environment, UCSB	**2021**
UCSB EEMB Graduate Research Symposium	**2020**
National Fish and Wildlife Fire Restoration Grantee Forum	**2019**

Mentorship

Undergraduate Researchers

Independent projects:

Aubrey Chuen Developed a non-destructive plant health survey method for greenhouse applications. This method uses remote sensing techniques and a multispectral camera for image analysis in R. *Worster Award Recipient*	**2022 -2023**
Bailey McKernan Conducted an experiment on how drought-conditioning might improve outplant success in the backcountry. *Schmidt Family Foundation Mentorship Award Recipient, URCA Recipient. Masters Student at SDSU.*	**2021 -2023**
Nicholas Haghani After the campus closure due to COVID, Nicholas pivoted from a lab-based project to bioinformatic and statistical analyses on microbe communities in a highly stratified marine system. *PhD student at UC Davis.*	**2019 -2020**

Additional mentoring (discipline, Graduate/Undergrad):
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Outreach and Teaching

Outreach

EEMB Undergraduate Research Open House — **2022-2024**

Developed and ran an open house event aimed at broadening access to underrepresented groups in undergraduate research. Led fundraising and co-led organizing each year.

High school research demos

Introduced high school students to university programs and facilities. Led students through a brief experimental project harvesting mycorrhizal seedlings. — **2023-2024**

Teaching Assistant

Ecological Modeling

Led a computer-based laboratory section introducing students to coding in R and working with calculus-based mathematical modeling using numerical simulations. — **2022**

Introduction to Ecology

Broad introductory course to many of ecology's foundational theories. Led discussion sections centered on literature interpretation. (2021 was via Zoom) — **2021, 2024**

ABSTRACT

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Forest outcomes are contingent upon the persistence of crucial symbioses between plants and soil fungi – mycorrhizae. To understand the vulnerability or resilience of these ecosystems, which are the dominant land cover type and house the bulk of terrestrial carbon, we must account for ecology of mycorrhizal symbioses. Temperate and boreal forests are primarily reliant on the guild known as ectomycorrhizal fungi which facilitate nutrient cycling and plant nutrient uptake under the canopies of more than 60% of the world's trees. In this symbiosis, both the host trees and mycorrhizal fungi often have the capacity to interact with multiple partner species, even at the same time. This interaction network creates a complex set of both direct and indirect interactions that can determine the dynamics of forest communities.

By combining field studies, mesocosm experiments, and theoretical modeling, my research interrogates how ectomycorrhizal fungi and their host trees interact to shape community structure and drive system-level ecological outcomes. In this dissertation, I share three research efforts conducted over the course of my graduate studies each of which use different techniques to scale from idealized ecological systems to the full complexity of the natural world.

In **Chapter 1**, I share a multi-year restoration effort working to better understand the ecology of an endemic Southern California conifer, the bigcone Douglas-fir (*Pseudotsuga*

macrocarpa). In this project, we planted 1,728 seedlings in into backcountry sites in the scar of the 2007 Zaca Fire. We combine fire severity, slope position, habitat type, soil inoculation, and summer watering in a mixed experimental design to inform USFS restoration efforts in the future, following the seedlings for more than two years to observe long-term outcomes. Using Bayesian mixed models, we assessed the direction and strength of each contributing factor, leading to insights into improved planting locations and conditions for bigcone Douglas-fir.

I build upon this field-based work in **Chapter 2** where I report on a greenhouse experiment looking at the interactions between host species and soil microbial inoculum. We planted seedlings of two species, the bigcone Douglas-fir (*Pseudotsuga macrocarpa*) and the canyon live oak (*Quercus chrysolepis*), into two live field soils and one sterile soil for a year. We then uprooted seedlings, washed them, and replanted them with a neighbor of variable backgrounds, allowing us to observe the influence of soil and neighbors together. We then incorporated a simulated drought to provide greater insights into ectomycorrhizal dynamics under current ecological stressors. We find strong, lasting effects of soil on seedling outcomes with negative plant-soil feedbacks under drought conditions. These results point to the importance of understanding soil microbial partner availability and the context dependence of stressors for mixed forest dynamics under climate change.

Finally, in **Chapter 3** I develop a mathematical model depicting host trees and ectomycorrhizal fungi in a shared network. In this model, we allow for identity-based and quality-based partner rewards in the mutualism network, integrating two common mechanisms for partner control in empirical studies. By specifically outlining and interrogating the combined effects of these pathways, we help build an understanding of the

impacts of different assumptions about the nature of the symbiosis. We then use this model to assess the role of ectomycorrhizal network connectivity in forest community stress responses, finding that agnostic partner allocation (without strong partner control mechanisms) leads to the most stable forest communities through stress. This result points to important questions for empiricists working to understand the evolutionary benefits of partner sanctions in the ectomycorrhizal symbiosis.

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1. Introduction

1.1 Motivation and background

The response of organisms and populations to environmental change is influenced by the concurrent changes to the external factors (e.g. resources, community dynamics) that determine their success (Hagen et al. 2012; Aslan et al. 2013; Åkesson et al. 2021). Community ecology examines how these external factors shape the composition, diversity, and resilience of ecological communities. Among these factors are the various species interactions – positive, negative, and neutral – that sum to determine species survival. With the growing influence of climate change-driven ecological change, these species interactions are likely to be widely destabilized (Hellmann, Prior, and Pelini 2012; Gilman et al. 2010; Lord, Barry, and Graves 2017). Not only will populations become more or less successful in their environments but species are also likely to be gained and lost under shifting environmental conditions (Beaury et al. 2020; Lewis 2005). A deeper understanding of how critical species interactions will respond to the impacts of climate change will help inform more successful ecological forecasting and lead to more successful conservation efforts. Mutualisms are positive-positive species interactions and have gained increasing interest in recent decades for their widespread role in structuring ecosystems (Chomicki et al. 2019; Hale, Valdovinos, and Martinez 2020; Fowler et al. 2023). Different mutualisms have also been found to have varying responses to environmental change, where some partnerships provide a buffering effect and others collapse (van der Heide et al. 2021). Perhaps the most iconic case of dysbiosis can be observed in the widespread bleaching of coral reefs as the ocean warms (Bosch and Miller 2016). As a result of corals expelling their symbionts, they are themselves left vulnerable to starvation and disease (Baird et al. 2009). Thus, the

foundation of the productive reef ecosystem is liable to collapse. In other cases, such as in many pollinator systems, redundancy in compatible species gives some hope in light of declining populations (Hale, Valdovinos, and Martinez 2020; Settele, Bishop, and Potts 2016). Such mutualisms may buffer environmental stress through functional replacement of partners and the network effect of support by alternate partners in fluctuating communities (van der Heide et al. 2021). The effort to anticipate the vulnerabilities of ecosystems should therefore be concerned with recognizing the structure of foundational mutualisms and how these symbioses might shape species interactions into the future.

1.2 Temperate forests and ectomycorrhizal fungi

Forests are among the ecosystems which cannot be fully understood without accounting for the critical nature mutualism, specifically the mycorrhizal fungi on the roots of the trees (Anthony et al. 2022; Averill et al. 2022; Bennett et al. 2017). These symbionts play a role not only in nutrient cycling and uptake, but also in driving the community dynamics of forests by shaping the establishment and competitive potential of their tree hosts (Kennedy 2010; Perry et al. 1989). The ecology of many mycorrhizal hosts have become so intertwined with their symbionts that they adapt their morphology and physiology to accommodate fungal partners (S. E. Smith and Smith 1990; Read 1996; Hobbie and Högberg 2012). Such investments are rewarded as research has shown that fungi can provide more than 75% of plant nitrogen in some cases (Hobbie and Högberg 2012). Further, the biogeography of different mycorrhizal host types is strongly predictable based on the climatic limitations on decomposition, pointing to the consistent influence of mycorrhizal fungi in forest outcomes (Steidinger et al. 2019).

Outside the tropics, the most common mycorrhizal guild are the ectomycorrhizal fungi known for their robust decomposition capacity and success in harsh environmental conditions (Steidinger et al. 2019; S. Smith and Read 2008). In fact, roughly eight out of ten trees beyond the tropics are ectomycorrhizal (Steidinger et al. 2019). Given the growing recognition of diverse, integral roles of these fungi and the current elevated rate of environmental change, building a deeper knowledge of ectomycorrhizal community ecology will empower a fuller understanding of forest dynamics and vulnerability.

Ectomycorrhizal fungi are a diverse assemblage of taxa grouped by a common growth morphology (S. Smith and Read 2008). These soil fungi grow on the outside of plant roots where they form a mantle, extending hyphae only shallowly in between the epidermal cells of the roots to make up the Hartig net where nutrient exchange occurs (Bonfante and Genre 2010; Halling 2001). The ectomycorrhizal growth habit has evolved more than 70 independent times from diverse saprotrophic taxa (H.-J. Hawkins et al. 2023). This origin story connects the symbionts to their functional capacities as nutrient cyclers; they remain as soil fungi decomposing and recycling decaying matter. But the carbon subsidy from plant hosts can provide an edge in the competitive soil environment (Yan et al. 2024). In return, ectomycorrhizal fungi provide their hosts with the resources necessary to survive far beyond the limitations of their inherent physiology (Karst et al. 2018; Van Nuland et al. 2024).

1.3 Soil microbes and forest dynamics

The composition of trees in a forest plays an important role in determining the structure and function of the soil microbial community belowground (Runte et al. 2021). Diverse tree stands contribute to the maintenance of diverse mutualists but can also dilute the effect

strength of host-specific pathogens (Bever, Broadhurst, and Thrall 2013; Bennett et al. 2017). A substantial body of literature has contributed to our understanding of what have been dubbed plant soil seedbacks (PSFs) dating back to – and named for – work done by Dan Janzen and Joseph Connell more than half a century ago (Janzen 1970; Connell 1971). The Janzen-Connell hypothesis, developed in tropical forests, states that forest diversity is maintained by low conspecific recruitment success (negative plant-soil feedback) under mature individuals due to the accumulation of pathogens. And while plant-soil feedbacks are understood to be less negative (or even positive) in ectomycorrhizal systems, the role of plant pathogens is also recognized to increase as plant stress grows (Kadowaki et al. 2018; Gómez-Aparicio et al. 2017; van der Putten et al. 2013). This influence is particularly acute for vulnerable seedlings which shape the successional trajectory of forests (Bennett et al. 2017; Gómez-Aparicio et al. 2017; van der Putten et al. 2013; Crawford et al. 2019). Thus, soil microbial populations can be a double edged sword, pointing to the importance of considering the breadth of plant-soil feedbacks when attempting to observe the role of mycorrhizal fungi.

The ectomycorrhizal communities found on the roots of trees are formed via two alternate pathways. The first is through spore colonization wherein compatible trees can induce the germination of fungal spores which then grow to form mycorrhizae with that host tree (Nara 2009; Kennedy et al. 2011). Alternatively, fungi already growing in the soil may colonize a tree through mycelial colonization, eliminating the need for the specific germination cues to be produced by a given host (Hubert and Gehring 2008; Bogar, Dickie, and Kennedy 2015). This second pathway can allow for novel associations between fungi and an alternate host/neighbor tree species. Such partnerships may provide novel benefits through access to

otherwise inaccessible resources or the maintenance of function through uncommon environmental conditions (Moeller and Neubert 2016).

By this mechanism, ectomycorrhizal fungi and their host trees also have the potential to shape the success of nearby ectomycorrhizal trees. These alterations of symbiotic outcomes among co-occurring trees are commonly referred to in the literature as neighbor effects (Bogar and Kennedy 2013; Moeller et al. 2016; Otsing et al. 2021; Seyfried et al. 2021). Neighbor effects can have consequences for the health and dynamics of forest stands via a number of distinct mechanisms (Lofgren, Nguyen, and Kennedy 2018; Hortal et al. 2017). In addition to the potential for novel associations, co-occurring hosts can also promote seedling recruitment by acting as a reservoir of symbionts for a dispersing seed (Bennett et al. 2017).

Alternatively, co-occurring trees may have incompatible fungal communities that instead lead to greater competition in the soil. Fungal competition for limited resources can lead to community filtering induced by host preference, niche preemption, physical displacement, or another competitive driver (Christian and Bever 2018; Kennedy 2010; Dickie 2007; Rasmussen, Busby, and Hoeksema 2018; Kennedy et al. 2011). Thus, benefits to a host tree are liable to change through time as fungal communities and their functional profiles shift. These changes to the structure and function of the mutualism and their context dependence in mixed forest stands add to the complexity of predicting ecosystem dynamics.

A more comprehensive understanding of ectomycorrhizal community ecology will provide insights not only into the underpinnings of world's forests but could also empower more strategic decision-making on managed landscapes. In the context of restoration, ectomycorrhizal fungi are gaining growing recognition as a potential tool to expand planting

success, though challenges remain in building funchal communities for variable ecosystems and climates (Policelli et al. 2020; Assad, Reshi, and Rashid 2022; B. J. Hawkins, Jones, and Kranabetter 2015). Additionally, in-situ inoculum sources have the potential to bolder seedlings but are accompanied by the possibility of pathogens, particularly in stressful abiotic conditions (Corbin and Holl 2012; Pugnaire et al. 2019). In existing mixed forest stands, deeper knowledge of ectomycorrhizal community ecology may provide insights into the vulnerability of tree species as their neighbors experience stress or the possibility of invasion by alternate hosts. Across tree life stages and landscape successional stages, ectomycorrhizal symbioses play an important role in driving the success or failure of forest communities.

1.4 Dissertation scope

In this dissertation, I contribute to these areas of inquiry by combining field studies, mesocosm experiments, and theoretical models to investigate the contributions of neighbor effects on forest outcomes. In Chapter 1, I explore the restoration potential of a threatened Southern California endemic conifer, the bigcone Douglas-fir (Pseudotsuga macrocarpa). In this work, we sought to better understand how this species interacts with the co-dominant canyon live oak (Quercus chrysolepis) and other landscape features to predict outplant success. Due in part to the difficulty of teasing apart microbial effects from additional complexity in the field, I follow this study with a greenhouse experiment.

In Chapter 2, I investigated the role of soil conditioning and neighbors on seedling growth and drought response. Using the same two species as in Chapter 1, we individually conditioned seedlings to live soils from under mature individuals of each species as well as a

sterile control soil. Over the following two years, seedlings were paired up into shared pots filled with sterile soil. Seedlings were combined across species and soil history combinations and allowed to establish a common microbial community from those remaining on the roots of constituent seedlings. Then, in the third year, half of all replicates were subjected to a simulated drought treatment to test how seedling histories affected health outcomes and growth.

To further interrogate the role of neighbors with overlapping ectomycorrhizal communities, in Chapter 3 I develop a mathematical model depicting trees and their fungal symbionts. Using this model, I study how variable partner controls on the allocation of resources in a common mycorrhizal network (CMN) shift community stability through stress. This model establishes a framework to observe the variable impacts of different assumptions of ectomycorrhizal network structure and develop testable hypotheses for future empirical research. By working across techniques, this dissertation aims to build a more complete understanding of this foundational symbiosis and the implications it may have for the future of temperate forests.

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2. Chapter 1

Restoring bigcone Douglas-fir post-fire in drought-stricken southern California: assessing the effects of site choice and outplanting strategies

2.1. Abstract

Forest restoration is a powerful tool to combat the dual threats of drought and fire, both of which have been increasing in frequency and severity in recent years in the Western United States. The hard-hit region of Southern California is home to the endemic bigcone Douglas-fir, *Pseudotsuga macrocarpa* (Vasey), whose abundance and range have been impacted by multiple large fires within the last two decades. To better understand the ecology of these trees, and thereby improve the potential for restoration in light of predicted future conditions, we outplanted 1,728 seedlings into burned areas with varying levels of pre-planting fire severity and proximity to water (near canyon bottom and upslope). Within each planting area, seedlings were planted into one of four microhabitats; under conspecifics, under the codominant oak species (*Quercus chrysolepis* (Liebm)), in the open (no woody canopy), or in the open within a microsite feature such as a log, rock or a small shaded hole. At each site and within each microhabitat, half the seedlings were treated with oak-soil amendments (soil from under the oak) and half with spring watering (4 months after planting). To better understand the influence of outplanting conditions, we tracked the survival of the seedlings over the next two years. Short-term (March to June) survivorship following planting was quite high and the most significant die-off of seedlings occurred during the first dry season (June to November) despite strong droughts in the second and third dry seasons. Overall, only 7.3% (127 of 1,728) of seedlings survived. Seedling success

depended strongly on the microhabitat and summer watering, though not in necessarily intuitive ways. Seedlings that received supplemental water during the first summer did worse than unwatered seedlings. The most successful microhabitats for planting were open sites with microsite features and sites underneath canyon live oak, while sites under mature bigcone Douglas-firs had the lowest rates of survival. Position on the slope had no effect on outcomes and soil amendment had a weak negative effect on seedling survival.

2.2. Introduction

Globally, conifer forests are experiencing the detrimental impacts of climate change in the form of increased aridity and large high-intensity wildfires (Breshears et al., 2005; Allen et al., 2015; Halofsky et al., 2020; Stephens et al., 2020). Many forests are storing tonnes of carbon in the form of tree biomass and soil organic matter, the loss of which could be catastrophic for the balance of carbon budgets at regional to global scales (Hurteau et al., 2008). Additionally, forests are home to a diverse set of animals and plants reliant on an intact canopy, help to provide clean air and water, and protect lands from erosion and flooding. The management of forests and fires are thus critical for maintaining these ecosystem services. Ecological restoration of canopy trees can be an effective tool for combating the loss of stored carbon and other resources that decline when forests are decimated (Kashian et al., 2006).

While ecological restoration has been proposed as a tool to assist regions in reaching climate-related goals (Harris et al., 2006; Suding et al., 2015; Bustamante et al., 2019), the reality is that forest restoration goals are challenging to achieve particularly at scale. Restoration of forest species is often hindered by the harsh conditions present in degraded forests and by the reality that sites may burn again before canopy trees have become well

established. Thus practitioners face site-specific hurdles and uneven success (Cruz-Alonso et al., 2019; Rodríguez-Uña et al., 2020; Nolan et al., 2021). Simultaneously, it is important to build broader frameworks that can save land managers and practitioners time and money in the planning and implementation of restoration projects. For these reasons, restoration studies should represent a balance between the testing and integration of ecological principles and the need for local but scalable management strategies.

Given the large footprints of recent wildfires (Juang et al., 2022), burned forests are often far from seed sources, necessitating active planting to recover the tree canopy. Fire-scorched areas are also frequently devoid of canopy cover, which can lead to increased soil drying, making it difficult for seedlings to establish and survive. Tools, such as irrigation, can help overcome barriers to seedling establishment, but they are impractical across large and in remote areas. Additional hurdles to seedling success include access to soil-derived inorganic nutrients, such as nitrogen and phosphorus, some of which influence the availability and establishment of crucial symbiotic microbial communities (e.g. ectomycorrhizae).

Integration of mycorrhizal inoculations into restoration is becoming more common, but there is yet to be a general consensus on best practices (Teste et al., 2004; Grove et al., 2019). Ectomycorrhizal fungi associate with many temperate forest species, particularly conifers, and are estimated to exist on the roots of more than 60% of trees across the globe (GFBI consortium et al., 2019). Thus, it may be beneficial to integrate symbiotic communities into restoration practices, particularly when seedling establishment is hampered by insufficient access to soil moisture and nutrients (Lu et al., 2016). Moreover, planted seedlings have been shown to benefit from established mycorrhizal communities in

the soil, such as those associated with conspecifics or alternate hosts with overlapping fungal compatibility (Bingham and Simard, 2011).

In this study, we report results of a restoration experiment after the 2007 Zaca Fire, which burned through multiple vegetation types, including chaparral, mixed conifer and hardwood (oak) forests (see Kibler et al. 2019) and left a large fire scar (972 km^2) in southern California. The Zaca fire burned through a region that is home to an endemic conifer, *Pseudotsuga macrocarpa*, which we hereafter refer to as bigcone Douglas-fir or simply bigcone. This drought-tolerant sister species of the common western USA Douglas fir (*Pseudotsuga menziesii* (Mirb.)) is endemic to the southern California mountains, a region that has experienced multiple severe wildfires over the last two decades. These fires, alongside recent historic droughts and the restricted distribution of the species, have led to the listing of this species on the IUCN Red List as a near threatened species (Farjon, 2011). The challenges facing bigcone Douglas-fir are indicative of broader impacts that are predicted to accompany climate change, large scale fires, and future restoration efforts.

The primary goal of this study was to identify both environmental features and outplanting treatments that promote the greatest seedling survival of this conifer. To investigate the optimum combination of landscape-level factors and planting strategies, we designed a study testing the effects of pre-planting fire severity (high, moderate, low), slope position (low and high elevation), microhabitat type (e.g., developed tree canopy, open habitat), planting condition treatments (e.g., soil inoculation, watering), and their interactions on the restoration success of bigcone seedlings. We selected planting locations and treatments that are practical for implementation by restoration practitioners and land managers. We did not include irrigation since that is not realistic for these remote settings. It

may be expected that sites with the greatest outplanting success would be those that experienced low mortality during fire and thus offer shade and soil microbial conditions (e.g., mycorrhizal networks) conducive to seedling growth rather than conditions in a large barren patch of ground. Yet planting into low-mortality burn sites does little toward the goal of restoring bigcone trees within their former range. Hence, it is critical to identify microsites and planting conditions that will lead to the greatest likelihood of survival within moderate- to high-mortality burn sites where substantial areas of forest canopy were removed by fire.

2.3. Methods

Site selection

This study was initiated in response to the Zaca Fire that burned over 97,124 hectares in southern California from July to September 2007 including >4000 stands of bigcone Douglas-fir (Parkinson et al., in press). Selection of study sites was confined to the Zaca Fire perimeter. Aerial imagery was used to evaluate the condition of bigcone stands before and after the fire. To better understand how fire influences the restoration potential of bigcone seedlings, we outlined three classes of fire severity based on the percent mortality of bigcone stands following the Zaca Fire. Areas were categorized as high-severity sites if greater than 75% of bigcone canopy died, moderate severity if between 75% and 25% bigcone canopy died, and low severity if less than 25% bigcone canopy died. We further refined the restoration areas by selecting planting sites outside of designated Wilderness Areas (due to permitting constraints), on north-facing slopes, accessible from trails or roads, and at least 0.12 hectares in size. From the list of criteria, two sites were selected from each of the three fire severity classes (high, moderate, and low; n = 6; Figure 1).

Within each site, four microhabitat treatments were identified: near mature bigcone Douglas-fir (hereafter "bigcone"), near canyon live oak ("canyon oak"), areas without canopy or large topographic features ("open"), and areas without canopy but with topographic features (e.g., boulder, log) to provide shade to seedlings ("open with microsite"). Although many reforestation efforts incorporate site prep and even continue to remove vegetation after planting (i.e. "release"), the arid portions of the bigcone Douglas-fir range, where this study was conducted, have little competing vegetation (see Appendix for photos). The only changes made to sites were to increase the number of microsites for the "open with microsite" treatment by bringing in nearby logs or other 'natural' shade structures. Canyon oaks were chosen as an alternate canopy site due to their abundance near bigcone stands. We also wanted to investigate a potential nurse plant relationship after anecdotal reports that naturally regenerating seedlings had been frequently observed near oaks. At the two high-severity burn sites, seedlings were planted below standing dead trees because there were no living bigcone trees remaining to plant under. These four microhabitat treatments were replicated at high (mid-slope to ridgeline) and low (toe slope closer to canyon) elevations on the slope to assess the effect of proximity to water source. These 48 locations – 6 sites x 2 slopes x 4 microhabitats – constitute our 'plots' for the study. This outplanting design, along with the information in the following section, is summarized in Figure 2.

Planting and measurements

Seedlings were purchased from the USDA Placerville Forest Service nursery in Camino, California, approximately four months prior to the start of outplanting. The two-

year-old seedlings were received as bare-root plants with the soil, a synthetic potting mix in plastic bags (five seedlings to a bag). They were held in waxed cardboard boxes at 4°C in a walk-in cooler between the time of receiving and the time of planting. Seedlings were planted within 18 hours after removal from the cooler.

Seedlings were planted plot by plot (n = 48 plots, 36 seedlings per plot) over the course of one month in March 2019. Southern California is characterized as having a Mediterranean climate with cool wet winters and hot dry summers. Planting in the late winter allowed for the avoidance of freezing conditions and the opportunity to capitalize on later winter-spring precipitation to aid in seedling establishment. The planting region received 8.48cm of rain in the month of planting with an additional 4.29cm coming in April and May combined. Only 2.31cm of rain fell between June and November of that year (Supplemental Figure S1). Due to a history of low survival in bigcone restoration efforts, techniques were incorporated in this study that, while unorthodox, were believed to hold potential as ecologically justifiable manipulations that could be used in remote settings.

A total of 1,728 seedlings were planted across our mixed design. Seedling holes were dug with a hoedad (Forestry Suppliers #69088), after which soil samples were collected from the bottom of each hole for soil fungal community analyses (see below). Half of the holes at each plot received 200mL of soil inoculum prior to the seedling being planted. This soil inoculum was collected from under a single canyon live oak (*Quercus chrysolepis*) from the same plot by first removing leaf litter and/or herbaceous vegetation. Homogenized soil from the first 15 cm was applied as inoculum to half of the seedlings. This low-cost, low-effort subsidy was intended as a potential restoration technique to introduce communities of soil symbiotic fungi to areas on the landscape that may have been devoid of such

communities. Inoculum used at each plot originated from within the same plot and was not homogenized across plots.

