Symbiont Community Reordering and Disassembly in a Pinon-Juniper Woodland

Introduction. Climate change induced shifts in ectomycorrhizal communities may impact forest regeneration and ecosystem function. As the frequency and severity of natural disturbances that impact biotic communities becomes more unpredictable, chronic environmental forcings (i.e. increased aridity) are also impacting these communities. Responses of ecological communities to predictable disturbance regimes have been widely studied, while responses to chronic forcings are less well understood. These forcings may alter abundances of dominant species in communities resulting in community reordering (changes in abundance without species loss or gain) or in extreme cases, community disassembly (progressive species decline & loss) [1,2]. This directional change may precede a state-transition, but it is unclear how reordering alters ecosystem function[1].

Communities of ectomycorrhizal fungi (EMF) are critical for establishment and success of forests, contribute to hydrological function, aid in carbon sequestration, and may mediate the impacts of drought on their hosts[3,4]. Communities of EMF are diverse, but generally a few species account for >50% of abundance[5]. Therefore, many of these species may be fulfilling similar ecological functions. Furthermore, there is not a strong link between EMF richness and ecosystem function[5]. Thus, I predict that changes in abundance of dominant EMF species will have greater implications for ecosystem function than loss or gain of rare species.