On June 30th, prior to the full onset of the first summer, half of the seedlings across all treatments received 500mL of water. Water was provided more than a month after meaningful levels of rainfall had ceased for the summer and soil was already dry by the time of watering. The goal of this strategy was to reach the seedlings before they had fully adapted to drought conditions, thereby extending the hospitable growing conditions of winter and spring. The volume of water was compromised due to the labor-intensive task of transporting large volumes of water up dangerous steep slopes where bigcones grow and was also intended to test a low-tech, scalable solution that could be realistically implemented by practitioners dealing with thousands of outplanted seedlings. This watering treatment resulted in nine replicates across each plot (36 seedlings per plot, crossed with soil inoculation and watering treatments).

The survival of seedlings was monitored five times over the next 26 months, including at the time of planting (March 2019), before (June 2019) and after the first summer (November 2019), and two summers (July 2020 and May 2021) following planting (Fig. 3A, Table 1). Seedlings were marked as dead when needles were completely brown or completely missing. Because this species has the capacity to resprout even after losing all of their green needles, it is possible for seedlings that appeared dead in one census to be alive at a later point in time. This scenario was rare ($n < 10$), but when it did occur the seedling was retroactively given a living health score for any previous time step in which it was considered dead.

Soil fungal analysis

To understand how soil fungal communities may influence the survivorship of outplanted seedlings, soil was collected from the bottom of every planting hole. Soil from each of the 36 seedlings was combined at the plot level and analyzed together. Following collection, soil was stored in a cooler (approx. 4-8°C), moved to a refrigerator (4°C) within 14 hrs, and a minimum of three homogenized subsamples were stored at -80°C within 5 days for molecular analysis.

DNA was extracted from 200-250 mg of soil using the Qiagen DNeasy Powersoil Pro Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. ITS1 region was amplified using the GoTaq Green mastermix (Promega, USA) with the ITS1 primer pair ITS1F-KYO1 and ITS2-KYO1 (Toju et al., 2012) and the following thermal cycler steps: 95◦C for 3 min, 35 rounds of 95◦C for 30 s, 50◦C for 30 s, 72◦C for 30 s, then a final elongation step of 5 min at 72◦C. Illumina indices were ligated with 3 min at 95◦C, 10 cycles of 95◦C for 30 s, 55◦C for 30s, 72◦C for 30 s, then a final elongation step of 72◦C for 5 min. Finally, samples were purified with AMPure XP beads and diluted to 10 ng/uL before multiplexing all samples for sequencing in two sequencing runs based on time of processing. Amplicons were sequenced on the Illumina MiSeq platform with 250-bp paired-end reads at the California NanoSystems Institute (UC Santa Barbara).

Amplicon sequences were processed using the DADA2 pipeline (Callahan et al., 2016). Adapter and primer sequences were removed using the *cutadapt* package and chimeras were removed before filtering (Martin, 2011). DADA2::filterandtrim settings removed any sequences with an "N" assignment, truncated reads at the first instance of a quality score at or below 2, and removed reads shorter than 50bp. Amplicon sequence

variants (ASVs) were given taxonomic assignments using the UNITE database for use in functional assignments with FUNGuild (Nguyen et al., 2016; Nilsson et al., 2019) (see Supplemental Figures S2:S3 for guild-specific PCoAs).

Fungal community turnover (beta diversity) across plots was evaluated based on Bray-Curtis dissimilarity (*vegdist()* function in *vegan*) (Oksanen et al., 2008). Community structures were visualized using a principal coordinates analysis (PCoA). Finally, community dissimilarity was used as the response variable in a series of PERMANOVA tests (*adonis()* in *vegan*) to evaluate how fungal community composition shifted across the study plots as well as to assess the correlation between fungal community dissimilarity and seedling survival at the plot level (n = 36). Included in these tests were all factors relating to the plot as a whole: fire severity, microhabitat, position on slope, and seedling survival along with relevant interactions.

Seedling survival analyses

Due to the low rates of survival in the seedlings, we analyzed only binary survival data for all time points across the two years. Our Bayesian model was developed using the *Rethinking* package in R (McElreath, 2021). This package is built upon the package RStan (Stan Development Team, 2022) which allows the user to implement Hamiltonian Monte Carlo sampling to calculate the posterior probabilities of model parameters. Survival was predicted as a function of whether or not the individual seedling was alive at the prior time step, with the addition of relevant experimental parameters. We used random effects to account for the levels of nestedness of our data, selecting from our models the relevant model with the lowest tested Watanabe–Akaike information criterion (WAIC) score (Equations 1:2). Models were iteratively built, incorporating all predictor variables (e.g.,

microhabitat, fire severity, water addition) and random effects (e.g., time, site, interactions) in a sequence corresponding to their level of expected impact, before WAIC-selection. If models were not improved by the addition of a predictor such that the model with additional predictors had a higher WAIC score, that predictor was removed prior to proceeding with further additions to the model. All models were run on four chains and checked for convergence by ensuring that all R-hat values were ≤ 1.1 (Andrew Gelman et al., 1995).

$$Survivali \sim Binomial(AliveLasti\ , pi\ )$$

$$\begin{aligned} logit(\ pi\ ) \ = \ \ & \alpha 1\ x\ Time\ +\ \alpha 2\ x\ Site\ +\ \alpha 3\ x\ SiteMicrohabitatSlope \\ & +\ \ \alpha 4\ x\ SlopeWater\ + \\ & \beta 1\ x\ Microhabitat\ +\ \beta 2\ x\ Fire\ +\ \beta 3\ x\ WaterAddition\ +\ \beta 4\ x\ SoilAddition \end{aligned}$$

In the model (Equations 1:2), random effects are denoted with an α (alpha) scaling parameter and predictors are denoted with a β (beta) scaling parameter. This model design accounts for the noise associated with the mixed design to focus directly on the independent effects of microhabitat treatments, fire severity in the area, water addition in the summer, and added soil inoculum. The predictive power of each of the parameters in the final model were evaluated using posterior comparisons, wherein the relative effects of treatments are compared by taking the difference between posterior samples from each of the microhabitats.

2.4. Results

Seedling survival

In May of 2021, after more than two years of monitoring, 7.3% (127 of the 1,728) of the seedlings were still alive. The greatest seedling survival was in the "open microsite" microhabitat treatment (39% of surviving seedlings) and the sites with low fire severity (50% of surviving seedlings) (Fig. 3B, Table 1). The best fit model describing seedling survivorship included the effects of microhabitat, fire severity, water, and soil inoculation. The effect of slope position was not included as a predictor in the model, though the interaction between slope and water was included.

Model posterior comparisons between tested microhabitats are shown in Figure 4A. The only statistically significant microhabitat treatment effect was the strong negative (predicting more die-off) effect of planting under mature or dead bigcone Douglas-firs. Among the remaining three treatments, open sites did the worst, with seedlings planted in open-microsites and those planted near canyon live oaks faring the best.

Fire severity showed no distinguishable effect on seedling performance (Fig. 4B). While seedlings in areas with low fire severity had the highest survival, the posterior comparisons between the three fire classes are centered around zero, showing no directional effect that was statistically significant.

The planting condition treatments (soil addition and summer watering) are also presented as posterior comparisons in Figure 4C-D. Soil addition had very little effect on the seedlings' survival, though it tended to have a slightly negative effect. Summer watering, on the other hand, was strongly associated with negative seedling survival outcomes.

Soil fungi

DNA sequences were successfully extracted from 154 soil subsamples which were roughly evenly distributed across the six planting areas and four microhabitat treatments. We retained 4,890,524 reads with a mean (and standard deviation) read count of 31,757 (10,894) reads per sample. Fungal community turnover was best predicted by the spatial distribution of the samples. The planting site was the single strongest predictor of fungal dissimilarity, with an R^2 value of 0.13 in the PERMANOVA. Because these sites are, by their nature, not replicated, and therefore have less relevance in connecting fungal community turnover to the ecology of regenerating forests, we chose to drop this factor and focus additional tests on experimental considerations, including fire severity, microhabitat, slope location, and survival. From these, we eliminated factors with R^2 values below 0.02, leaving us with the strongest PERMANOVA that included fire severity, microhabitat treatment, and their interaction, which explained 17% of the total variation among samples. Fire severity, with the strongest predictive power apart from planting area, had an R^2 of 0.07. Similarly, in the PCoA the most distinct pattern was a differentiation of communities based on fire severity (Fig. 5).

2.5. Discussion

Putting ecology into practice

The aim of this study was to expand our understanding of seedling survival in post-fire environments and to better inform restoration of degraded landscapes, particularly those facing drought and fire. Many of the treatments with positive outcomes from our study can be put into practice, although uncertainties on specific recommendations still exist. Notably,

this study was conducted during a period of prolonged drought in California and Southwestern North America more broadly (Williams et al., 2022). Planning for and studying these conditions is imperative for land managers, and that may mean planning for low rates of survival in some forest restoration projects. Importantly, the low survival in this study is higher than other known restoration efforts of the same species. Other studies have shown total die-off in censused seedlings (Guiterman et al., 2022) and personal communications with Forest Service employees reiterate these challenges. Literature describing dryland forest restoration is sparse, and we found low rates of survival (even below 10%) are not uncommon in studies with little to no seedling management post-planting (Holl and Brancalion, 2020; Höhl et al., 2020). Moreover, to provide greater nuance to our understanding of optimal seedling conditions, it was necessary to plant into a range of more and less suitable habitats leading, in part, to increased seedling mortality. Nonetheless, we argue that studies such as this are an important step in ground-truthing restoration theory and recentering academic pursuits on feasible restoration targets for practitioners interested in reforesting after disturbance. Within favorable sites, bigcone seedling survival was high.

Planting after fire

This study took place in what might be considered a model system for the predicted trajectory for forests across the temperate Western United States; a forest type defined by limited water and a history of fire (see Lombardo et al., 2009 for fire history of bigcone across the region prior to 2007). Arid regions are anticipated to get even drier over the coming decades (Putnam and Broecker, 2017) and large-scale high-severity fires will become more frequent (Moritz et al., 2012). We found that high fire severity alone is not a barrier to successful restoration. There is little measurable difference between sites that

experienced varying fire severities, although the sites with the lowest severity had the greatest survivorship. Importantly, other factors, such as the microhabitat and post-planting treatment, were stronger predictors of the establishment success of this drought-adapted conifer.

Seedling microhabitat was a critical determinant of survivorship. Seedlings planted underneath mature bigcone Douglas-fir experienced the greatest mortality. This was counter to our expectation that open sites would be the most challenging to restore because of a lack of shade and litter. One possible explanation for this pattern is that the presence of host-specific pathogens outweighed the benefits of any host-specific mutualistic symbionts, such as mycorrhizae, as in the Janzen-Connell hypothesis (Janzen, 1970; Connell, 1971). We did not have the sampling resolution to assess the role of pathogen buildup in seedling die-off, but this is a common mechanism in observed negative plant-soil feedbacks (van der Putten et al., 2013). Microhabitats without a canopy or additional cover, referred to here as *open* sites, had greater seedling survival than under *bigcone* but not more than the *open with microsites* or the *canyon live oak* sites. The greater survivorship in open areas with microsites was likely associated with shading and, possibly, micro-topographical features with the potential to increase water availability to the seedlings. While we do not have soil moisture data, the intention of the experimental manipulation was to reduce water stress. Finally, the *canyon live oak* sites provided a successful habitat for bigcone seedlings to grow, which was statistically comparable to *open with microsite* sites. The benefits for a seedling under an oak could be some shading effect as in *open with microsite* sites, though this should have been true also of the *bigcone* sites. Similarly, the roots of the oaks may increase the pool of available soil water by hydraulic lift, effectively increasing the access of

nearby plants to deep soil water (Caldwell et al., 1998). It is also possible that the seedlings under oaks were able to benefit from compatible symbiotic microbial communities (Runte et al., unpublished) without the negative effects of host-specific pathogens, which would have been present under mature bigcone trees. Previous greenhouse work by collaborators has also shown that bigcone seedlings in proximity to canyon live oaks are better able to withstand drought stress (Rodolf et al., 2019) and a previously unpublished survey found that natural seedlings in the field were almost always associated with canyon live oaks (Moritz et al, unpublished).

Elevation

Location on the slope was not a strong predictor of seedling survival or fungal community turnover. This effect was dropped in the process of model selection as any predictive power added to the model was outweighed by overfitting. We chose to still add slope into the soil fungal community PERMANOVA, but it was also removed due to a low R^2 value. Nonetheless, it is remarkable that mature bigcone trees, particularly at low elevations, are frequently distributed in canyon, riparian settings as reported by the UFSF (Howard, 1992). Other studies have also found strong effects of hillslope position on seedling survivorship and forest composition, leading us to expect that our manipulation simply may not have captured the full strength of these differences between ridges and canyon bottoms (Frey et al., 2007; O'Brien and Escudero, 2022). Our downslope plots were lower but by no means equivalent to a riparian planting.

Summer watering

The increased mortality of watered seedlings was an unexpected outcome but one that may be highly relevant for restoration practitioners. There is great interest in irrigating seedlings in semi-arid environments with extreme interannual variation in precipitation, like southern California. Given the remote location and steep slopes of many restoration projects in the region, watering seedlings is labor intensive and a significant financial investment. In some cases, water addition has led to improved seedling survivorship in shrublands not far from the bigcone restoration sites (Dewees, unpublished). However, our work shows that early summer irrigation of bigcone was deleterious and ultimately resulted in substantial mortality. What remains to be understood is when and where additional water will be a benefit to seedlings and when it might be a hindrance.

Multiple explanations exist for the mechanism by which watering might negatively affect seedlings. A number of these mechanisms relate to plant hydraulics and rely on the assumption that bigcone Douglas-fir is an isohydric species, meaning it closes its stomata to conserve water as soil moisture decreases. Studies of *Pseudotsuga menziesii,* a close relative that is less drought tolerant, has confirmed this physiological approach (Kerhoulas et al., 2020). In our study, watering occurred after the end of the growing season, as soil moisture was likely dropping too low to allow for plant productivity. The addition of supplemental water to the surface soil may have induced a reactivation of the plants' hydraulic system, leading to root embolisms lower in the soil. If this led to enough root die-off, the plant would have been more likely to die in the next dry season. Similarly, even if all roots were able to take up water, the water provided most likely evaporated, infiltrated, or was taken up quickly. If the plants were not able to respond quickly enough by re-closing their stomata,

stems might have experienced severe cavitation, leading to irreparable damage. Another explanation that could have played a role in the demise of watered seedlings is an activation of dormant pathogens in the soil. Increased moisture may have made the environment temporarily more hospitable to dormant pathogens that could have devastated already weakened plants.The last notable factor related to watering would be the promotion of non-beneficial growth. Water in the topsoil could encourage seedlings to grow shallow roots over the deep roots that may be better equipped to seek out naturally available water. Watering could also promote greater leaf area, which would contribute to greater transpiration and vulnerability during droughts. However, in this and other unpublished work, we have observed that bigcone seedlings primarily grow in the late winter and spring, producing far less above-ground material after the onset of summer. Hence, it is unlikely that this mechanism is responsible for the increased die-off of the seedlings after watering.

Soil inoculation

While compatible fungal partners have been shown to increase the growth and success of seedlings in controlled environments (Lu et al., 2016; Moeller et al., 2016), the addition of soil inoculum collected from a co-occurring plant species did not benefit seedling survival in this field study. Previous field studies also found insignificant effects of inoculation on seedling survivorship (Teste et al., 2004; Grove et al., 2019). Many possible explanations exist for this phenomenon, including failure to form mycorrhizas or the negative effect of plant carbon loss in the subsidized development of new fungal networks that offsets any mycorrhizal benefits in the short term. Furthermore, seedlings in this field study may have all benefited equally from a mycorrhizal fungal network that was already in place and any effects of the inoculum may have been negligible. In greenhouse studies of the same species,

we have found that we cannot discern mycorrhizas on root tips until 4-6 months from inoculation, suggesting that the symbiosis forms over longer periods of time even under well-watered conditions of a greenhouse. Seedlings would only gain significant benefits via mycorrhizae after the roots come into contact with compatible fungal spores, the spores germinate and grow hyphae out into the soil. In this study, we saw that the vast majority of seedling die-off came in the first six months and would likely, therefore, not have been impacted by any beneficial fungal partners. This, in addition to the relatively small sample size of surviving seedlings, makes it difficult to parse ectomycorrhizal-specific plant outcomes.

One potentially confounding factor in this treatment is the differences in soil inoculum between planting areas. This was both a practical and ethical decision. Firstly, it would have been a large hurdle to bring soil from all areas to all other areas in order to make a single uniform inoculum. Sites are miles apart and that volume of soil would have been extremely heavy. Secondly, from the point of view of careful land management, we did not want to unintentionally introduce possible pathogens across a broader range than the single hillslope on which they already existed. Nonetheless, while the replication is low, we did see clustering of fungal DNA-profiled communities from the bulk inoculum within the two high severity sites and the two low severity sites (Fig. S4) (moderate severity sites did not cluster with each other or either of the other severity levels). Moreover, fungal community profiling work in fire-burned areas shows changes in fungal composition in response to varying severity (Glassman et al., 2016; Pulido-Chavez et al., 2021).

A potential alternative for restoration practitioners would be to facilitate the formation of mycorrhizas with drought-adapted fungi in the greenhouse prior to outplanting.

Previous work has shown that fungi differ strongly across water availability and other stress gradients in the field (Moeller et al., 2014; Bui et al., 2020). Stress-adapted fungi may better withstand harsh conditions of fire scars and other degraded areas, thereby providing substantial nutrient and water benefits to their host seedlings. Additionally, allowing seedlings to jumpstart fungal networks in the greenhouse, may bypass the initial carbon costs of establishing fungal partners in a field setting.

Limitations

The challenges to establishing bigcone Douglas-fir seedlings in burned sites are numerous. Among the hurdles to conducting this research were issues related to site selection, seedling propagation and preparation, and whether or how much to alter potential planting sites. The seedlings in this study were held in refrigeration for four months prior to outplanting. While this may be cause for concern for some, we expect that any mortality due to prolonged refrigeration would have been observable in the first few months after outplanting. Yet we saw almost no mortality prior to the first summer and therefore expect that any effect from refrigeration was small in comparison to field-experienced stresses.

Synthesis

Bigcone Douglas-fir is considered a drought adapted conifer (Lombardo et al., 2009). Across its range it occurs from within mixed conifer forests to mesic chaparral, and in some cases xeric open shrubland habitats. In more mesic chaparral sites, where chaparral is adjacent to or comprises the understory of bigcone stands, mature bigcones suffer very high mortality during fire (Parkinson, Moritz, D‘Antonio in press). Hence restoration in those habitats makes little sense: conditions might appear to be more favorable initially due to

somewhat more mesic conditions, but the ultimate fate of bigcone in chaparral vegetation is very poor due to the inevitabiliy of stand-killing fires. By contrast, Parkinson et al, found that association with *Q. chrysolepis* and with more open understory increased the likelihood of at least partial stand survival during largescale fires. Stands that suffered some mortality during fire but are in these refugia (open understory, or associations with oaks) could be selected as targets for postfire population enhancement through outplanting as done here. Because microsite manipulations are easy to conduct in the field and can dramatically improve survival, we recommend that the selection of outplanting sites be done to maximize both short term (adding protective microsites) and long term population survival (avoiding regions with greater potential for high fire intensity).

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2.7. Figures and figure captions

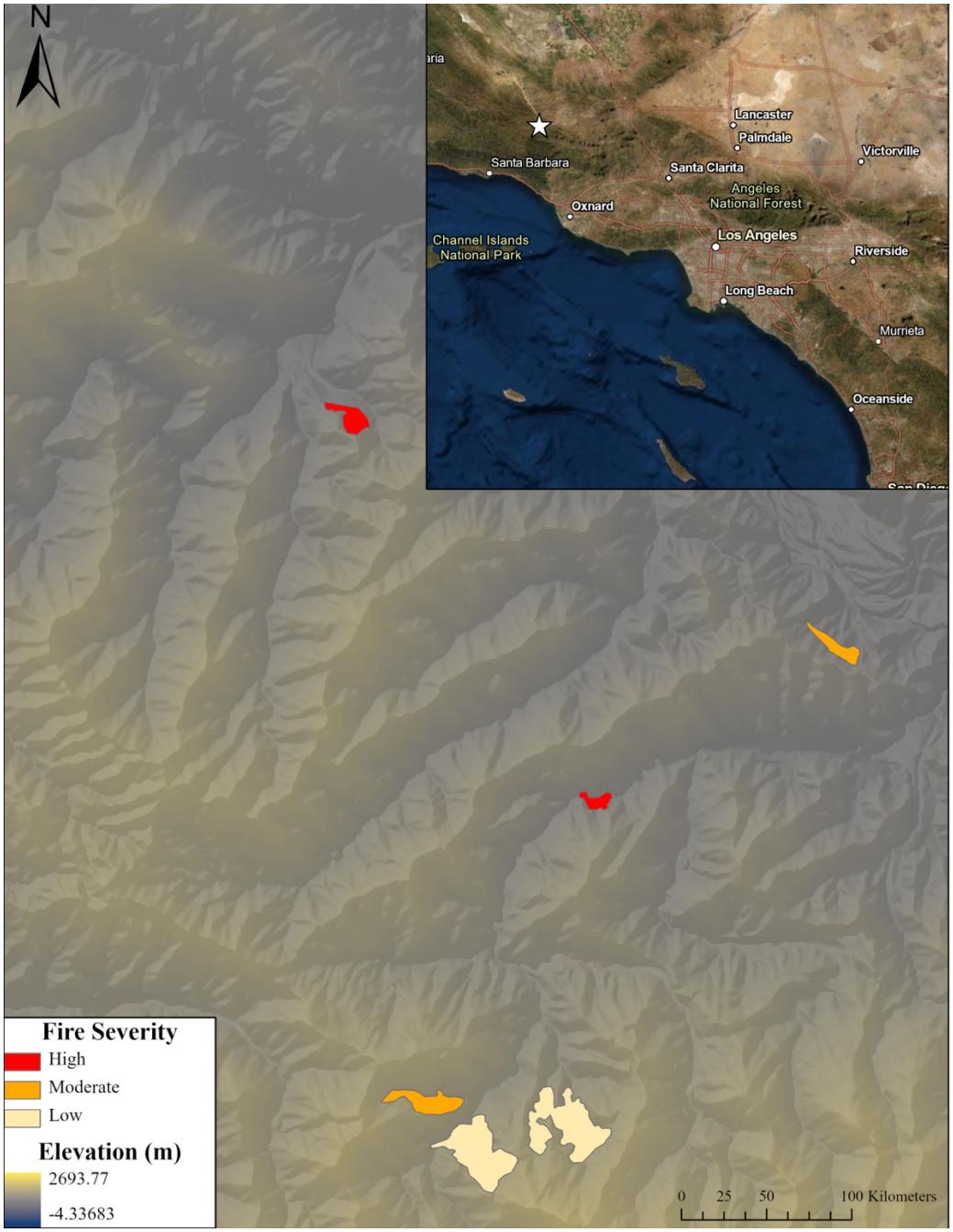

Figure 1: Map of study locations. Planting areas were chosen to be as close to one another as possible to constrain climate, elevation, and parent material. They follow CA HWY 33 on the north side of the Pine Mountain saddle (34.649917, –119.385418). Sites are colored by the severity of fire experienced in the area, calculated from percent die-off (see above). Shading represents elevation and is overlaid on a hillshade layer to display topography. Shapefiles are available in the appendix. Inset map shows the location of sites (white star) within a broader geographic context.

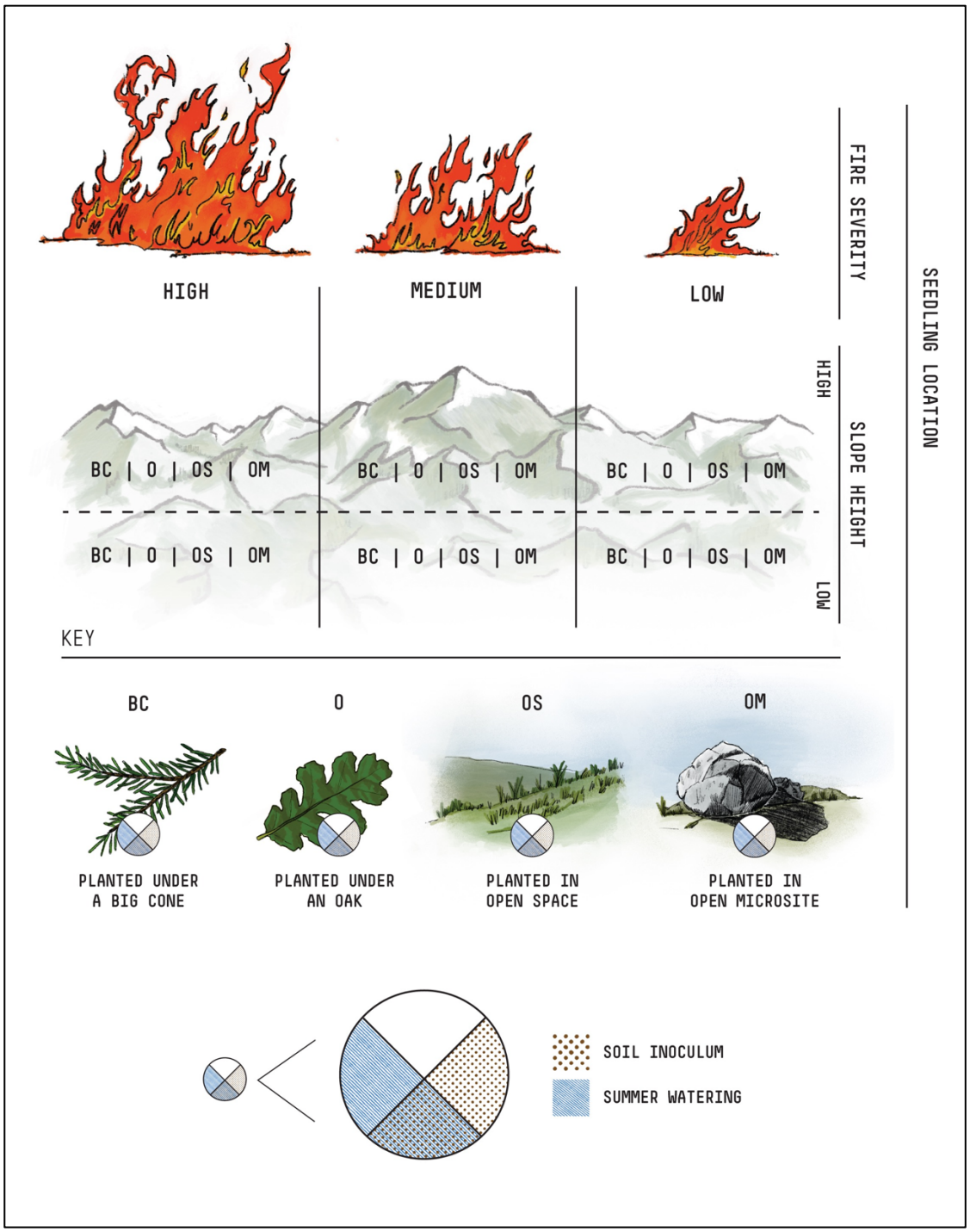

Figure 2: Outplanting design. Breakdown of seedling planting locations across the landscape. Three fire severity levels were replicated twice in planting areas shown in Figure 1. Within these areas, there were high and low elevation planting locations across which the microhabitat treatments were replicated (under bigcone Douglas-fir, under canyon live oak, in the open, and in the open with microsites). These sites (48 sites, 36 seedlings per site) were further broken down by inoculating half of the seedlings with live soil for mycorrhizal associates and watering half in June of the planting year such that nine seedlings had each of four combinations of soil inoculum or not and watering or not.

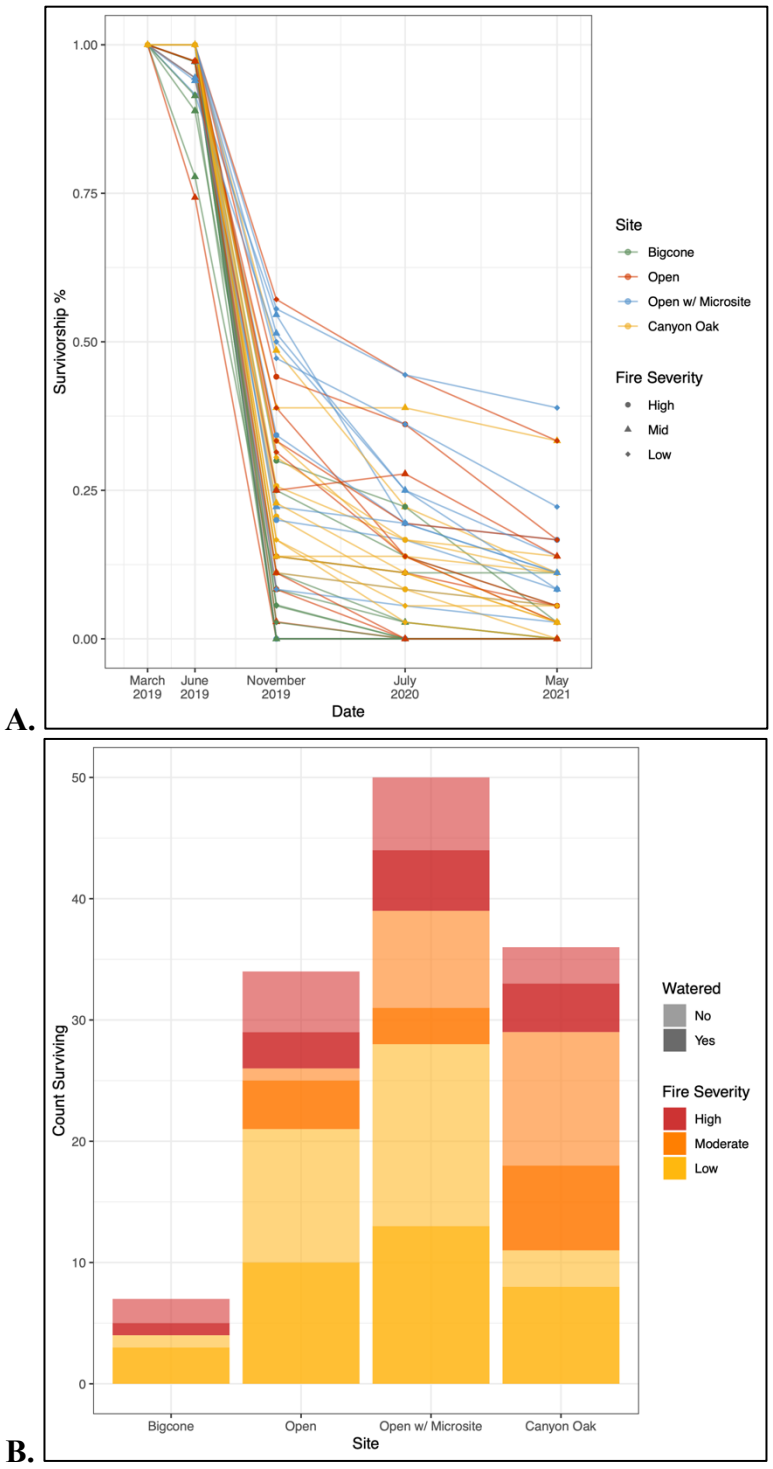

Figure 3: Seedling survival. Each of these panels depict the overall survival of seedlings throughout the study (March 2019–May 2021). (A) Shows the proportion of seedlings surviving within each planting area, with each microenvironment given a different color and each fire severity level given a different shape. (B) Shows the final count of seedlings surviving after 26 months. Here, seedlings are organized by microenvironment and stacked by fire severity. Within each fire severity bin (red, orange, yellow), seedling counts are shaded by whether or not they received a first summer watering (darker) or not.

Table 1: Number of surviving seedlings throughout the study

Site	March 2019	Loss March to June	June 2019	Loss June to Nov.	Nov. 2019	Loss Nov. to July	July 2020	Loss July to May	May 2021
Low Severity									
Bigcone	143	0.7%	142	93.0 %	10	60.0%	4	0.0%	4
Open	142	0.7%	141	59.6 %	57	42.1%	33	36.4%	21
Open w/ Microsite	144	2.1%	141	58.9 %	58	31.0%	40	30.0%	28
Canyon Oak	144	0.0%	144	80.6 %	28	50.0%	14	21.4%	11
All	573	0.9%	568	73.1 %	153	40.5%	91	29.7%	64
Moderate Severity									
Bigcone	143	11.2%	127	94.5 %	7	71.4%	2	100.0 %	0
Open	142	8.5%	130	89.2 %	14	28.6%	10	50.0%	5
Open w/ Microsite	139	3.6%	134	67.2 %	44	47.7%	23	52.2%	11
Canyon Oak	142	1.4%	140	68.6 %	44	31.8%	30	40.0%	18
All	566	6.2%	531	79.5 %	109	40.4%	65	47.7%	34
High Severity									
Bigcone	137	1.5%	135	86.7 %	18	27.8%	13	76.9%	3
Open	142	0.7%	141	83.7 %	23	26.1%	17	52.9%	8
Open w/ Microsite	142	0.0%	142	83.8 %	23	30.4%	16	31.2%	11
Canyon Oak	141	0.0%	141	77.3 %	32	46.9%	17	58.8%	7
All	562	0.5%	559	82.8 %	96	34.4%	63	54.0%	29

These data show the number of seedlings surviving and the percent die-off between censusing events. Data are binned by the microenvironment and fire severity (n=144 seedlings per row).

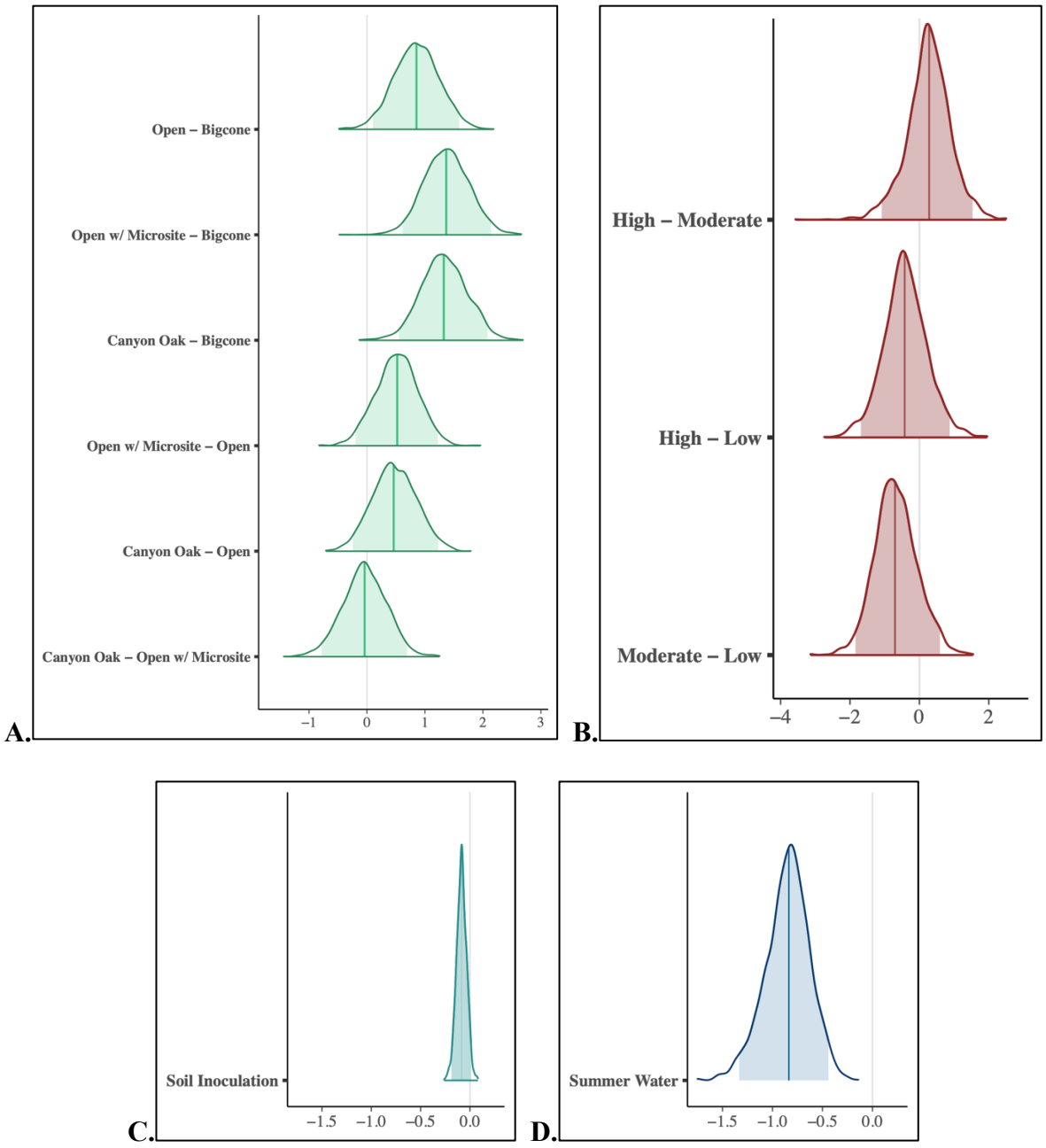

Figure 4: Posterior distributions of parameters in seedling-survival model. Shaded regions depict 95% probability in the distribution and the vertical line shows the median estimate. For categorical terms, microhabitat (A) and fire (B), the distributions show the contrasts between treatments. Binary terms, soil inoculation (C) and summer water (D), are centered at zero with +1 for treated seedlings and –1 for untreated seedlings. Positive x-axis values are related to increased survival of seedlings in our model (Eqs 1, 2).

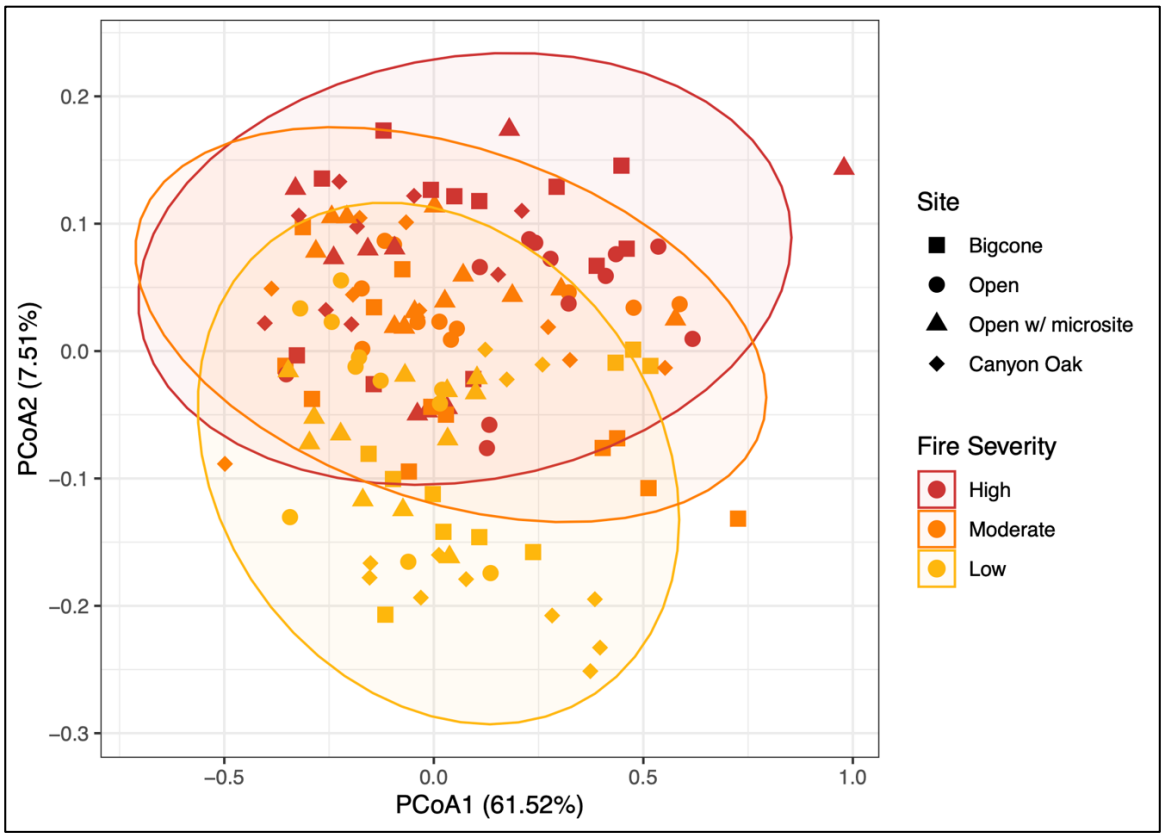

Figure 5: Principal coordinates analysis (PCoA) of soil fungal communities. Soil fungal community data from within each of the planting sites. Bray–Curtis dissimilarity was used to differentiate the community compositions. Shading and point shapes are used to distinguish fire severity and microenvironment, respectively. Ellipses represent the 95% confidence interval for each fire severity level.

2.8. Appendix

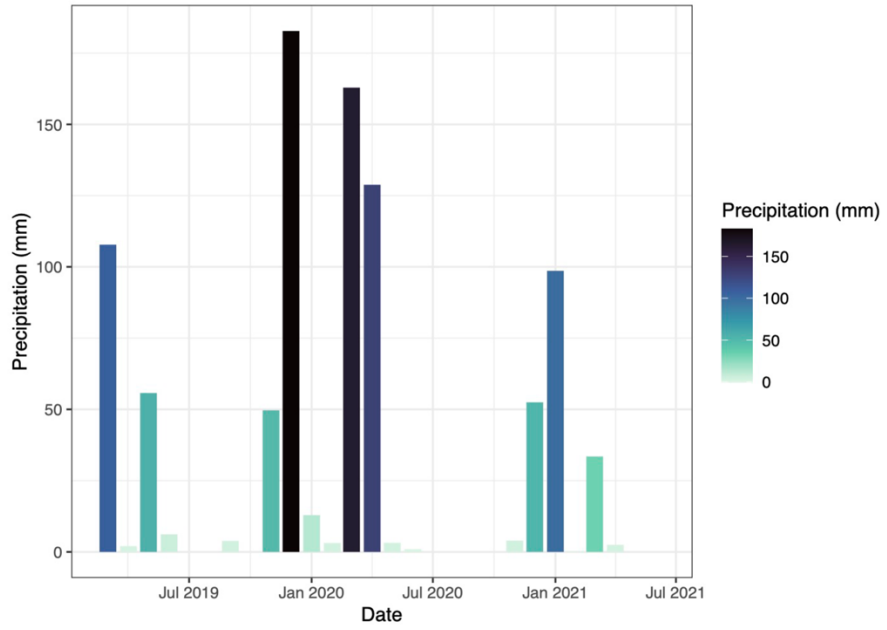

Figure S1: Precipitation at planting sites
Cumulative monthly rainfall at planting sites plotted from the time of planting until after the final census. Data is downloaded from PRISM (Hart and Bell, 2022).

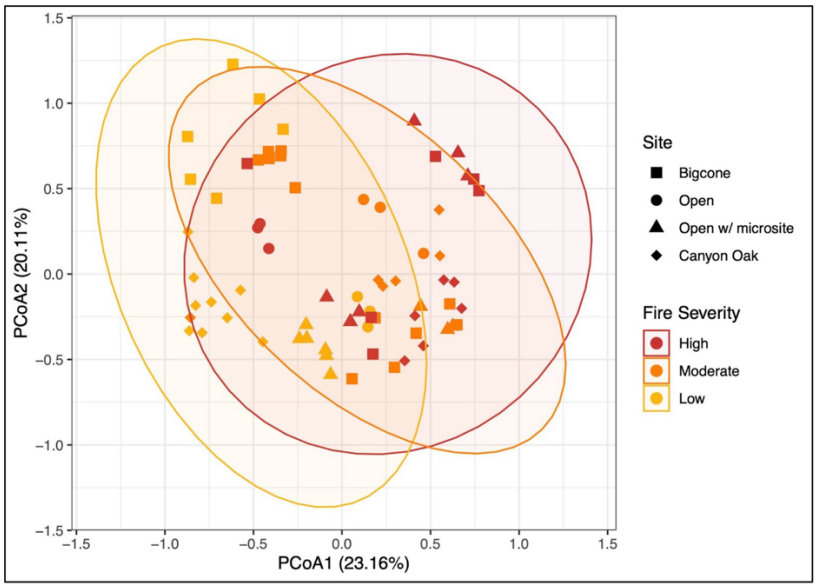

Figure S2: PCOA of plant-pathogenic soil fungal communities
Fungal community data from planting plots was subsetted based on FUNGuild assignments, here showing any fungal ASV with a guild assignment containing "Plant Pathogen". Bray-Curtis dissimilarity was used to differentiate the community compositions. Shading and point shapes are used to distinguish fire severity and microenvironment, respectively. Ellipses represent the 95% confidence interval for each fire severity level.

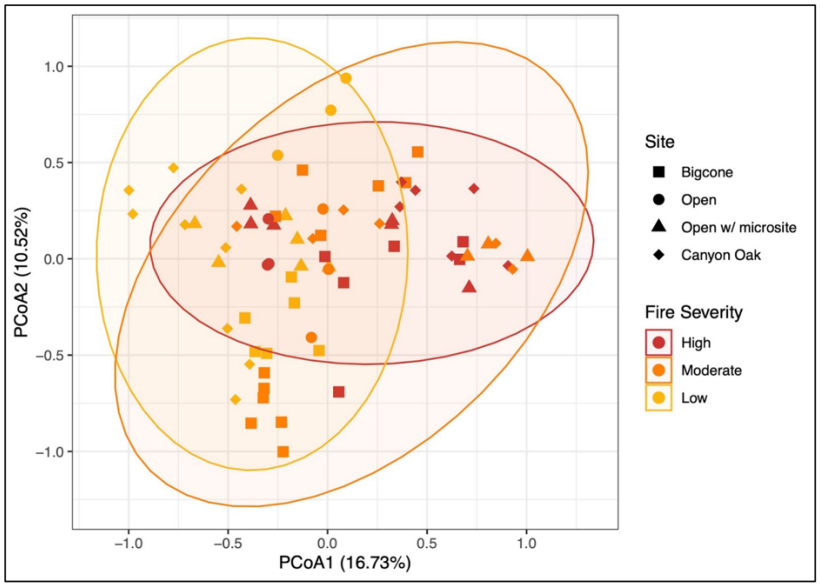

Figure S3: PCOA of ectomycorrhizal soil fungal communities
Fungal community data from planting plots was subsetted based on FUNGuild assignments, here showing any fungal ASV with a guild assignment containing "Ecto". Bray-Curtis dissimilarity was used to differentiate the community compositions. Shading and point shapes are used to distinguish fire severity and microenvironment, respectively. Ellipses represent the 95% confidence interval for each fire severity level.

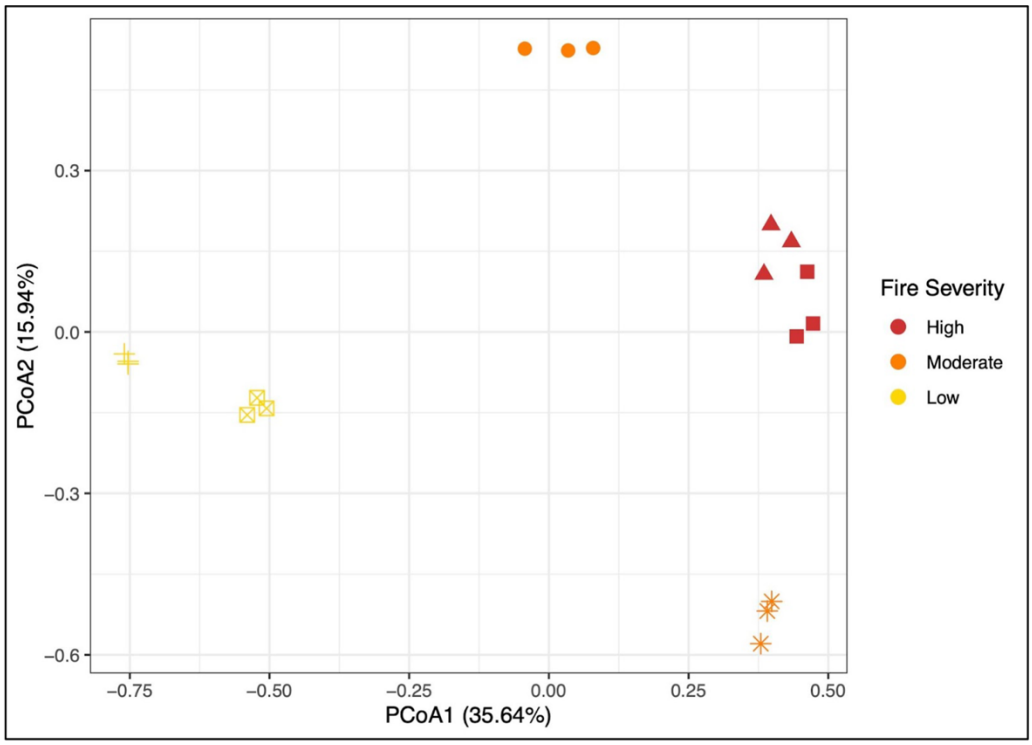

Figure S4: Soil Inoculum PCoA
Soil fungal community data from each of the bulk inoculum sources, shown from triplicate samples. Bray-Curtis dissimilarity was used to differentiate the community compositions. Color is used to differentiate fire severity with shape showing the different planting areas.

Figure S5: Planting conditions 1

Figure S6: Planting conditions 2

Figure S7: Planting conditions 3

Figure S8: Planting conditions 4

Site	March 2019	Loss March to June	June 2019	Loss June to November	November 2019	Loss November to July	July 2020	Loss July to May	May 2021
Low Severity									
Bigcone	143	0.7%	142	93.0%	10	60.0%	4	0.0%	4
Open	142	0.7%	141	59.6%	57	42.1%	33	36.4%	21
Open w/ Microsite	144	2.1%	141	58.9%	58	31.0%	40	30.0%	28
Canyon Oak	144	0.0%	144	80.6%	28	50.0%	14	21.4%	11
All	573	0.9%	568	73.1%	153	40.5%	91	29.7%	64
Moderate Severity									
Bigcone	143	11.2%	127	94.5%	7	71.4%	2	100.0%	0
Open	142	8.5%	130	89.2%	14	28.6%	10	50.0%	5
Open w/ Microsite	139	3.6%	134	67.2%	44	47.7%	23	52.2%	11
Canyon Oak	142	1.4%	140	68.6%	44	31.8%	30	40.0%	18
All	566	6.2%	531	79.5%	109	40.4%	65	47.7%	34
High Severity									
Bigcone	137	1.5%	135	86.7%	18	27.8%	13	76.9%	3
Open	142	0.7%	141	83.7%	23	26.1%	17	52.9%	8
Open w/ Microsite	142	0.0%	142	83.8%	23	30.4%	16	31.2%	11
Canyon Oak	141	0.0%	141	77.3%	32	46.9%	17	58.8%	7
All	562	0.5%	559	82.8%	96	34.4%	63	54.0%	29

3. Chapter 2

A reflection of their roots: How soil conditioning and neighbor effects shift seedling outcomes and drought response.

3.1. Abstract

Seedlings, like all of us, are a product of their environment. The conditions in which they grow can have lasting effects on their future outcomes through factors such as nutrient or water conditions, access to important symbiotic communities, or the accumulation of enemies. This project, motivated by in-situ observations, questions the nature of a potential nurse plant relationship between an endemic fir (Pseudotsuga macrocarpa) and a co-dominant oak (Quercus chrysolepis). To better understand the multiple interacting drivers existing in natural contexts, this project investigates how soil conditioning and neighboring plants interact to drive seedling outcomes and whether these effects are dependent on environmental conditions. In a greenhouse setting, seedlings of each of the two species were conditioned for a year in individual pots containing home inoculum, away inoculum, or solely sterile potting mix before being washed of all soil and co-planted in a mixed design with another seedling of the same or different species. Neighbor seedlings were then tracked for a year before being subjected to a simulated drought for an additional 8 months. To assess the distinct and interacting roles of conditioning and neighbor context, seedling size, mycorrhization, and health were tracked throughout the course of the experiment.

Seedling conditioning had strong effects on physiology going into the shared pots. Each species exhibited clear responses to the availability of inoculum, pointing to the role that microbial communities play in their success. This conditioning continued to drive changes in shared pot communities where neighbor species as well as neighbor soil

conditions were found to have clear impacts on the aboveground growth of seedlings. Importantly, the effect of initial soil conditioning remained a strong predictor of seedling health and growth throughout the experiment with neighbor conditions largely acting to scale these effects. When subjected to drought, the firs were far more likely to decline in performance with a history of home soil conditioning (negative plant soil feedback), particularly when their neighbor was an oak. Oaks did not show such strong productivity responses to drought but did show a clear cost of home soil conditioning when potted next to a fir. By outlining these stark context-specific differences in seedling outcomes, this study highlights the complexity of predicting plant-soil feedbacks as well as forest successional dynamics in such a rapidly changing world.

3.2. Introduction

Approximately 80% of temperate trees rely on ectomycorrhizal fungi for the uptake of nutrients and water in nutrient-scarce and drought conditions (Steidinger et al. 2019; Smith and Read 2008; Yin et al. 2018). In this symbiosis, tree sugars fixed in photosynthesis are traded to soil fungi in exchange for mineral nutrients and water obtained by fungi (Kiers et al. 2011; Klironomos et al. 2011). By subsidizing the growth of these fungi, trees are able to functionally extend their rooting breadth, taking advantage of the higher surface area of fine fungal hyphae (Cheng et al. 2016; Marschner and Dell 1994; Lindahl and Tunlid 2015). Furthermore, often both trees and fungi are compatible with multiple partner species. This can lead to a complex network of interactions when, for example, trees support ectomycorrhizal fungi that may also benefit potentially competing tree individuals (Bruns, Bidartondo, and Taylor 2002; Ferlian et al. 2021; Rasmussen, Busby, and Hoeksema 2018).

Alterations of symbiotic outcomes among co-occurring trees at very local scales are commonly referred to as neighbor effects (Bogar and Kennedy 2013; Moeller et al. 2016; Otsing et al. 2021; Seyfried et al. 2021). Neighbor effects can have meaningful consequences for the health and survival of individuals and are driven by multiple distinct mechanisms (Lofgren, Nguyen, and Kennedy 2018; Hortal et al. 2017). One mechanism relies on the fact that ectomycorrhizal communities found on the roots of trees are formed via one of two pathways. The first is through spore colonization wherein compatible trees can induce the germination of fungal spores which then grow to form mycorrhizae with that host tree (Nara 2009; Kennedy et al. 2011). Alternatively, fungi already growing in the soil may colonize a tree through mycelial colonization, eliminating the need for the specific germination cues to be produced by a given host (Hubert and Gehring 2008; Bogar, Dickie, and Kennedy 2015). This second pathway can lead to novel interactions between hosts and symbionts when in the presence of an alternate host/neighbor tree species (Lofgren, Nguyen, and Kennedy 2018). On the other hand, co-occurring ectomycorrhizal fungi regularly compete for common resources, potentially leading to community filtering via competitive exclusion by host preference, niche preemption, or physical displacement (Kennedy et al. 2011; Christian and Bever 2018; Kennedy 2010; Dickie 2007; Rasmussen, Busby, and Hoeksema 2018). Finally, while not as often considered in the context of neighbor effects, mixed forest environments can dilute the effect of host-specific pathogens and provide refuges for seedlings by maintaining beneficial symbionts nearby alternate host trees (Bennett et al. 2017; Gómez-Aparicio et al. 2017; van der Putten et al. 2013; Crawford et al. 2019). These multi-species interactions complicate the exchange of resources between trees

and fungi, and few studies explore the consequences of multi-species interactions for host tree performance, particularly under resource stress.

The outcomes of host-microbe interactions are dictated by the conditions in which the symbioses occur. Drought is among the most prevalent forest stressors and is expected to increase as a driver over the coming decades (Zhao et al. 2020; Anderegg et al. 2018; Trugman et al. 2021). While drought directly affects tree productivity and can lead to large scale die offs of individual species (W. R. Anderegg et al. 2019; Adams et al. 2017), it can also impact the relative abundances of microbes in the soil leading to losses of mycorrhizal benefit and an increased role of bacteria and pathogens (de Vries et al. 2012; van der Putten et al. 2016). On the other hand, many mycorrhizal plants have been shown to experience reduced water stress under drought in the presence of specific mycorrhizal partners (M. F. Allen 2007; Marjanović and Nehls 2008; Bowles, Jackson, and Cavagnaro 2018), with recent work further demonstrating the facilitation of water uptake by mycorrhizal fungi (Kakouridis et al. 2022). These patterns may lead to changes in mutualist selection towards fungi that are better adapted to maintain function when water is limiting (Moeller, Peay, and Fukami 2014; Bui et al. 2020). Understanding how these patterns hold across different host-soil matches is important for understanding how and where extreme events may shift forest outcomes.

In this study, we investigated how ectomycorrhizal trees and their neighbors are influenced by soil microbial communities, aiming to gain insight into the dynamics of mixed forest stands. We chose to work with a pair of co-dominant canopy trees which grow in the mountains of Southern California, USA. Bigcone Douglas-fir (Pseudotsuga macrocarpa, hereafter ‘bigcone’) is an endemic fire-adapted conifer and canyon live oak (Quercus

chrysolepis, hereafter ‘oaks’) is an evergreen hardwood which grows across a broader range of Western North America but substantially overlaps with bigcone populations in southern California (Parkinson, D’Antonio, and Moritz 2022). Previous work has observed higher rates of bigcone seedling recruitment under oaks as compared to conspecifics, though the mechanism by which this ‘facilitation’ occurs is yet unknown (Runte et al. 2022; Parkinson, D’Antonio, and Moritz 2022; Minnich 1977; McDonald and Littrell 1976). In particular, it is unclear whether the facilitation effect is driven directly by the neighboring tree species, or indirectly via the below-ground microbial community associated with heterospecific neighboring trees.

In a greenhouse setting, we conditioned seedlings to different natal soils, and then combined seedlings from different natal soils into shared pots. Later, half of the pots were subjected to a prolonged drought. Using this design, we addressed the following specific questions: 1. Do natal soil and neighbor species have distinct or redundant effects on ectomycorrhizal community composition? 2. How is seedling growth affected by microbial partners from natal soil as well as those contributed by neighbor seedlings? 3. Are the sign and strength of neighbor effects influenced by drought? We hypothesized that fungal diversity would increase with both inoculum diversity and host diversity, and that neighboring host species would facilitate one another's growth by supporting a more diverse fungal community. We also expected that increased mycorrhizal colonization rate and diversity would ameliorate the effects of drought while competition between conspecifics would increase.

3.3. Methods

To quantify the relative importance of natal soil and neighboring hosts on focal tree performance and mycorrhizal community composition, we conducted a two-phase greenhouse experiment using Bigcone Douglas-fir (Pseudotsuga macrocarpa, 'bigcone') and canyon live oak (Quercus chrysolepis, 'oak'). In the first phase of the experiment, we conditioned seedlings in three different natal soil types (bigcone, oak, and sterile control) in order to establish microbial associations. In the second phase of the experiment, we co-planted seedlings and measured their subsequent growth and photosynthetic performance; by subjecting half of the seedlings to drought, we also quantified the impacts of water limitation on seedling performance.

Greenhouse experiment

We collected seed stock for this experiment from a single site in Santa Barbara County, Southern California USA (34.743682, -119.985252). Simultaneously, we collected soils to use as fungal inoculum. After removing litter and herbaceous ground cover, we removed approximately 6 liters of soil from the top 15cm from three replicate holes under 100% bigcone or 100% oak canopy cover. We sieved soils to 4mm and homogenized them within each canopy type before storing at 4°C until use. We cold stratified the seeds in trays of wet sand at 4°C for 4 weeks before moving them to the climate-controlled greenhouses at the University of California, Santa Barbara for germination. The greenhouse was covered with a 50% pass-through shade cloth and held to a temperature range of approximately 15 to 25°C.

To observe the influence of natal soil (Fig. 1a), we created three soil treatments: a sterile potting mix, 4:1 potting mix to sieved field soil from an oak stand (‘oak soil’), and 4:1 potting mix to sieved field soil from under a bigcone (‘bigcone soil’). The potting mix

was composed of pumice, vermiculite, compost, hydrated coconut coir, and sand (in a 4:2:2:1:1 ratio). To minimize any microbial inoculum in the greenhouse mix, we autoclaved the soil twice at 120℃ and 1.4kg/cm2 pressure for 45 minutes (allowing it to cool for a minimum of 24 hours between autoclaving runs). We planted germinants into individual 0.4L pots (Stuewe and Sons AB35) filled with one of our three soil types. Once potted, we grew seedlings in their respective soils, allowing the formation of mycorrhizas with compatible fungi, for 13 months. This duration was necessary to allow for slower-growing mycorrhizas to form (Erland and Finlay 1992; Pruett, Bruhn, and Mihail 2008) and to time the following co-planting just prior to spring leaf-out. Watering was done with deionized tap water by saturating the pots then allowing the surface soil to dry to the touch before a subsequent watering. During this time, and throughout the project (approximately every 3 months), we measured seedling height, additional branching lengths, basal stem diameter, and leaf count (oaks) or chlorosis score (bigcones). The chlorosis score is a 1 to 5 ranking of the visual greenness of the plant as a proxy for health (Moeller et al. 2015; 2016).

After the first 13 months of growth, we unpotted the seedlings, washed off all soil, and measured total seedling size using size-scaled photos and assessed percent colonization rates of root length by ectomycorrhizal fungi under a dissecting microscope. We destructively harvested, dried, and weighed 10 seedlings from each soil x species combination to verify the correlation between photos and dry biomass. At this repotting step, we also assessed mycorrhization (see Molecular and bioinformatic methods below). To minimize stress and root/fungal mortality, we re-planted all seedlings within 4 hours, and typically fewer than 2 hours during which time root systems were kept in damp towels to maintain moisture.

To quantify how neighboring hosts would alter performance, we repotted seedlings into shared pots (Fig. 1b). We co-planted seedlings into 2.8-liter pots filled with sterile potting mix, such that any soil microbes coming into the pot would be in the form of mycorrhizas, epiphytes, or endophytes associated with one of the two plants going into the pot. These plant pairs were of variable species and natal soil combinations. We allowed neighbor seedlings to grow in their 'shared pots' with regular watering for the next 14 months.

To test the interaction of drought with EMF background and neighbor presence, we moved the shared pots onto a drip irrigation system for more fine-scale control of water inputs in the final stage of the study. We divided the shared pots into watered and drought groups. We restricted drought pots to 50% shorter irrigation times, with all pots receiving water every other day. We intended these frequent, brief watering events to maintain a consistent and prolonged disparity in soil water availability between treatments. We monitored soil moisture (Figure S1) regularly using an electrical conductivity soil moisture probe to ensure effective treatments (Bluelab Pulse Multimedia EC/MC Meter; Bluelab, Tauranga NZ). We continued this treatment for 8 months, measuring seedling health throughout (see NDVI surveys below). Soil moisture in drought pots was consistently lower than soil moisture in control pots, irrespective of seedling composition ($p < 0.001$, Fig. S1). During the first month of irrigation, all pots dropped to low soil moisture levels. After an increase in irrigation duration for the watered pots, soil moisture leveled out between weekly measurements and remained relatively constant for the duration of the experiment.

After 8 months of drought treatment, we harvested all seedlings and washed the seedlings clean of soil and then separated them into root, aboveground woody, and foliar

subsamples. We scanned each of these samples for area and dried (min. 72 hr at 60°C) and weighed them for biomass. Prior to drying, we observed the root systems under the microscope for percent colonization and fungal root tip dissection following the same protocol as the repotting step.

Molecular and bioinformatic methods

In order to understand how soil inocula change seedling ectomycorrhizal communities, we first developed a method for characterizing ectomycorrhizal communities by associating observed root tip morphologies with Sanger sequenced material. At both the repotting and harvest timepoints, we observed seedling root systems under a dissecting scope and quantified the relative abundance of ectomycorrhizal fungi by scoring colonized and uncolonized root tips. To do so, we initially verified colonization then, for seedlings with colonized tips, scored the entirety of small root systems or a representative subsample of larger root systems (mean: 129 (bigcone) to 133 (oak) tips). We classified observed ectomycorrhizal root tips into different morphotypes, and sampled root tips representing each morphotype from each seedling by placing root tips into 10µL of Extraction Solution (Sigma E7526, Burlington, MA). We extracted DNA by incubating root tips for 10 minutes at 65°C then 10 minutes at 95°C, and then diluted in 10µL Neutralization Solution B (Sigma N3910, Burlington, MA). We amplified fungal DNA following Moeller et al. 2016 (Moeller et al. 2016) and sent samples for Sanger sequencing at Molecular Cloning Laboratories (South San Francisco, CA USA).

We manually trimmed sequences then clustered them into contigs using the software program Geneious (Biomatters Inc., Auckland NZ) at high sensitivity with default thresholds. We used consensus sequences in taxonomic assignment of clustered samples and

trimmed sequences for samples that did not cluster. To apply taxonomic assignments, we used the *assignTaxonomy*() function from the *dada2* package in R, referencing the UNITE database (release information) (Callahan et al. 2016; Nilsson et al. 2019). We further filtered samples to include only known ectomycorrhizal taxa using FUNGuild assignments (Nguyen et al. 2016). From each morphotype, we sequenced at least 12 tips, and then assigned taxonomy to unsequenced tips. Specifically, when all sequences indicated correspondence between a morphotype and taxonomy, we assigned all tips of that morphotype to that OTU. When sequences indicated that taxonomy varied by seedling, we sequenced a tip from each seedling. To fill out our root tip identities, we recorded non-ectomycorrhizal sequences and failed amplification attempts and included them in the assignment of morphotypes to fungal taxa – or lack thereof – as well as adjusting colonization rates accordingly.

Seedling size image analysis

Because dry biomass requires destructive sampling, we used a size-to-biomass conversion analysis to estimate seedling dry biomass at the repotting time point.. In order to be minimally damaging to the seedlings, we photographed the seedlings against a white background alongside a scale bar. We then used ImageJ (Schneider, Rasband, and Eliceiri 2012) to visually split seedlings into above and belowground compartments at the root collar and then quantify above- and belowground area in cm2. Using the masses of the 60 destructively sampled seedlings, we fit linear regressions ($R2 >= 0.93$, Fig. S2) to predict above- and belowground biomass for all photographed seedlings.

NDVI surveys

During the drought treatment, we measured the health of seedlings using Normalized Difference Vegetation Index (NDVI) values, an accepted plant productivity reflectance metric (Pettorelli et al. 2005; Zeng et al. 2022). Throughout the 8 month drought treatment, we collected images of the seedlings approximately every two months using a multispectral survey camera (Survey 3 by Mapir). By the final harvest, we had photographed each seedling four times. We positioned a tripod holding the camera approximately 20 cm above the pots to capture top-down images of the seedlings. To minimize background noise and to exclude the neighboring seedling, we positioned a gray felt cone around the subject seedling. The images were composed of three optical bands (red, green, and near-infrared). Using the rembg image background removal tool, initiated through Docker and R, we segmented the seedlings from any background noise (Gatis [2020] 2024). Using pixel reflectance values, we calculated NDVI by dividing the difference between NIR and red reflectance by the sum of the two bands.

Data Analysis

We conducted all analyses in R, version 4.4.0, through the RStudio environment (RStudio Team 2021). We considered p-values at or below 0.05 to be significant across our significance tests. We computed differences between treatment groups in response to experimental design elements using Welch's t-test for direct two-sample comparisons and one-way ANOVAs with Tukey’s Honest Significant Difference test for comparisons of more than two groups. We fit linear regressions and used AIC-selection to investigate the individual and interacting effects of multiple experimental treatments.

We assessed the initial soil effects, co-planting effects, and cumulative seedling growth effects independently. Thus, depending on the focal test, we calculated seedling size

metrics separately for the initial soil conditioning phase (masst1), the relative growth in shared pots (masst2/masst1), and total biomass by the end of the study (masst2). Moreover, when considering the effects of neighbors and neighbor soil independent of drought, we used only the biomass of those seedlings that did not experience a drought.

In order to improve interpretability of our health data, we took the mean NDVI value of all sampling timepoints on an individual seedling. This allowed us to capture a more complete representation of the decline of seedlings over the duration of the treatment and minimized sampling bias. We then considered NDVI values across drought treatments in relation to whether a seedling was grown in home soil (seedling species matched to natal soil) versus the alternate live soil in a metric we term 'home soil match'. In these analyses, we evaluated only seedling pairs which had both been conditioned in a live natal soil. Because seedlings were potted with a neighbor, we considered home soil match highest when both a focal seedling and its neighbor had been potted in the focal seedling's home soil. We defined this match as decreasing as first the neighbor soil did not match, then if the neighbor soil did but the focal seedling soil did not match, then if neither matched.

To further understand the relative importance of different aspects of the experimental design in relation to growth and health outcomes, we fit generalized boosted regression models endpoint data. We ran the *gbm.step*() function from the *dismo* package on species-specific datasets including the factors: natal soil, neighbor species, neighbor soil, drought treatment, and fungal colonization rate (bigcone only). We evaluated total seedling biomass at harvest and seedling NDVI as dependent variables. We conducted model selection by fitting models across a range of tree complexities and learning rates and comparing the

cross-validation deviance explained scores as well as their variance, as is outlined in the appendix of Elith et al. 2008 (Elith, Leathwick, and Hastie 2008).

3.4. Results

Soil inoculum drove strong and persistent growth effects

At the end of the first year of growth, seedling sizes and relative investment into above- and below-ground biomass were markedly different based on natal soil. For both species, the root systems of seedlings conditioned in sterile soil were significantly larger than root systems from either live soil treatment (Fig 2.a,d). Aboveground biomass of bigcone seedlings was significantly greater in oak soil than in either bigcone soil or sterile soil (Fig 2.b,e). By contrast, in oak seedlings there were no distinguishable differences in aboveground growth among soil types. In total biomass, bigcone seedlings were larger in sterile soil than in either live soils while there were no distinguishable differences in total oak biomass between soil treatments. Notably, the root-to-shoot ratios of both species were significantly different from one another by soil type; root-to-shoot ratios were highest in sterile soil, moderate in bigcone soil, and lowest in oak soil (Fig 2.c,f).

In shared pots that were not subjected to drought, seedlings originally conditioned in live soil grew more (mass at harvest/mass at repotting) than seedlings conditioned in sterile soil regardless of who they shared a pot with (Fig S4). For both bigcones and oaks, root growth and total growth were significantly higher for bigcone soil and oak soil-conditioned seedlings than for sterile soil-conditioned seedlings. In bigcone seedlings, this trend continued for aboveground growth, though for oaks, bigcone natal soil conditioning led to

greater aboveground growth than sterile natal soil conditioning but oak natal soil conditioning was indistinguishable from either other treatment.

Natal soil effects were also persistently strong predictors of final bigcone seedling biomass where live soil-conditioned seedlings were significantly larger in roots, shoots, and in total (Figure S5). Bigcones were also significantly smaller in total size when grown next to an oak neighbor, though this pattern was mediated by the relative sizes of the seedlings to one another. Oaks, by the end of the study, showed no significant residual effect of natal soil (Figure S5). Oaks were overall smaller when planted next to conspecifics, with a seedling's size relative to its neighbor – at the time of repotting – significantly predicting the strength of that effect. This response was observable across roots, shoots, and total oak seedling size. Among the bigcone seedlings, all pots with solely sterile natal soil seedlings had complete mortality by the end of 3 years. Only seedlings from sterile natal soil which were co-planted with a live soil-conditioned seedling survived. Oaks did not exhibit the same pattern, nor did any other soil type have significant influence on survival. Each species experienced significant increases in seedling mortality under drought, though these effects were not driven by any specific soil or neighbor effect.

Oak neighbor species were negative but neighbor oak soils were positive

Neighbor identity affected bigcone seedling growth: individuals planted next to a conspecific grew significantly more roots and had greater total growth than when planted next to an oak neighbor, even when accounting for the relative size of the neighbor and either of the seedling's natal soil (Supplemental Table S6). Additionally, biomass of bigcone seedlings with neighbors conditioned in live natal soil grew significantly more than those with neighbors conditioned in sterile natal soil. Those with oak soil-conditioned neighbors

grew more shoots than those with bigcone natal soil or sterile natal soil. Drought treatments did not have any significant effect on bigcone growth when assessed in best-fit models alongside other independent variables.

Total oak growth in shared pots showed no clear effects of neighbor species (Supplemental Table S7). Yet, greater relative size of the seedling, as compared to its neighbor, drove an increase in growth overall alongside second and third-order interactions (e.g. Oak soil:Drought treatment, Oak soil:Drought treatment:Neighbor soil oak). Oak root growth was significantly greater with neighbors conditioned in oak natal soil than with neighbors conditioned in sterile natal soil. Oak root growth was also greater, though not significantly with neighbors conditioned in bigcone natal soil than with neighbors conditioned in sterile natal soil ($p = 0.056$, F-stat. = 1.768). Oak root growth also increased with drought. Shoot growth in oaks was significantly reduced next to oak neighbors when compared to bigcone neighbors, though this effect was mediated by relative size and drought.

Ectomycorrhizal community composition

At the repotting time point, bigcone seedlings from oak natal soil had significantly higher rates of mycorrhization than those from bigcone natal soil. Oaks, on the other hand, showed no difference in colonization rates between live soil treatments. Across both seedling species, sterile soil-conditioned seedlings were observed to be devoid of any mycorrhizae apart from a single oak seedling (out of 40 sterile soil individuals observed) which had a colonization rate of 0.01.

Sanger sequencing efforts showed distinct host species and soil effects. Bigcone seedlings were found to be in symbiosis with the same fungal partners across both live soil

treatments, primarily being colonized by Rhizopogon hawkerae with lesser rates of Pustularia sp. and Suillus lakei (Fig 3). By contrast, oak seedlings showed strong effects of natal soil; seedlings in oak-soil were found with a range of species, including Pustularia sp., Tuber candidum, Cenococcum sp., and Peziza sp. but only with Pustularia sp. in bigcone soil.

Drought effects were strongest in home soils

Overall, seedling health declined in response to drought, as was expected. Health values declined by 21.4% (sd = 4.8%) and 23.0% (sd = 3.6%) in bigcones and oaks, respectively. Importantly, among live soil-conditioned seedlings, there was a strong effect of home soil on seedling outcomes. For both species, drought had a stronger negative effect on seedling health with increasing home soil match. Here we consider an increasing match from no seedlings in the pot conditioned in a focal seedling's home soil, to just the neighbor, just the focal seedling, and finally both seedlings in the pot. For each seedling species, the strongest negative effect of drought was observable when both seedlings in a pot were conditioned in a focal seedling's home soil (Figure 4). In bigcone seedlings, there was a significant dip in NDVI in these contexts.

Soils outweighed neighbors and drought in seedling biomass but not always health

Our generalized boosted regression models showed that over the course of the study, bigcone growth and health were both most strongly predicted by natal soil (Fig 5). Moreover, neighbor natal soil outweighed either neighbor species and drought as a contributor to seedling outcomes, with a strong interaction with natal soil. These results predict the impact of neighbor soil to be of the greatest importance for seedlings initially conditioned in sterile soil .

Oak seedlings, on the other hand, showed divergent patterns in which factors predicted growth versus health (Fig 5). As with bigcone seedlings, oak biomass was best predicted by natal soil. Moreover, oak biomass was better predicted by neighbor-driven factors than by drought treatment, though neighbor species had greater predictive power for oak biomass than for bigcone biomass. The health of the oaks, measured as NDVI, was far better predicted by the drought treatment than by any other factor. This was in contrast to the bigcone seedlings wherein drought never outweighed natal soil or neighbor-driven influences. Similarly to oak growth, oak health was predicted also by natal soil and neighbor soil, though neighbor species was very minimally predictive.

3.5. Discussion

Seedling success is shaped by environmental context, including neighboring trees and the microbial communities that they host. Our work demonstrates that early exposure to microbial communities can alter seedling growth and physiological trajectories, even more so than the subsequent presence of live neighboring seedlings. In this way, our results are consistent with other evidence for priority effects–in which early-arriving species determine the trajectory of a system's composition and/or function (Fukami 2015; Fukami et al. 2010; Stroud et al. 2024)–in mycorrhizal systems (Werner and Kiers 2015; Kennedy, Peay, and Bruns 2009) . Thus, at least at the seedling stage, our findings suggest that neighbor effects primarily arise from microbial propagule supply (Amaranthus and Perry 1987; Dixon et al. 1984) rather than direct competition among hosts for resources (Peay 2018; Moeller et al. 2016).

In our study, plant-soil feedbacks were largely neutral or positive, with seedlings grown in "home" natal soils performing roughly as well as seedlings in "away" soils and

outperforming seedlings in sterile soils. Numerous studies have demonstrated the beneficial effects of "home" conditioned soils on ectomycorrhizal plants through the propagation of mutualistic partners (Nash, Laushman, and Schadt 2020; Grove et al. 2019; Amaranthus and Perry 1987; Dixon et al. 1984), and the consistency of mycorrhizal colonization and community composition across both live soil types suggests that fungal propagules are likely well-dispersed across the field landscape that we studied (Teste, Simard, and Durall 2009; Kennedy 2010). However, we did observe increasingly negative plant soil feedbacks under drought conditions. These patterns were consistent across seedling species with a surprising effect of increased declines when both seedlings in a pot were initially grown in a focal seedling's home soil. Such stress-induced plant-soil interactions have been previously identified and are hypothesized to grow in relevance with the onset of climate change (Gómez-Aparicio et al. 2017; Pugnaire et al. 2019; van der Putten et al. 2013). Whether these effects are induced by pathogens or the absence of an alternative mutualist from an alternate soil is unclear under our study design.

Our ability to identify specific ectomycorrhizal drivers of observed seedling responses was limited by the relatively low-diversity communities that we observed in the greenhouse. Similar community simplifications are not uncommon in greenhouse studies of Douglas-fir seedlings (Dučić et al. 2009; Hagerman and Durall 2004), with a few genera (e.g. Rhizopogon, Canococcum) often dominating in controlled environments (Borchers and Perry 1990; Kazantseva et al. 2009; Parke, Linderman, and Trappe 1984). These results may be in-part explained by differences in fungal colonization methods in the field, with soil translocation benefitting species spore-based colonizers with stress-tolerant spores (Smith and Read 2008; Nara 2009). Nevertheless, we also observed transfer of ectomycorrhizal

partners between seedlings in the greenhouse, as shown in previous studies (Moeller et al. 2016). It remains uncertain how well ectomycorrhizal community dynamics in a greenhouse predict the dynamics in the field, though previous studies have argued that strongly-overlapping fungal communities between the two settings support the utility of greenhouse bioassays (Jones et al. 1997).

Although in our study, we did not find strong effects of neighboring seedling identity or neighbor natal soil on seedling performance, living neighbors' effects may become more pronounced as seedlings age. Numerous field studies demonstrate the importance of host neighborhood to mycorrhizal community composition (Grove et al. 2019; Ramsfield et al. 2020), and one other greenhouse study on co-potted Douglas-fir seedlings showed convergence of the fungal communities of co-planted seedlings over time (Moeller et al. 2016). In a field setting, as seedlings become larger over time, it is reasonable to expect an intensification of resource competition (Pitkänen, Bianchi, and Kangas 2022; Berkowitz, Canham, and Kelly 1995; Vandenberghe et al. 2006) as well as larger root systems on which more fungi can coexist (Birch et al. 2023) allowing for more comingling of the fungal community (Kennedy et al. 2011; Kennedy, Peay, and Bruns 2009). Indeed, trees in mixed forest stands often host a higher diversity of ectomycorrhizal fungi than those in monotypic stands (Ferlian et al. 2021; Gao et al. 2013; Dickie 2007). Previous work in mixed conifer-oak systems show significant symbiont overlap but strong host preference and abiotic influence on species interactions (Rasmussen, Busby, and Hoeksema 2018; L.M. Suz et al. 2017).

Our findings have direct implications for the maintenance of mixed forest trees including the Bigcone Douglas-fir, a Southern California endemic species confronted with

intensifying fire and drought stress due to anthropogenic climate change (Parkinson, D'Antonio, and Moritz 2022; Runte et al. 2022). As with other Douglas-fir species, a reservoir of ectomycorrhizal inoculum is likely essential for successful recruitment (Barker et al. 2013). In this study, strong soil-driven seedling outcomes point to the importance of considering the limited or shifted inoculation potential of post-disturbance landscapes (Glassman et al. 2016; Barker et al. 2013; Bent et al. 2011). In summary, integrating ectomycorrhizal community ecology into landscape management strategies has clear potential to improve conservation and restoration outcomes (Policelli et al. 2020; Hawkins, Jones, and Kranabetter 2015; Corbin and Holl 2012; Laura M. Suz et al. 2015).

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3.6. References

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3.7. Figures and figure captions

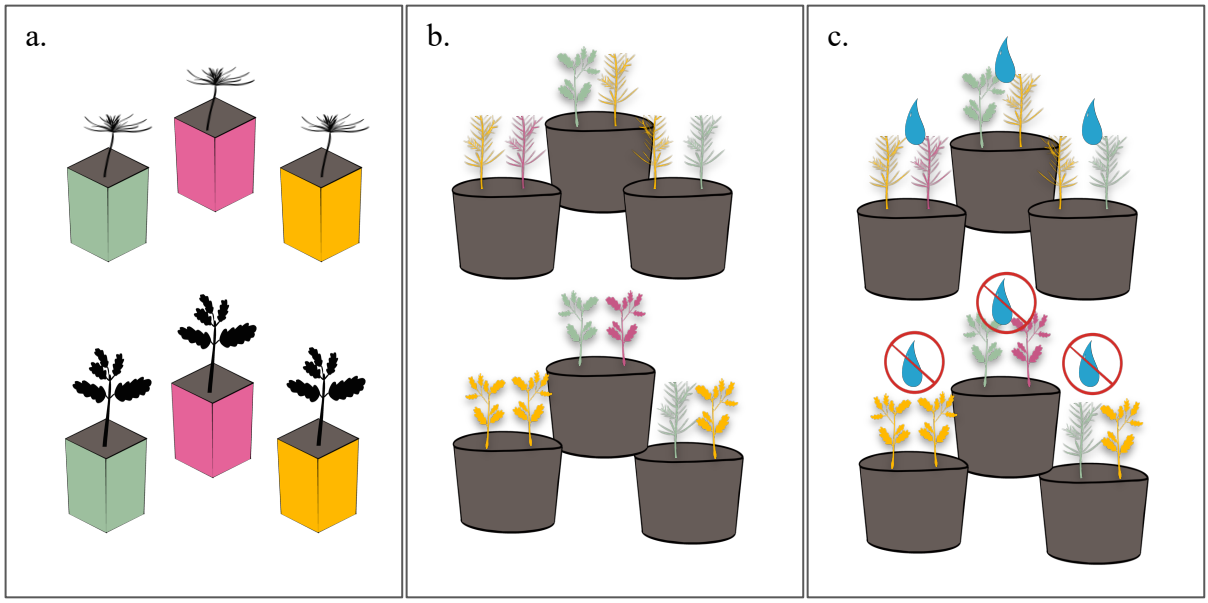


Figure 1: Experimental design. (a) Individual seeds were germinated in sterile soil before being potted into 0.4L pots with either sterile potting mix or a sterile 4:1 potting mix to field-collected live soil inoculum mix. Seedlings were grown in individual pots for approximately one year before being washed clean of all soil and (b) co-potted with a neighbor of the same of different species and soil conditioning (see Fig. SX for full design and replicates). Paired seedlings grew for another year before (c) half were subjected to a 10-month simulated persistent drought.

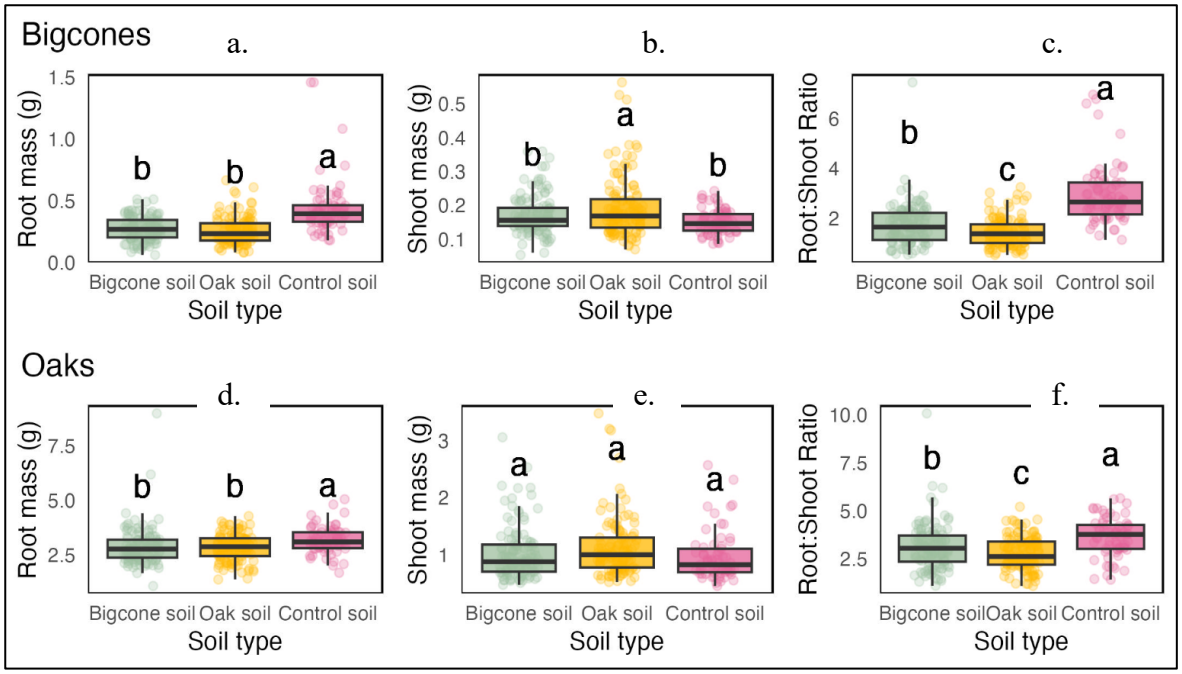


Figure 2: Bigcone biomass at repotting. Biomass values are fitted estimates from a linear model fit on a subset of destructively sampled seedlings and plotted on a $\log_{10}$ scale (see *Methods*). (a,d) Control soil seedlings had higher root biomass than live soil seedlings. (b) Bigcone oak soil seedlings had greater aboveground biomass than bicone soil and control soil seedlings while (e) oaks showed no effect. (c,f) Control seedlings had the highest root to shoot ratio and bigcone soil seedlings had higher root to shoot ratios than oak soil seedlings. All reported differences are based on significance of $p < 0.05$ using a Tukey HSD test following a two-way ANOVA.

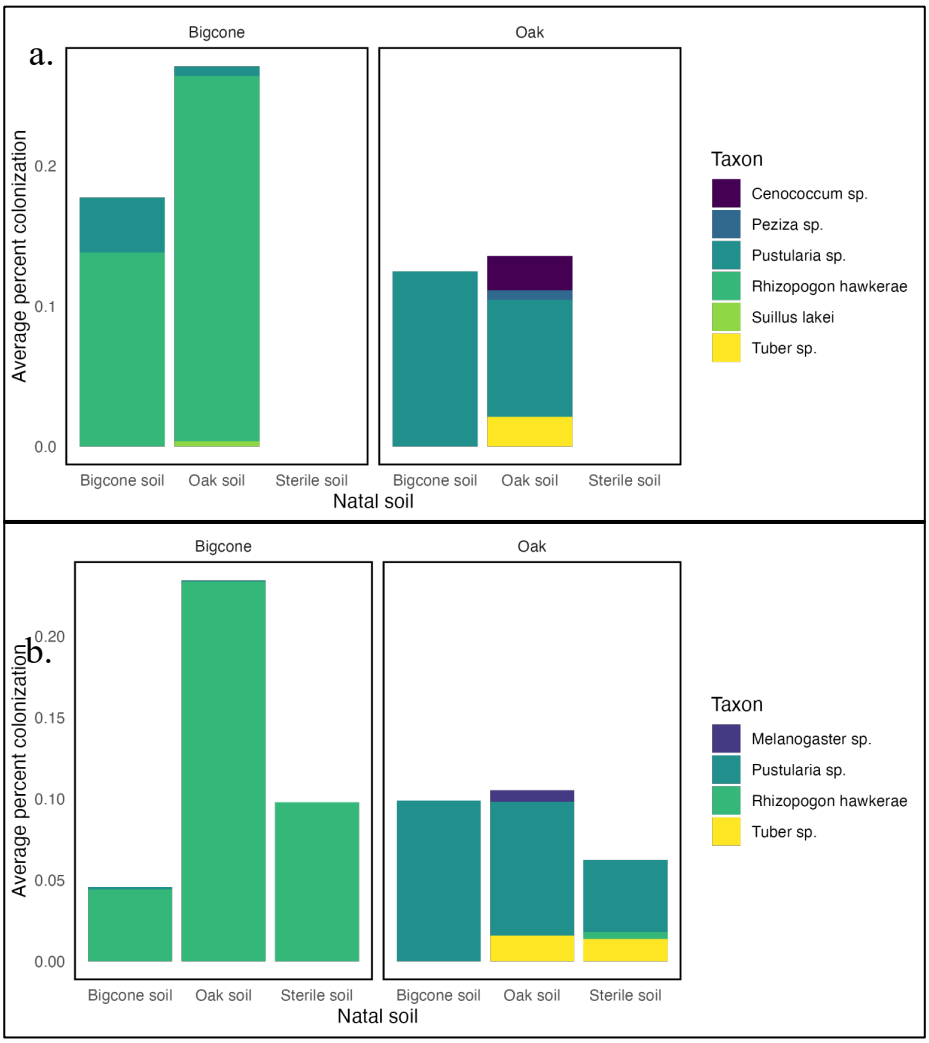


Figure 3: Seedling mycorrhizal communities and colonization rates. (a) At the repotting time point, sterile seedlings across species were not found to have any mycorrhizal partners. Bigcones has significantly higher colonization in oak soil than in bigcone soil. Oaks showed no significant difference in colonization rate but did have greater alpha diversity in oak soil. (b) At final harvest, seedling colonization rates were approximately the same as at repotting with a decrease in bigcones in home soil. Sterile seedlings of each species were found to have taken up mycorrhizal partners from their neighbors. A decrease in diversity was observable across species.

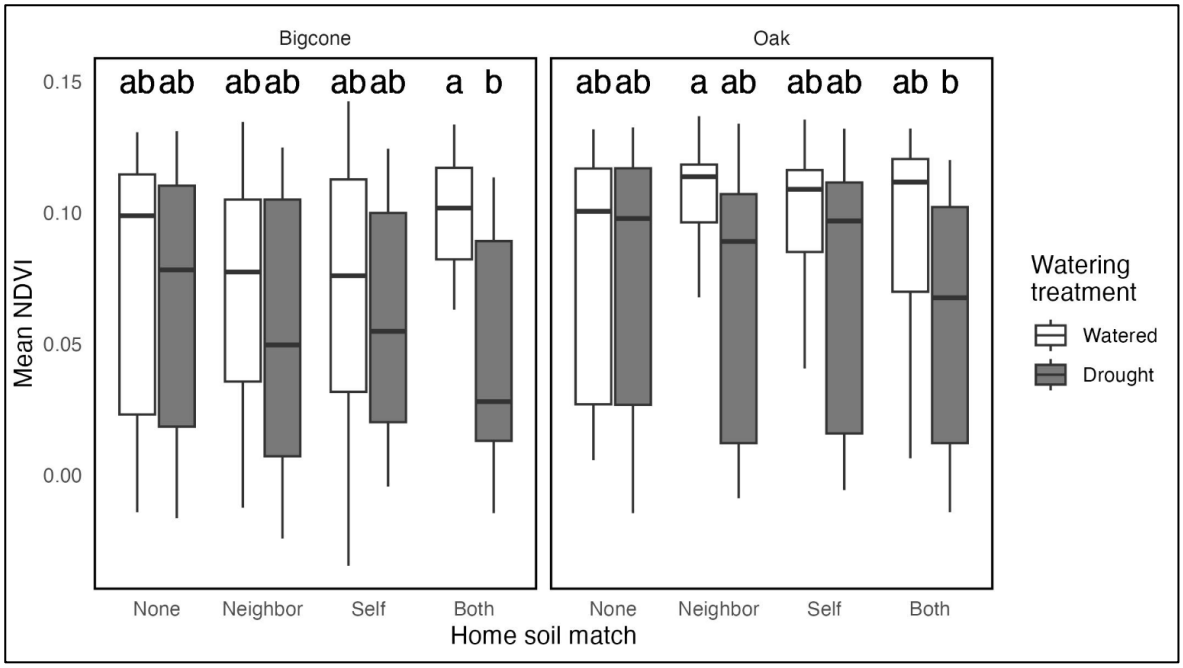


Figure 4: Increasing match between a focal seedling and it's home soil led to declines in health under drought conditions. Considering only seedlings conditioned in live soil, home match increases from left to right. 'None' considers a case in which neither seedling in a pot was conditioned in a focal seedling's home soil. 'Neighbor' considers the case where only a neighbor seedling was conditioned in a focal seedling's home soil. 'Self' considers just the focal seedling matching it's home soil. Finally, 'Both' considers the case in which both seedlings were conditioned in the focal seedling's home soil. Letters indicate Tukey's Honest Significant Differences within panels.

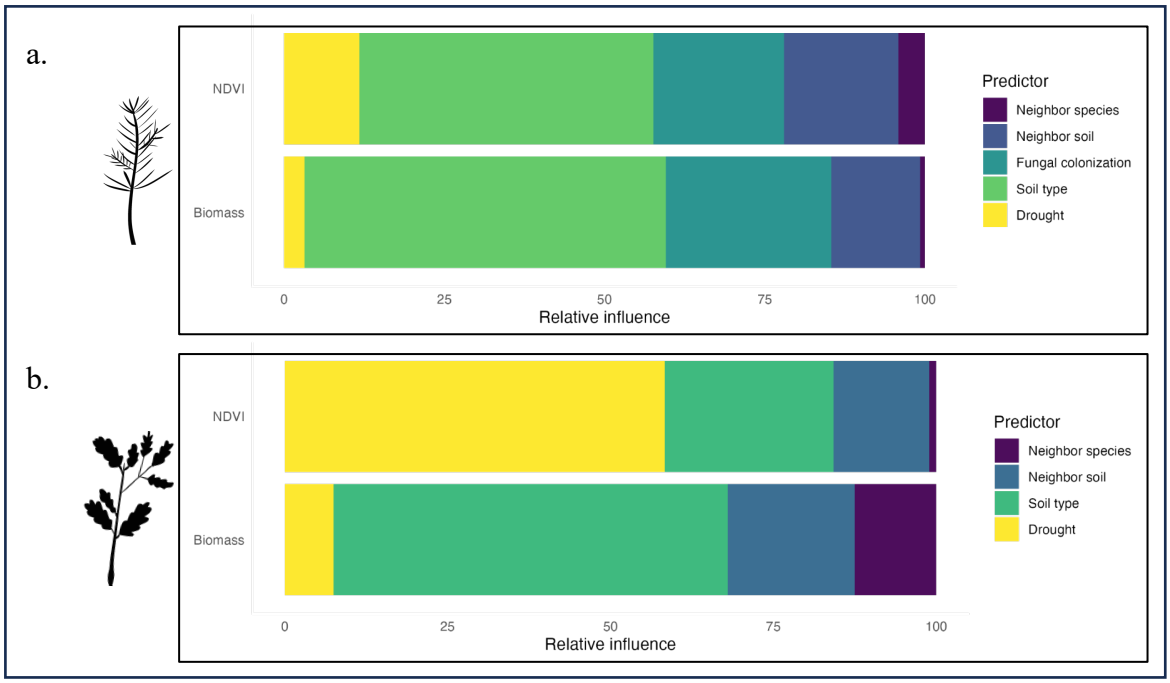
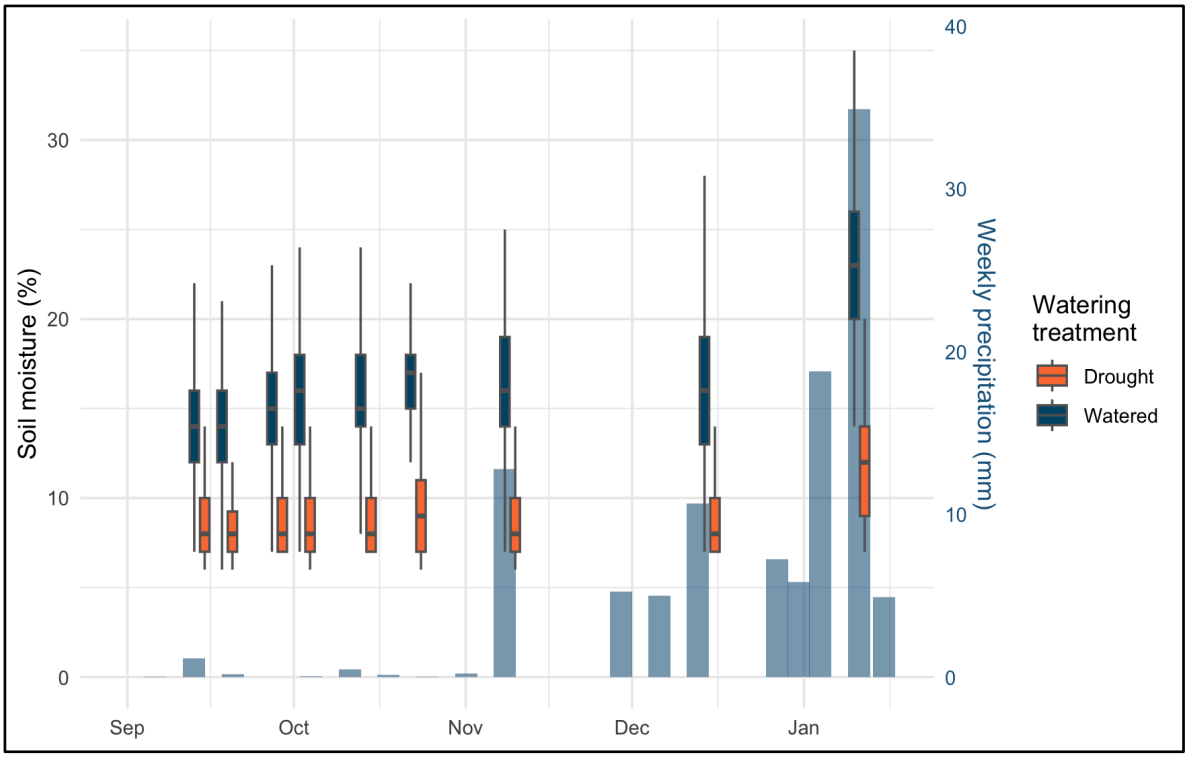
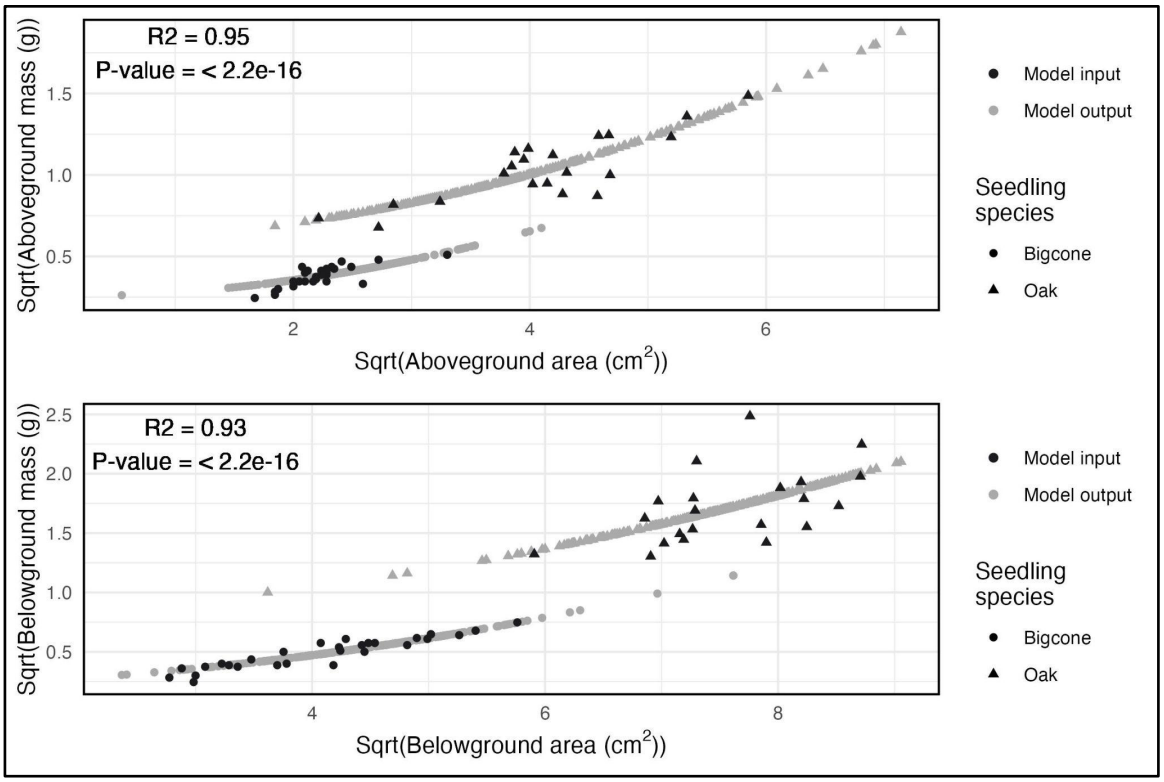


Figure 5: Results of gradient boosted regression models. (a) Bigcone seedling biomass and NDVI were predicted similarly by soil type most strongly, followed by colonization at repotting, then neighbor and drought effects. (b) Oaks, on the other hand, varied in the predictors of their biomass and NDVI. NDVI is well-predicted by drought while drought had little effect on biomass outcomes. Instead, biomass is best predicted by soil type then neighbor effects.

3.8. Appendix



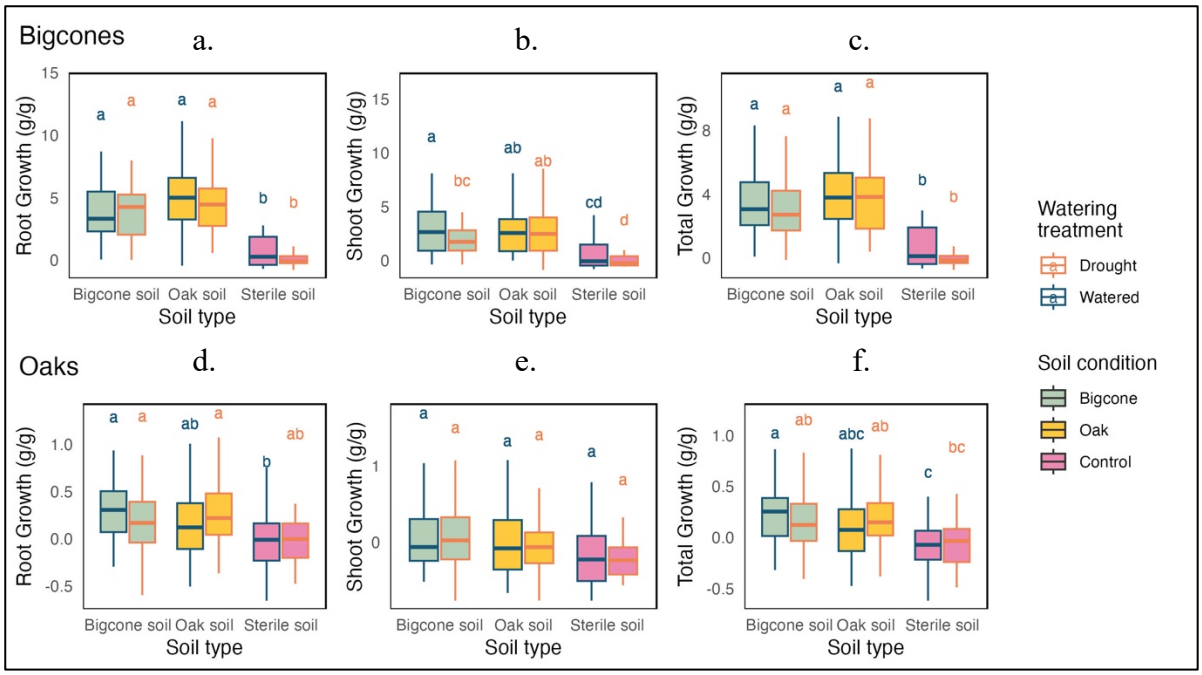
Supplemental Figure S1: Soil moisture data showing consistent soil moisture with a slight uptick in soil moisture corresponding to local precipitation and the expected increase in local humidity.



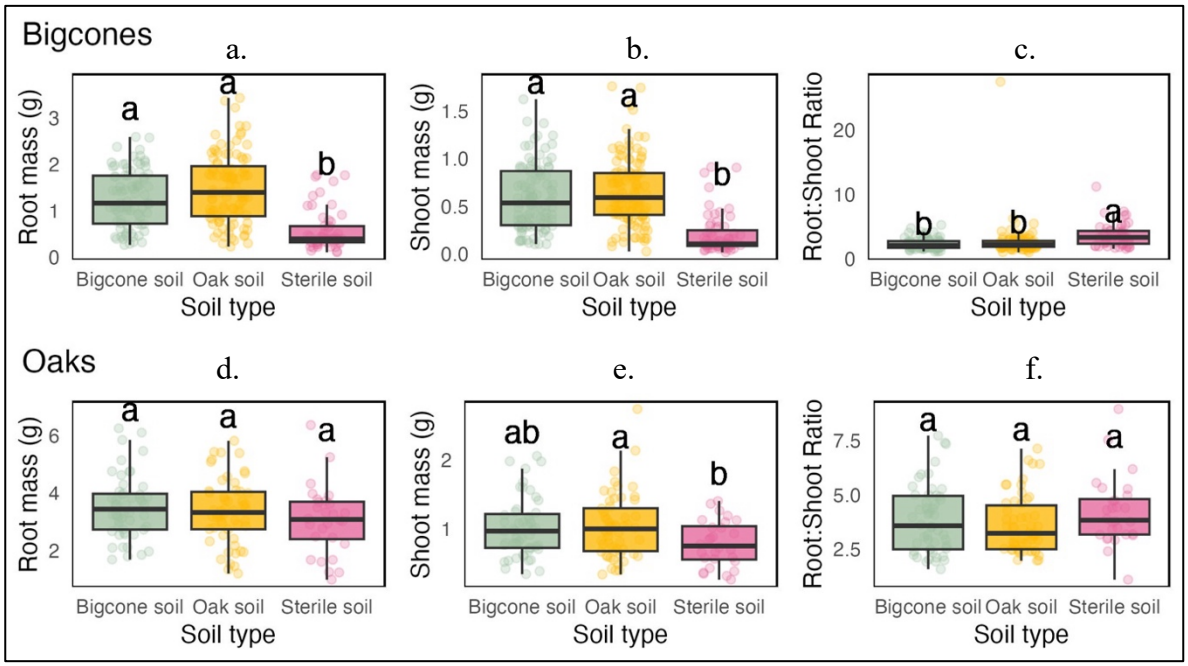
Supplemental Figure S2: Predicted biomass of seedlings at the repotting time step. Included are raw image area data and predicted biomasses of bigcone and oak seedlings in the above and belowground compartments.

Supplemental Table S3: Survival table with replicates by treatment.

Seedling sp.	Soil type	Neighbor sp.	Neighbor soil	n Watered	% Surv. Watered	n Droughted	% Surv. Droughted	Total % survival
Bigcone	Bigcone soil	Bigcone	Bigcone soil	12	0.917	12	0.583	0.75
Bigcone	Bigcone soil	Bigcone	Oak soil	14	0.857	13	0.769	0.815
Bigcone	Bigcone soil	Bigcone	Sterile soil	14	0.643	11	0.636	0.64
Bigcone	Bigcone soil	Oak	Bigcone soil	12	0.917	13	0.692	0.8
Bigcone	Bigcone soil	Oak	Oak soil	14	0.929	13	0.692	0.815
Bigcone	Bigcone soil	Oak	Sterile soil	--	--	--	--	--
Bigcone	Oak soil	Bigcone	Bigcone soil	14	0.786	13	0.846	0.815
Bigcone	Oak soil	Bigcone	Oak soil	12	0.917	14	0.857	0.885
Bigcone	Oak soil	Bigcone	Sterile soil	11	0.818	12	0.417	0.609
Bigcone	Oak soil	Oak	Bigcone soil	15	1	13	0.769	0.893
Bigcone	Oak soil	Oak	Oak soil	13	0.846	13	0.846	0.846
Bigcone	Oak soil	Oak	Sterile soil	--	--	--	--	--
Bigcone	Sterile soil	Bigcone	Bigcone soil	14	0.786	11	0.818	0.8
Bigcone	Sterile soil	Bigcone	Oak soil	11	0.818	12	0.667	0.739
Bigcone	Sterile soil	Bigcone	Sterile soil	12	0	14	0	0
Bigcone	Sterile soil	Oak	Bigcone soil	--	--	--	--	--
Bigcone	Sterile soil	Oak	Oak soil	--	--	--	--	--
Bigcone	Sterile soil	Oak	Sterile soil	--	--	--	--	--
Oak	Bigcone soil	Bigcone	Bigcone soil	12	0.833	13	0.462	0.64
Oak	Bigcone soil	Bigcone	Oak soil	15	0.733	13	0.615	0.679
Oak	Bigcone soil	Bigcone	Sterile soil	--	--	--	--	--
Oak	Bigcone soil	Oak	Bigcone soil	12	0.833	10	0.8	0.818
Oak	Bigcone soil	Oak	Oak soil	14	1	14	0.714	0.857
Oak	Bigcone soil	Oak	Sterile soil	11	0.818	10	0.7	0.762
Oak	Oak soil	Bigcone	Bigcone soil	14	0.929	13	0.615	0.778
Oak	Oak soil	Bigcone	Oak soil	13	0.615	13	0.692	0.654
Oak	Oak soil	Bigcone	Sterile soil	--	--	--	--	--
Oak	Oak soil	Oak	Bigcone soil	14	1	14	0.714	0.857
Oak	Oak soil	Oak	Oak soil	14	0.929	12	0.5	0.731
Oak	Oak soil	Oak	Sterile soil	12	0.833	12	0.833	0.833
Oak	Sterile soil	Bigcone	Bigcone soil	--	--	--	--	--
Oak	Sterile soil	Bigcone	Oak soil	--	--	--	--	--
Oak	Sterile soil	Bigcone	Sterile soil	--	--	--	--	--
Oak	Sterile soil	Oak	Bigcone soil	11	1	10	0.8	0.905
Oak	Sterile soil	Oak	Oak soil	12	1	12	0.833	0.917
Oak	Sterile soil	Oak	Sterile soil	14	0.929	12	0.917	0.923



Supplemental Figure S4: Seedling growth repotting to harvest. Differences in growth for bigcone seedlings (a-c) are most strongly predicted by natal soil with no significant effect of drought in the shared pots. Total growth of bigcone seedlings was comparable across live natal soil treatments and significantly greater than in sterile natal soil seedlings. Oak seedlings show no weaker signals of growth differences with roots (d) showing greater differences that aboveground biomass (e) summing to significantly different total growth (f) between bigcone natal soil and sterile natal soil seedlings in the watered treatment.



Supplemental Figure S5: Seedling biomass at harvest. Soil-driven differences in seedling size observed at the repotting time point are largely consistent for bigcone seedlings (a-c) with live soil seedlings growing larger in total with lower root:shoot ratios. Oak seedlings showed minimal differences by the end of the study. Only shoot biomass was significantly different between treatments with oak natal soil seedlings being larger aboveground than sterile natal soil seedlings (e).

Supplemental Table S6: Reported are the best fit models predicting bigcone seedling growth of roots, shoots, and total biomass between the repotting and harvest timepoints. All models were selected from the most complex model incorporating natal soil, neighbor species, neighbor soil, drought, and the relative sizes of the seedlings in the pots as well as their interactions. Best fit models were chosen by AIC-selection. Only predictors with p-value < 0.05 are shown in the table.

Predictor	**Estimate**	**Std. Error**	**t-value**	**p-value**
Root (Adj. R^2: 0.358, F-statistic: 13.38 on 10 and 212 DF, p-value: < 2.2e-16)				
(Intercept)	1.492	0.683	2.185	0.0300
Bigcone soil	2.979	0.688	4.328	0.0000
Oak soil	4.731	0.675	7.012	0.0000
Neighbor oak	-2.152	0.735	-2.927	0.0038
Shoot (Adj. R^2: 0.1833, F-statistic: 6.406 on 11 and 254 DF, p-value: 2.281e-09)				
Bigcone soil	2.351	0.429	5.487	0.0000
Oak soil	2.244	0.426	5.260	0.0000
Neighbor bigcone	1.164	0.413	2.819	0.0052
Neighbor soil oak	0.921	0.411	2.240	0.0259
Total (Adj. R^2: 0.3481, F-statistic: 10.12 on 13 and 209 DF, p-value: < 2.2e-16)				
Bigcone soil	2.418	0.575	4.205	0.0000
Oak soil	3.496	0.569	6.148	0.0000
Neighbor oak	-1.394	0.654	-2.132	0.0341

Supplemental Table S7: Reported are the best fit models predicting oak seedling growth of roots, shoots, and total biomass between the repotting and harvest timepoints. All models were selected from the most complex model incorporating natal soil, neighbor species, neighbor soil, drought, and the relative sizes of the seedlings in the pots as well as their interactions. Best fit models were chosen by AIC-selection. Only predictors with p-value < 0.05 are shown in the table.

Predictor	Estimate	Std. Error	t-value	p-value
Root (Adj. R^2: 0.1728, F-statistic: 2.59 on 31 and 205 DF, p-value: 3.721e-05)				
Neighbor soil oak	0.670	0.296	2.267	0.0244
Drought treatment	0.572	0.263	2.176	0.0307
Oak soil:Neighbor soil bigcone	-0.717	0.232	-3.083	0.0023
Oak soil:Drought treatment	-0.692	0.238	-2.906	0.0041
Neighbor soil oak:Drought trt.	-0.666	0.238	-2.798	0.0056
Oak soil:Neighbor soil bigcone:Drought trt.	0.960	0.320	3.004	0.0030
Oak soil:Neighbor soil oak:Drought trt.	0.885	0.317	2.794	0.0057
Shoot (Adj. R^2: 0.1783, F-statistic: 2.543 on 35 and 214 DF, p-value: 2.284e-05)				
Neighbor oak	-0.999	0.345	-2.895	0.0042
Neighbor oak:Relative size	0.788	0.242	3.252	0.0013
Neighbor oak:Drought trt.	1.062	0.483	2.201	0.0288
Total (Adj. R^2: 0.1675, F-statistic: 2.979 on 24 and 212 DF, p-value: 1.348e-05)				
Relative size	-0.019	0.010	-2.001	0.0467
Oak soil:Neighbor soil bigcone	-0.563	0.204	-2.766	0.0062
Oak soil:Neighbor soil oak	-0.447	0.203	-2.205	0.0286
Neighbor oak:Relative size	0.193	0.074	2.611	0.0097
Oak soil:Drought treatment	-0.594	0.215	-2.765	0.0062
Neighbor soil oak: Drought trt.	-0.603	0.215	-2.806	0.0055
Oak soil:Neighbor soil	0.785	0.281	2.791	0.0057
Bigcone soil:Neighbor soil	0.587	0.281	2.087	0.0381

4. Chapter 3

Is the friend of my friend my enemy? The maintenance of multiple hosts and symbionts in a mutualism network.

4.1. Abstract

Understanding the role of mutualisms in ecological community dynamics is complicated by the existence of multispecies networks in which indirect interactions can dictate the outcomes of symbiosis. For example, in the symbiosis between ectomycorrhizal fungi and woody plants, trees can host multiple species of fungi which may provide variable benefits in different conditions. The fungi, in turn, may associate with multiple trees or tree species. Currently, variable expectations of the degree and type of partner control mechanisms in the symbiosis present a challenge to the development of theory-backed hypotheses of the role of mutualism networks in community outcomes.

In this study, we develop and analyze a mathematical model consisting of a system of ordinary differential equations describing the exchange of resources between trees and fungi. We first outline a set of system assumptions before building from a single tree-fungal pair to a network of two tree hosts and two fungal partners all exchanging resources and with a shared soil nitrogen pool. We integrate two partner control strategies – identity-based allocation and quality-based allocation – to assess the role of, and interactions between, both empirically-observed phenomena. This framework allows us to quantify the benefits (or costs) of shared partners and determine how partner control mechanisms influence the system outcomes, focusing particularly on community stability (evaluated as resistance to stress, resilience after stress, and total evenness through a stress event). We find first that total control over partner resource allocation leads to the degradation of network-mediated

effects and the isolation of host-partner pairs. We also find that weak partner control, wherein resources are shared more evenly amongst all partners, lead to the most stable mutualism networks. There was strong agreement amongst our three distinct metrics on the benefit of these *agnostic* partner allocation strategies for whole-community stability. This modeling effort presents a series of useful expectations to inform empirical studies and shows how variable assumptions about mutualism network structure lead to meaningfully different outcomes for ectomycorrhizal communities.

4.2. Introduction

Symbiotic interactions are fundamental to the structure and function of some of the most productive and widespread ecotypes (Stachowicz 2001; Hay et al. 2004). While symbioses can vary in their strength and direction, here we focus on those generally considered to be evolutionarily mutualistic. Examples of such symbioses include coral-dwelling zooxanthellae, ant-acacias, legume rhizobia, and many others (Dimond and Carrington 2008; Stanton and Palmer 2011; Masson-Boivin and Sachs 2018). In many of these instances, understanding the dynamics of an interaction between host and symbiont can be complicated by the existence of multiple compatible partners and the way in which a community of hosts shapes the pool of potential symbiotic partners. By these phenomena, communities of hosts and partners can form what becomes a network of interaction wherein the dynamics of a system can be dependent on indirect interactions such as the support of suboptimal symbionts by competing hosts (Nuismer, Jordano, and Bascompte 2013; Hammarlund et al. 2021). Moreover, our limited understanding of the extent of host and

partner controls in many of these systems restricts our ability to make predictions about the cascading effects of stressors on a system.

Among the most influential global symbioses is that between plant roots and mycorrhizal fungi which forms the foundation of the bulk of terrestrial primary productivity (Mohan et al. 2014; Rillig 2004). Mycorrhizal fungi are important symbionts to over 90% of land plants, providing them with scarce resources such as mineral nutrients and water, as well as pathogen defense, in exchange for plant carbon resources including sugars and lipids (Smith and Read 2008). The fungal members of this symbiosis are categorized into functional guilds, of which ectomycorrhizal fungi are particularly prevalent in temperate and boreal ecosystems, being found on the roots of approximately 80% of tree roots outside the tropics (B. S. Steidinger et al. 2019). Ectomycorrhizal fungi have evolved more than 70 independent times from saprotrophic clades and therefore have a broad phylogenetic distribution. Similarly, they have a diverse set of host compatibilities from single host species or families to more cosmopolitan host ranges. This leads to complex community dynamics in forests with multiple host tree and fungal species.

At the core of the symbiosis is an exchange of goods: plant carbon resources for fungal-derived nutrients, commonly nitrogen. While mycorrhizal symbioses are considerably more complicated than this simple trade, reduction of the interaction to a basic quid pro quo relationship retains the critical nature of exchange needed for a broadly-applicable model. Ectomycorrhizal fungi are primarily recognized for their conferred nitrogen nutritional benefits which they can either scavenge from inorganic sources or liberate from organic molecules. These fungi are obligately reliant on host plants for their

carbon resources and plants have been estimated to receive more than 75% of their nitrogen from ECM uptake (Hobbie and H¨ogberg 2012).

In ectomycorrhizal systems, an individual host plant may associate with multiple mycorrhizal fungi at the same time. Similarly, ectomycorrhizal fungi may associate with multiple hosts at the same time. These systems, wherein a fungus connects two or more plants are referred to as Common Mycorrhizal Networks (CMNs) and have garnered growing attention in recent years. In particular, questions about the degree of resource redistribution across CMNs as well as the degree of partner control on each side of the symbiosis spark spirited debate (Karst, Jones, and Hoeksema 2023; Henriksson et al. 2023; Rillig et al. 2024). Nevertheless, it is broadly agreed upon that co-occurring hosts can share mycorrhizal fungal partners and thereby influence the symbiont communities of their neighbors. Because of the crucial role of the symbiosis in both plant and mycorrhizal fungal success, this connectedness has important implications for the dynamics of plant communities, the competition therein, and community stability as a whole.

For example, during stress events (e.g. drought, pathogen outbreaks), these interaction networks have the potential to buffer or exacerbate individual and community impacts, though the type and degree of controls within the symbiotic network would dictate such outcomes. Due to the fact that observation and experimental control of partner interactions is largely intractable, mathematical models have been a useful tool in the exploration of mycorrhizal resource exchange and community dynamics. Many models in the field attempt to explain the maintenance of symbiotic function by focusing on the control on resources exchanged (Bever 2015; Bachelot and Lee 2018; Brian S. Steidinger and Bever 2014). Here, biological market models have been a common tool but disagreement remains

about the ideal representation of fluxes (L. M. Bogar 2024). Other models attempt to explain the persistence of low-quality – providing less benefit per cost – partners by accounting for alternate environmental conditions under which they would be beneficial (Moeller and Neubert 2016; Stevens et al. 2024). One possible mechanism that remains contentious is the potential benefit stressed individuals may receive by sharing a symbiotic network with unstressed competitors. These impacts of ectomycorrhizal networks on community-level stress responses have yet to be explored in a framework that accommodates the range of theorized allocation mechanisms in the symbiosis. Here, we develop a mathematical model to explicitly represent variable ectomycorrhizal network structures in order to investigate how different assumptions about partner control and connectedness shape community stress outcomes.

4.3. The Model

We model the ectomycorrhizal symbiosis by tracking the exchange of two core resources: carbon and nitrogen. Carbon is the resource in the model that makes up the biomass of the host tree, B_i, and symbiotic fungal biomass, F_j. This resource comes into the system through photosynthesis in the host tree and becomes fungal biomass through the conversion of carbon allocated by the tree(s) to their symbionts. The biomass of these trees and fungi scale their effect on the network by dictating the amount of resources they can contribute to the exchange. Additionally, these biomass pools are the metric by which we calculate system diagnostics, such as stress response, and evaluate community stability. Nitrogen, on the other hand, is modeled as an environmental resource, N_E, which fungi uptake on the behalf of host trees, becoming host Nitrogen, N_i. This nitrogen then dictates the photosynthetic capacity of the tree and the tree's potential as a symbiotic partner.

Trees are crucially reliant on the resources ectomycorrhizal take up for them (Marschner and Dell 1994; Rousseau, Sylvia, and Fox 1994; Harley 1997). In particular, in environments where decomposition is energetically or chemically limited, ectomycorrhizal fungi are consistently found as the dominant tree symbiont strategy (B. S. Steidinger et al. 2019). In our model, where the total nitrogen is finite, fungi take up whatever environmental nitrogen remains in the system on behalf of the trees. We assume that each host tree receives a proportion of the nitrogen taken up by each fungus in the system, receiving these benefits by the fungus- and host-specific uptake function, μ_{ji}. Tree nitrogen is lost at a constant rate, ℓN, to account for maintenance and mortality of nitrogen-holding biomass. In total, tree nitrogen dynamics are represented as:

$$\dot{N}_i = N_E \sum_j \mu_{ji} F_j - l_N N_i \quad (1)$$

In our model, nitrogen is the growth-limiting resource for trees, whose photosynthetic capacity depends on their nitrogen content, N_i (where i = 1 or 2 , representing the two trees modeled in our system). Nitrogen is a key element in chlorophyll, directly influencing a tree's capacity to grow more biomass (Wu et al. 2006). We model photosynthesis as scaling linearly with nitrogen at a growth rate g. Host biomass is lost at a constant rate representing respiratory and maintenance costs. Hosts may also allocate biomass to their belowground symbionts at a rate a. Thus, the dynamics of a tree's carbon biomass are given by:

$$\dot{B}_i = gN_i - (a + l_B)B_i \quad (2)$$

Ectomycorrhizal fungi use allocated host carbon to meet their metabolic demands and produce fungal biomass, F_j (where $j \in 1,2$). Although ectomycorrhizal fungi can gain some carbon benefit through decomposition, most carbon is thought to be obtained from host trees (Smith and Read 2008). We assume that each fungus receives an allotment of carbon from each host tree based on a tree- and fungus-specific allotment function, α_{ij}, and that fungi lose biomass to respiration and maintenance at a constant rate ℓ_F:

$$\dot{F}_j = \sum_i \alpha_{ij} a B_i - l_F F_j \quad (3)$$

Nitrogen in temperate forest systems is finite in the absence of nitrogen-fixing organisms which we do not consider in this work. In our model, we depict a fixed patch of forest in which the total sum of nitrogen does not change but can be moved between environmental (N_E) and host (N_i) pools (i.e. $N_E + N_i = N_{Total}$). This representation is based on the ecological reality that nitrogen resources tend to be zero-sum for competing ectomycorrhizal communities, imposing a limit to the total productivity of a system. We model this cycling of nitrogen by accounting for the available environmental nitrogen dynamics as the inverse of all host nitrogen dynamics, simply written as

$$\dot{N}_E = -\sum_i \dot{N}_i \quad (4)$$

where we use a negative summation to account for the addition or loss of nitrogen from the environment into host pools. Here and throughout, host individuals are indexed with an i while fungal individuals will be indexed with a j. Matching subscripts (e.g. B_1 and F_1)

represent primary partnerships whereas unmatched subscripts (e.g. B_1 and F_2) represent alternate partnerships.

Partner-specific sharing

To explore how preferential resource allocation be trees and/or fungi altered community responses, we used a series of allotment functions that allowed for varying degrees of partner specificity. Partner controls can take a number or mechanistic forms. (1) Researchers have found evidence of identity-based partner discrimination in the ectomycorrhizal symbiosis through differences in resource sharing (L. Bogar et al. 2019) or colonization rates (Rasmussen 2018) irrespective of partner quality. (2) Studies have also found ample evidence for discrimination of both plants and fungi based on partner quality, usually defined by resource exchange ratios (Werner and Kiers 2015; L. M. Bogar and Peay 2017). Critically, these two mechanisms are not necessarily mutually exclusive. Because each side of the symbiosis is under selective pressure to maximize its own fitness, the ability to sanction a once-high-quality partner remains vital even within evolved partnerships.
In this study, we integrate both of these two mechanisms along with the ability to weight the relative investment from completely identity-based allocation to totally quality-based allocation. In the equations above, we use α_{ij} and μ_{ji} to encompass these sharing patterns for tree-to-fungus and fungus-to-tree rewards, respectively. Within these functions, we define identity-based rewards (1) using the parameters p_{ij} (host tree i preference for fungus j) and q_{ji} (fungus j preference for host i). These values do not change over the course of a model run. Quality -based rewards (2) are represented by ϕ_{ij} and ψ_{ji}, the values of which do change based on dynamic partner quality (described below). The value of the *r* term dictates the proportion of rewards that are allocated based on quality. For example, when $r = 1$, all

resources are allocated based on quality. When $r = 0$, all resources are allocated based on identity. Thus, (1-r) represents the proportion of identity-based rewards. Because α represents the transformation of host carbon into fungal carbon, e_{ji} is the partner pair-specific conversion efficiency. In the case of μ, u_{ji} is the specific uptake rate of fungus j for host i. Taken together, the α and μ equations take the form:

$$\alpha_{ij} = e_{ji}\left[r\phi_{ij} + (1-r)p_{ij}\right] \quad (5)$$

$$\mu_{ji} = u_{ji}\left[r\psi_{ji} + (1-r)q_{ji}\right] \quad (6)$$

The functions ϕ and ψ allow symbiotic partners to preferentially award Carbon and Nitrogen, respectively, according to perceived partner quality. We integrate this framework by assuming that a symbiont with multiple partners will allocate resources to those partners in proportion to the resources they receive from each of them. For example, if a tree receives two units of nitrogen from fungus A and one unit of nitrogen from fungus B, fungus A will receive two thirds of this tree's allocated resources while fungus B receives one third (Figure 2). Each of these dynamic parameters are partner pair-specific and are calculated as the resources received from a partner divided by the total resources received. In the case of ϕ_{ij}, this evaluation integrates the pair-specific uptake rate while in ψ_{ji}, the partner evaluation does not include conversion the pair-specific conversion efficiency. This decision is based on the modeled pathways wherein uptake is considered to take place prior to resource delivery and conversion (efficiency) is considered to take place after resource delivery. The values of ϕ_{ij} and ψ_{ji} are recalculated each time step:

$$\phi_{ij} = \frac{u_{ji}F_j\left[r\psi_{ji}+(1-r)q_{ji}\right]}{\sum_j u_{ji}F_j\left[r\psi_{ji}+(1-r)q_{ji}\right]} \quad (7)$$

$$\psi_{ji} = \frac{B_i[r\phi_{ij}+(1-r)p_{ij}]}{\sum_i B_i[r\phi_{ij}+(1-r)p_{ij}]} \quad (8)$$

Here, it is important to build our intuition for what it would mean to have different combinations of these parameter values (Fig. 3. To do so, we define and name a series of archetypes that we will return to when working through our analyses. Beginning with the partner preference values (p&q), the further these preferences get from 0.5, the greater percentage of an individual's identity-based allocation goes to one partner (at 0, all identity-based allocation goes to the alternate partner and at 1, all identity-based allocation goes to the primary partner). At p&q = 0.5, identity-based allocation is split evenly between the two partners irrespective of quality or relative size. These outcomes are most clear when the percent investment based on quality is 0 (r = 0). In this case, when identity allocation is split evenly and no resources are directed based on quality, the network acts as an ultimate safety net to a stressed individual, as they will continue receiving half of the resources from each of their partners. We can think of this investment strategy as Agnostic because no preferences are made for identity or quality. From here, if the network continues to ignore quality but increases in partner preference, we consider this strategy to be Loyal since no matter the situation, these symbiotic pairs will choose to share only with a single partner. This strategy canlimit the impacts of stress by maintaining symbiotic function but also frees the unstressed partner pair to take advantage of whatever advantage they gain during a stress period. Notably, the case where a network has maximized both partner preferential allocation and investment in quality results in a very similar outcome. Because we initiate the quality scores (ϕ & ψ) as congruent to the preferences, members of the network continue to reward only a single partner because they initially receive the most benefit from them. We name

this strategy the True to roots strategy. Finally, we consider the case with no partner preference (p&q = 0.5) and 100% quality-based rewards (r = 1). Here, again, the network results in pairs of symbionts pulled apart from one another with nearly 0 exchange with alternate partners. This is the result of the host-specific uptake rates being higher for primary hosts, leading to a feedback of increased investment within the primary symbiont pairs. Because the framework is built on pragmatism rather than preference, we refer to this as the Practical strategy. In addition to varying partner preference and quality parameters, we can also simply model a system with only identity-based partner rewards (r = 0), only quality-based rewards (r = 1), or both (0 < r < 1).

Total model

Taken together, our full tree-fungal model is given by:

$$\dot{B}_i = gN_i - (a + \mathrm{l}_B)B_i \tag{9}$$

$$\dot{F}_j = \sum_i e_{ji}\left[r\phi_{ij} + (1-r)p_{ij}\right]aB_i - \mathrm{l}_F F_j \tag{10}$$

$$\dot{N}_i = N_E \sum_j u_{ji}\left[r\psi_{ji} + (1-r)q_{ji}\right]F_j - \mathrm{l}_N N_i \tag{11}$$

$$\dot{N}_E = -\sum_i \dot{N}_i \tag{12}$$

4.4. Analysis

This model was developed to better understand the impact of network connectivity on stress outcomes. In our analyses, we work toward that goal by first developing an understanding of the behavior of the model in steady conditions. We build in model complexity piece-wise, ensuring intuitive outcomes along the way. We then conduct stress tests by integrating ecologically-relevant testing scenarios (see Stressing the system below)

and evaluating the response of the network using common, interpretable diagnostics. These tests help us to build a set of hypotheses for the pressures driving symbiotic controls in the ectomycorrhizal symbiosis.

The simplest network

We first explored the model's dynamics in a single-tree-single-fungus system. In this case, p = q = 1 and $\phi = \psi = 1$ and our equations simplify to:

$$\dot{B} = gN - (a + l_B)B \tag{13}$$

$$\dot{F} = eaB - l_F F \tag{14}$$

$$\dot{N} = uN_E F - l_N N \tag{15}$$

$$\dot{N_E} = -\dot{N} \tag{16}$$

When simulating this simplistic model the B, F, and N pools all equilibrate to positive real values $\{B = B*, F = F*, N = N*, NE = N_E = N_{Total} - N*\}$ anytime that at least one of the pools has an initial condition greater than 0. There is an additional unstable equilibrium at $\{B = 0, F = 0, N = 0, N_E = N_{Total}\}$. Rates of equilibration and relative equilibria are determined by the specific set of parameter values used, though overall dynamics remain consistent across the range of reasonable parameter values. If we include either an acute (ex. loss of biomass) or prolonged (ex. temporarily increased mortality), the system will rebound to the same equilibrium after the stress has subsided. Here, again, the rate of decline under stress and the rate of recovery are both determined by rates of growth and mortality.

A more complex network

Scaling up to multiple partners on one or both sides of the symbiosis allows us to investigate the impact of allocation patterns between nodes within the mutualism network. The two-tree-two-fungus model equilibrates across all ecologically-relevant parameter values (Table 1). The model has one stable equilibrium at $\{B_1 = B_1^*, B_2 = B_2^*, F_1 = F_1^*, F_2 = F_2^* N_1 = N_1^*, N_2 = {}_2^*, N_E = N_{Total} - N_1^* - N_2^*\}$ and an additional trivial unstable equilibrium at $\{B_1 = 0, B_2 = 0, F_1 = 0, F_2 = 0, N_1 = 0, N_2 = 0, N_E = N_{Total}\}$. Encompassed in the stable equilibrium are the case where all pools are positive and eight additional combinations where one or more tree or fungal pool is zero, based on the value of the allocation parameters (see Appendix). We assessed equilibrium pool sizes as a response metric, starting both hosts at the same size as well as both fungi at the same size as one another (Figure 4). In each of the following responses, all but the focal parameter was held at its default value (Table 1). Increasing the tree growth rate, g, leads to larger equilibrium biomass in the host and fungal pools even while host nitrogen asymptotes near full utilization (>99% of total nitrogen in host pools). Increasing allocation rate, a, leads to declining host biomass and increasing fungal biomass as more photosynthetic production goes to fungal biomass rather than host biomass, though each of them retain positive equilibrium values. Conversion efficiency on primary host allocated carbon, with the trade-off of lower uptake rates and higher alternate host efficiency, leads to a peak in fungal biomass at relatively high values (around e = 0.35) before declining toward the highest efficiency values where uptake rates limit fungal biomass. Both investment in quality, r, and partner preference, p and q had no effect on host biomass and nitrogen equilibria but led to lower fungal equilibrium values at very high r and q values where relatively lower conversion efficiency on primary hosts leads to decreased fungal growth. Importantly, these

parameter investigations affirm the stability of the default model and can give some insight into edge cases as we analyze model dynamics through perturbation.

Stressing the system

To better understand the influence of CMNs on system outcomes, we applied simulated environmental stress to members of the network across partner control values. The stress we applied was to temporarily increase the loss (l_x) rate which can represent a wide range of ecological drivers including pathogen outbreaks, disturbance, increased competition costs, and others. This elevated loss rate lasted for only 3% of the simulation before the stressed individual was allowed to recover, if the partner control values allow. The stress periods were initiated four times throughout the simulation, alternating between different stressed individuals (Fig. 5). This fluctuation in partner quality is important for capturing the quality-driven changes in partner allocation which, in some cases, can have continued conditioning effects beyond the duration of the stress.

We quantified the ecological impacts of these simulated stress events by calculating three common ecological diagnostics: resistance, resilience, and evenness (Van Meerbeek 2021). Resistance is commonly defined as the ability to resist change in response to a stressor which we calculate as the percent decrease in biomass through the stress event. We calculate resilience, or the rate of recovery from stress, by assessing how long it takes a stressed individual to return to within 2% of its original size. To calculate evenness, we average the squared difference in biomass between the individuals of a given class (trees or fungi) from the onset of stress to the end of the simulation. Together, these response metrics give a concrete idea of how CMNs and the rules governing exchange shape long-term community stability.

To begin to understand community stress responses at default parameter values, we first observed the impact of varying who was stressed and at what times. In the simplest implementation, we stressed one tree or fungus at a time before adding in overlapping stresses between hosts and fungi (Figure 7). Fungi were largely unaffected when solely host trees were stressed and similarly trees had very high resistance and resilience (but not evenness) when only fungi were stressed (Fig. 7). When we stressed both trees and fungi at the same time, the pairing of whether or not the trees and their primary fungal partner were stressed together or sequentially critically changed the systemic response. In-fact, when stressing hosts and their primary partners at the same time, tree resistance to stress was lower and tree resilience was much lower (longer recovery period) than when the tree was stressed alone. Notably, these patterns were reversed when trees were stressed alongside alternate fungi in the system. In this case, the tree had greater resistance and resilience than when stressed alone due to their primary fungal partner doing relatively better while the alternate fungus was stressed.

These patterns can be understood as finite space being taken up in the absence of a vigorous competitor. When both host and partner are stressed together, the alternate pair can maximally take advantage of the more available nitrogen resources. This leads to exacerbated declines and slower recoveries as the stressed individuals work to gain back the 'ground' they've ceded. Alternatively, when host 2's primary partner is stressed at the same time as host 1 is stressed, host 1's primary partner can help them to limit their decline and rapidly recover. Here, it helps that host 1's primary partner is not at a disadvantage to fungus 2 (who is getting a healthy host's carbon) due to the stress on that alternate partner. Together, these patterns show how different conditions can change the cost or benefit of

being tied into a mutualism network and point to the structure of CMNs as being an important driver of forest stress response.

Beyond the default structure and partner interactions, we were interested also in how variable representations of the symbiotic controls on resource exchange impact the patterns observed above. To pursue this, we investigated the stress-induced repercussions of variable partner preference (p&q) and different percent allocation based on quality (r). When we simulated stress, here on a single member of the network, across a range of possible partner preferences (p&q = 0 : 1) and quality investment rates (r = 0 : 1), systems with low preference and low quality investment rates had greater stability. Critically, maximizing either of these parameters allows a stressed system to stabilize into a new equilibrium where recovery to pre-stress biomass will not occur (Figure 8). Additionally, when holding the other parameter constant, decreasing partner preference (closer to 0.5) or investment in quality toward the minimum value allows for increasingly rapid recovery – higher resilience (Figure 8). Across these two parameter ranges, the more Agnostic the resource distribution in the network, the better the system fared overall where stress recovery is concerned.

These patterns are compelling and led us to investigate more thoroughly across parameter space. We ran simulations across both partner control parameters and initiated stressed on host trees and fungi in isolation and together, just as in Figure 7. In each case, we evaluated our resistance, resilience, and evenness diagnostics to more holistically understand stability in the system (Figure 10). To create a more interpretable dataset, stability diagnostics were averaged across stress initializations (who was stressed in what sequence) for a single value at each combination of p and r. Resistance patterns showed that declines through stress are minimized at very low values of r, where partner identity is the only

determinant of allocation, and when either r or p is a maximum value. In this latter instance the trees and fungi are functionally paired up, not sharing any resources with alternate partners. We saw already that this benefit in resistance comes with a strong resilience trade-off (Figure 8). Resilience is maximized at the low partner control values where resources flow through the network most evenly and are least responsive to host identity or stress-induced declines. Conversely, resilience is minimized in cases where both partner control parameter values are high and recovery is slower. Evenness is similarly maximized at low partner control values. The lowest evenness values are in cases with very low partner preference ($p\&q \approx 0.5$) and low investment in quality. Evenness incorporates the severity of stress effect captured by resistance as well as the duration of effect captured by resilience.

4.5. Discussion

In this study, we consistently found that strong partner controls in the ectomycorrhizal symbiosis led to more unstable communities when exposed to stress events. This outcome was true irrespective of the mechanism by which partner controls were initiated with both strong partner preference and strong quality-based rewards leading to increased instability. Each of these partner control strategies decreased the connectedness of the network by increasing the proportion of resource exchange with a single, 'primary', partner. This finding runs counter to proposed explanations for observed partner sanctions in previous works. This divergence raises important questions about which selective pressures drive network structure in different contexts.

Also in this work, the nature of stress and its effects on different nodes in the network resulted in altered recovery patterns with a generalizable pattern. Although stresses were imposed on trees and fungi in various sequences, there remained a discernible stability

refuge at low partner control. This outcome is in-part shaped by the differences in severity of stress response when stressed are imposed on primary or alternate partners (Fig. 9). There is a particularly strong effect when a host tree and it's preferred fungal partner are stressed synchronously, an effect that outweighs the stress minimizing effect of asynchronous stress.

Networks-mediated stress responses collapse in the edge cases of total partner control. When partner preference is at 0 or 1, the network functionally ceases to exist. Exchange of resources only occurs within partner pairs and the system will find a new equilibrium through stress rather than fully recovering. Similarly, maximization of quality rewards (r = 1) allows individuals to eliminate all exchange with one potential partner, simplifying the network. This perfect control on resource distribution is not at all supported by the empirical work on the ectomycorrhizal system. Whether findings support identity or quality-based rewards, leakiness is a consistent aspect of the network architecture.

Despite the diverse partner controls we allow for in this study, we do not consider the inclusion of "cheaters" – partners that do not provide benefits in return for their costs – in the symbiosis. In a network controlled by the high stability conditions of this study – low partner preference and low-quality reward – cheaters would thrive. Such partners may shift the selective pressure away from an agnostic system toward some unknown level of partner quality control. This caveat represents an important consideration for researchers working to further this area of study.

Future work

The structure of this model framework has the potential to be expanded to many more network members and to include unique symbionts. So far, we have only used the two-tree-two-fungus system and have mirrored the pairs of symbionts as similarly invested in identity

and quality. Exploration of these parameters via a game theoretic may better address the selective pressure acting on individuals within the network and could provide insights into the evolution and exclusion of low-quality partners.

Models such as ours are useful tools for testing our expectations and highlighting the areas of uncertainty in the field that lead to meaningfully different system outcomes. This study provides new insights into the implications of different network structures for community stress responses by developing a novel partner control architecture. Such tools will be useful in anticipating the potential community outcomes in a period of increasing ecological volatility. In ectomycorrhizal ecology, as with many mutualistic systems, defining partner controls remains a gruelingly difficult task. Nevertheless, this study highlights just how important these controls can be in predicting community stress responses.

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4.7. Figures and figure captions

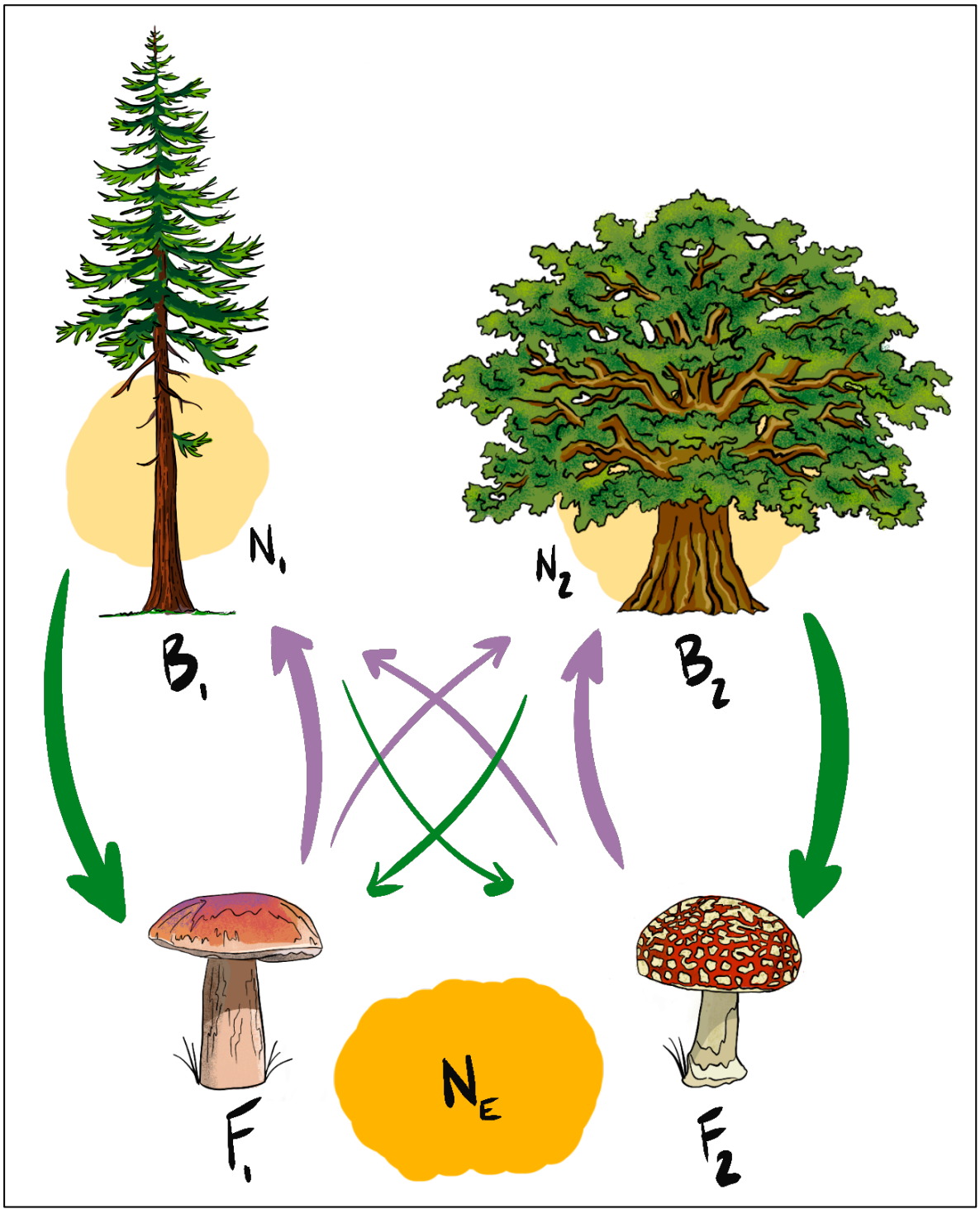
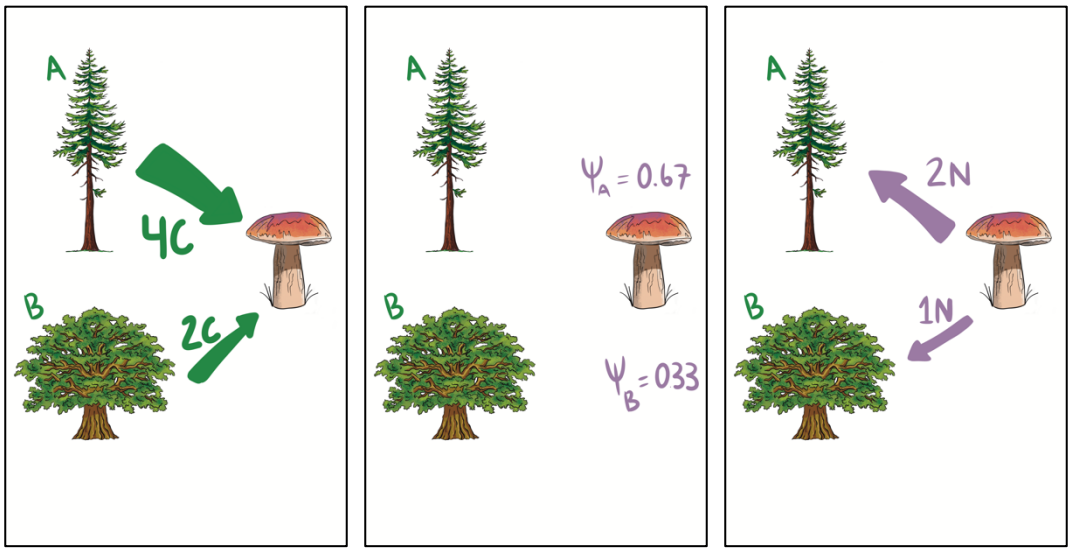
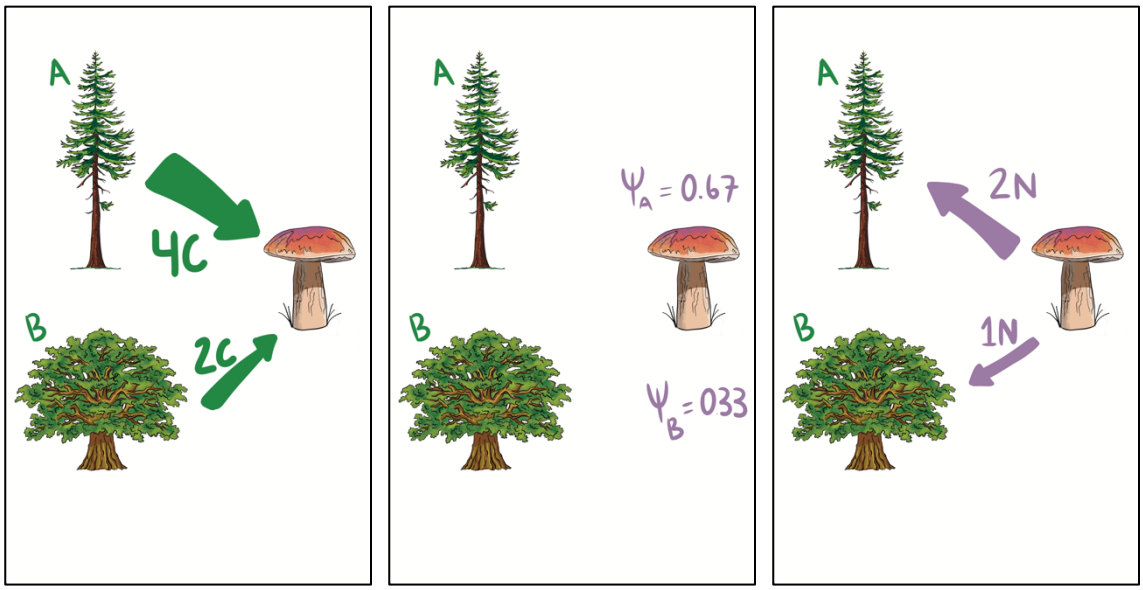


Figure 1: Model diagram. A depiction of the two tree, two fungus system with sharing between all partners in the network. Environmental nitrogen (N_E) is a common pool that is accessed by both fungi.


A
4C
B
2C
A
$\Psi_A = 0.67$
B
$\Psi_B = 033$
A
2N
B
1N



a. **b.** **c.**

Figure 2: Quality-based rewards. In (a), the trees pay out different amounts of carbon to the fungus. In (b), the fungus evaluates the trees' quality, which we calculate as ψ. In (c), the fungal payout is in proportion to the benefits received from the trees. This diagram does not consider identity-based rewards.

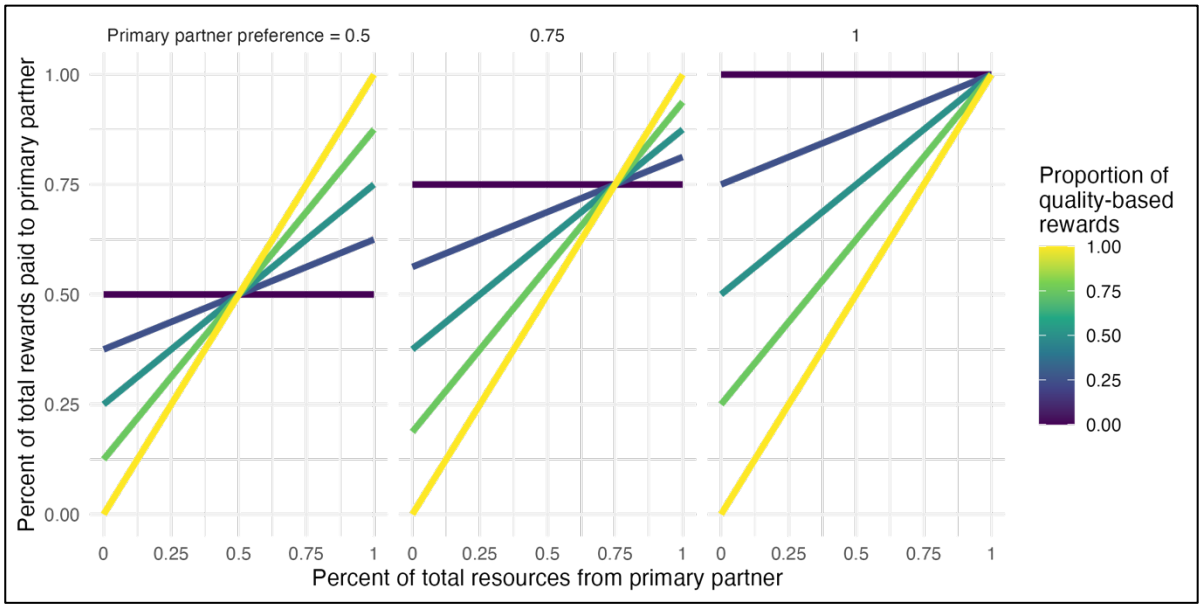


Figure 3: Partner payout based on benefits received. When quality-based rewards are above zero, resources paid out increase as a function of resources received. Benefits received when quality rewards approach zero are dictated most strongly by partner preference (p & q). When investment in quality rewards (*r*) are zero, resources received have no relationship with resources paid.

Symbol	Units	Description	Value range
State variables			
B_i	kg B Carbon	Host tree biomass	≥ 0
F_j	kg F Carbon	Fungal biomass	≥ 0
N_i	kg Nitrogen	Host tree nitrogen	0 ≤ x ≤ 100
N_E	kg Nitrogen	Environmental nitrogen	0 ≤ x ≤ 100
Allocation functions			
α_{ij}	unitless	Allocation percent from host$_i$ to fungus$_j$	0:1
μ_{ji}	unitless	Allocation percent from fungus$_j$ to host$_i$	0:1
Dynamic parameters			
ϕ_{ij}	unitless	Quality reward host$_i$ to fungus$_j$	0:1
ψ_{ji}	unitless	Quality reward fungus$_j$ to host$_i$	0:1
Static parameters			
g	$\frac{\text{kg Carbon}}{\text{kg Nitrogen}\cdot t}$	Host growth rate	0:1
a	time^{-1}	Host carbon allocation	0:1
u_{ji}	F_{-1} t $_{-1}$	Fungal N_e uptake	0:0.5
e_{ji}	$\frac{\text{kg } F \text{ Carbon}}{\text{kg } B \text{ Carbon}\cdot t}$	Fungal conversion efficiency	0:0.5
r	unitless	Relative investment in quality	0:1
p_{ij}	unitless	Host$_i$ preference for fungus$_j$	0:1
q_{ji}	unitless	Fungal$_j$ preference for host$_i$	0:1
l_x	time^{-1}	Loss of state variable 'x'	0:1
N_{Total}	kg Nitrogen	Total nitrogen	0

Table 1: Model state variables and parameters

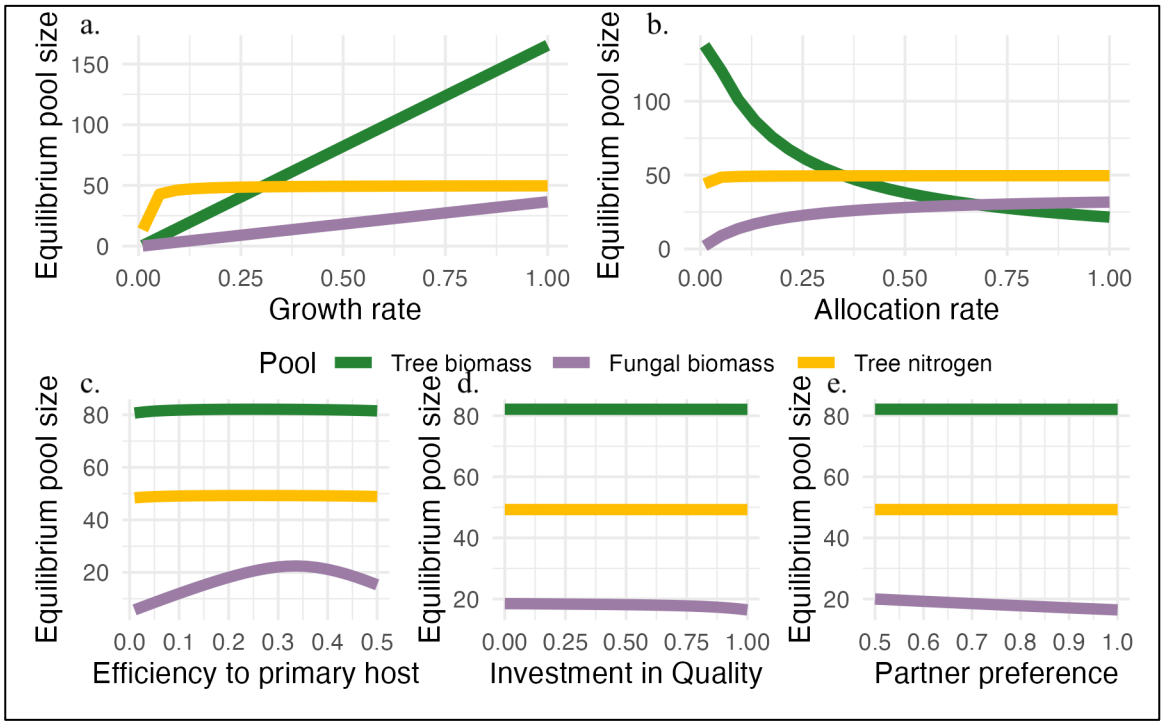


Figure 4: Model bifurcations for each parameter. In each panel, we show equilibrium pool sizes for the tree and fungal biomass as well as the tree nitrogen pool. Nitrogen consistently asymptotes, consistent with the limited pool in the system. In (a), higher host tree growth rates lead to larger equilibrium biomass of both hosts and fungi. Allocation (b) increases at a cost to the host biomass, though it benefits fungal biomass. Panels (c, d, and e) all relate to partner-specific interactions. Increased growth efficiency of fungi leads to a peak in fungal biomass before declining near its max value due to a trade-off with uptake rate. Similar efficiency trade-offs lead to lower fungal biomass at high quality investment (d) and partner preference (e).

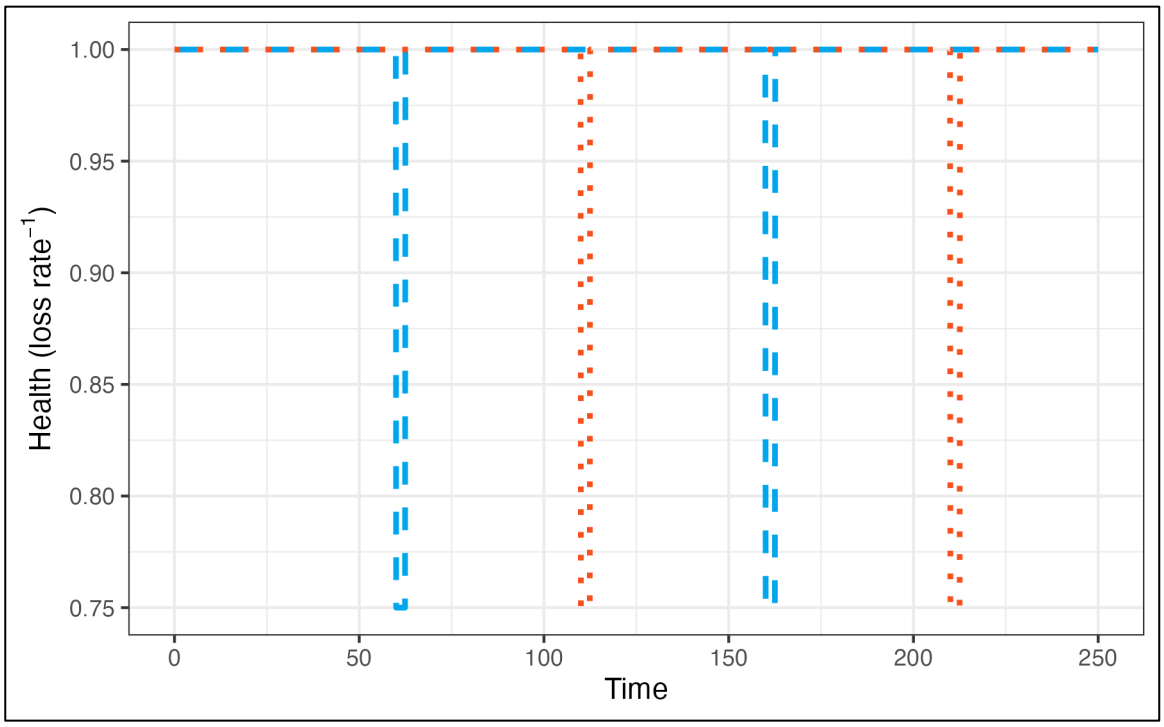


Figure 5: A visualization of the stress forcing in the model. Blue and red lines represent staggered stresses, each occurring twice throughout the simulation. Stress is implemented by increasing the loss rate in trees and/or fungi for brief instances then allowing them to recover.

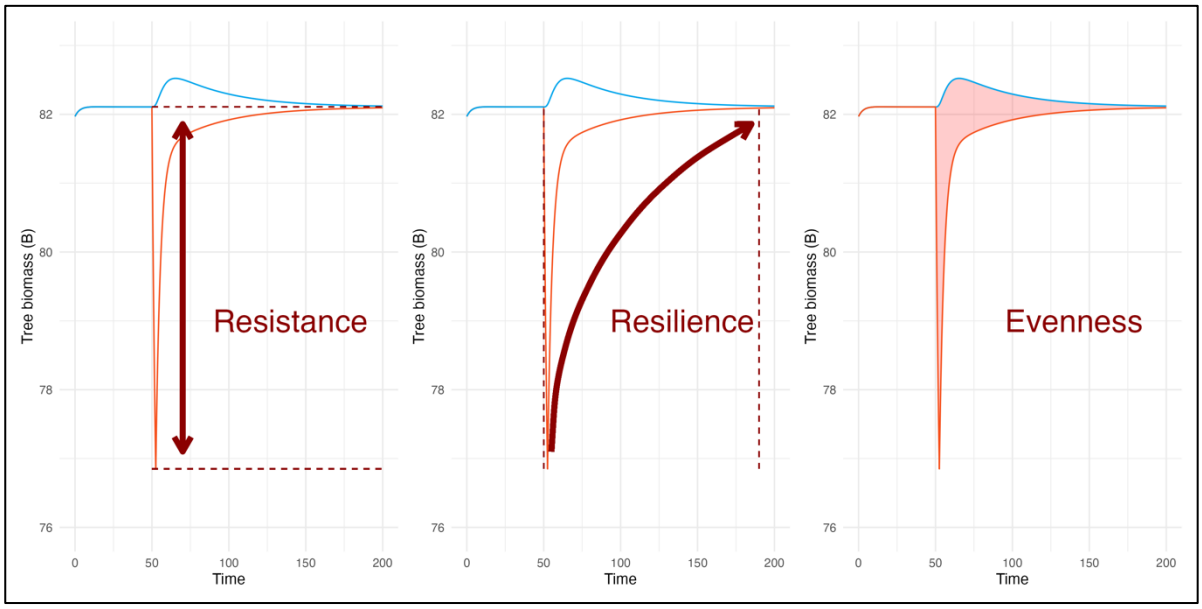


Figure 6: System stability diagnostics. Shown is a representative host stress event where the red line is a stressed host and the blue is an unstressed host. The shaded regions in the subplots illustrate how each metric is calculated. Resistance is the difference between the pre-stress biomass and the minimum biomass through stress. Resilience is calculated as the time to recovery. Evenness describes the relative difference between the host biomasses throughout the duration of the stress simulation.

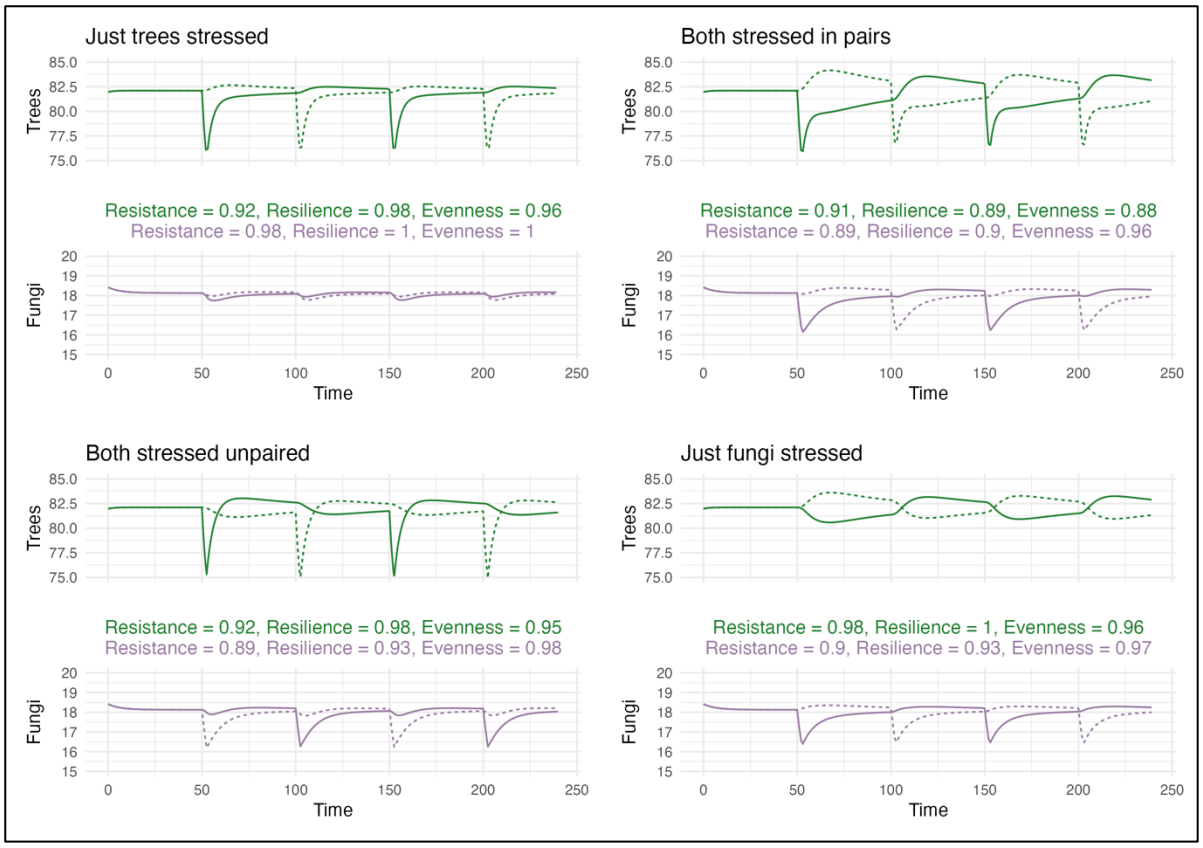


Figure 7: Stress simulations. Stressing trees and fungi independently, together in pairs, or together but on alternate partners. Simulations show strong influence of stress synchrony on system outcomes. Stresses on only one member at a time minimize impacts on partners. Stresses on alternate partners produce a more stable system (mostly by means of more rapid recovery, or higher resilience) than when primary partners are stressed together.

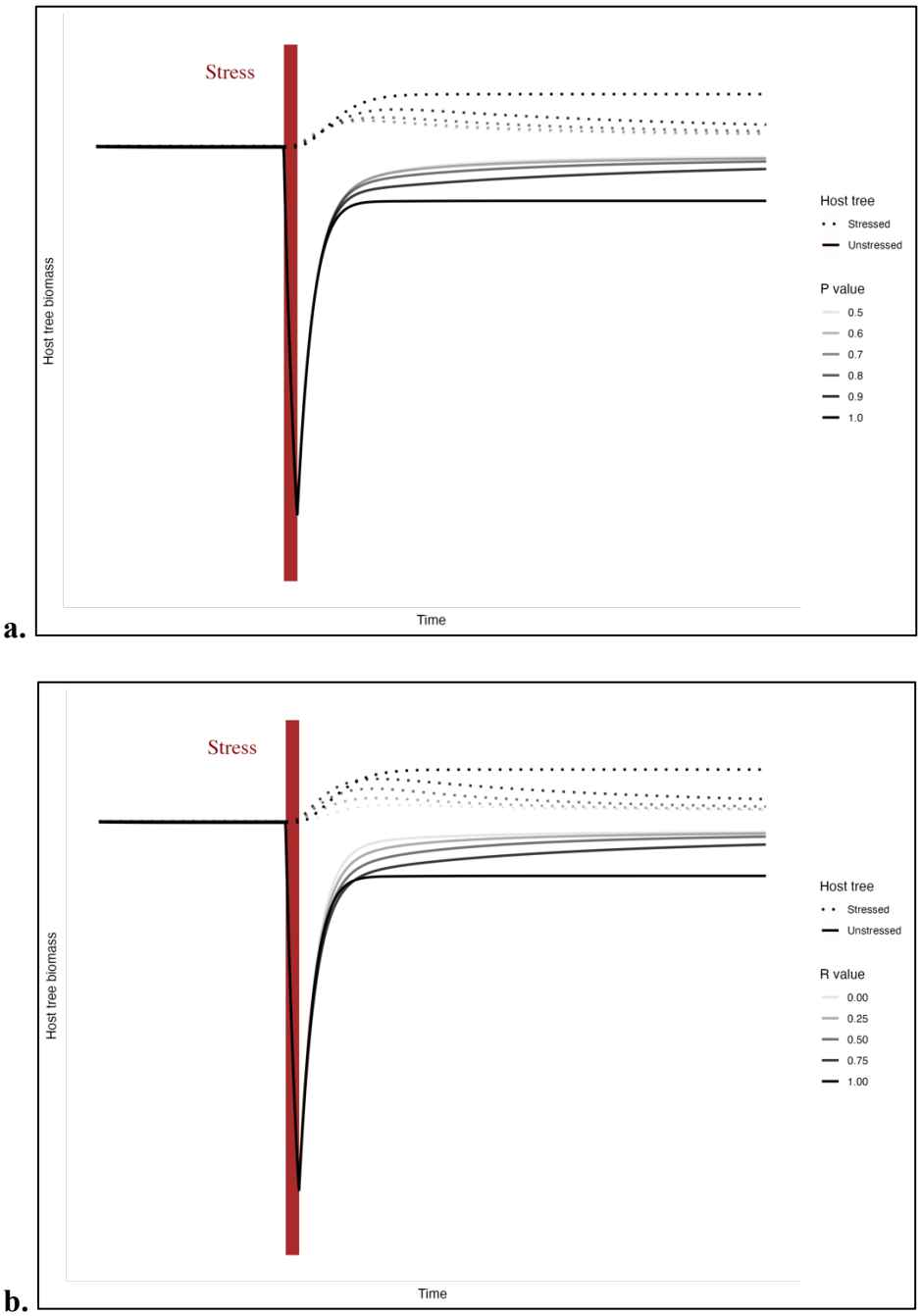


Figure 8: Variance in host tree biomass across partner-specific sharing values. Solid lines and shaded regions depict average biomass values and interquartile ranges through a single stress event. Columns vary based on which member(s) of the network were stressed in the simulation. The top row of plots shows responses to different partner preference values. The bottom row of plots show responses to different rates of investment in partner quality. Here, divergent host pools are indicative of lower stability in the system.

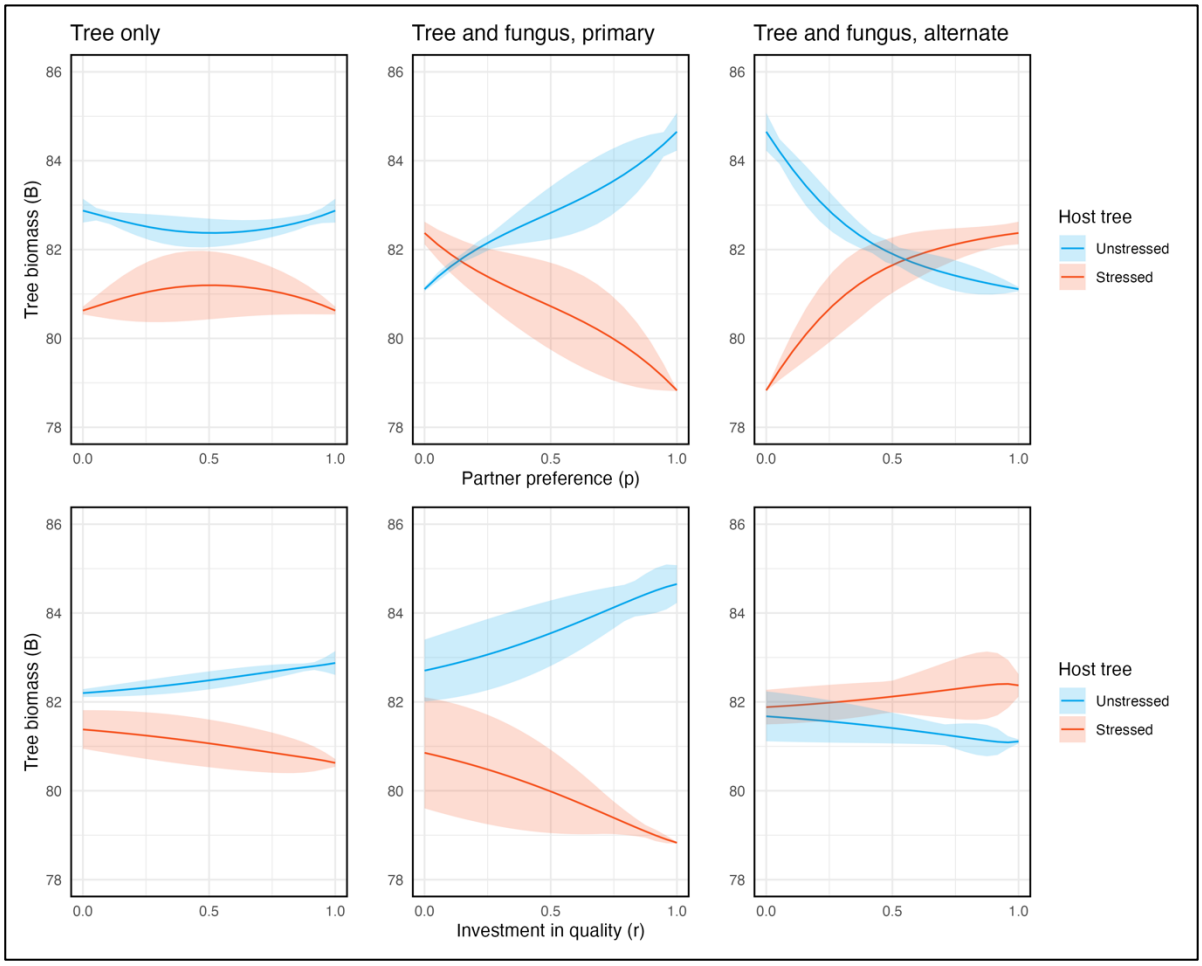


Figure 9: Variance in host tree biomass across partner-specific sharing values. Solid lines and shaded regions depict average biomass values and interquartile ranges through a single stress event. Columns vary based on which member(s) of the network were stressed in the simulation. The top row of plots shows responses to different partner preference values. The bottom row of plots show responses to different rates of investment in partner quality. Here, divergent host pools are indicative of lower stability in the system.

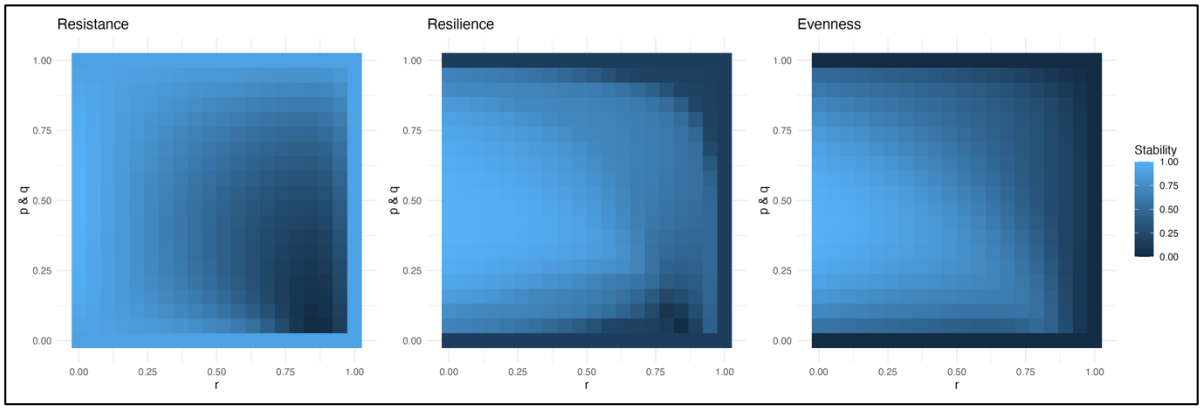


Figure 10: Network stability. While there remains a resistance refuge at maximized parameter values, all three metrics show agreement for stability maximization at medium (*agnostic*) partner preference and low investment in quality. These heat maps are generated across stress synchrony scenarios and averaged by parameter combination for overall patterns. Within metrics, values are rescaled from 0 to 1 using the minimum and maximum values across all simulations.

4.8. Appendix

Two-tree-two-fungus additional equilibrium states

{B1 = B1∗, B2 = 0, F1 = 0, F2 = F2∗, N1 = N1∗, N2 = 0}

{B1 = B1∗, B2 = 0, F1 = F1∗, F2 = F2∗, N1 = N1∗, N2 = 0}

{B1 = B1∗, B2 = B2∗, F1 = F1∗, F2 = 0, N1 = N1∗, N2 = N2∗}

{B1 = B1∗, B2 = B2∗, F1 = 0, F2 = F2∗, N1 = N1∗, N2 = N2∗}

{B1 = 0, B2 = B2∗, F1 = F1∗, F2 = 0, N1 = 0, N2 = N2∗}

{B1 = 0, B2 = B2∗, F1 = 0, F2 = F2∗, N1 = 0, N2 = N2∗}

{B1 = 0, B2 = B2∗, F1 = F1∗, F2 = F2∗, N1 = 0, N2 = N2∗}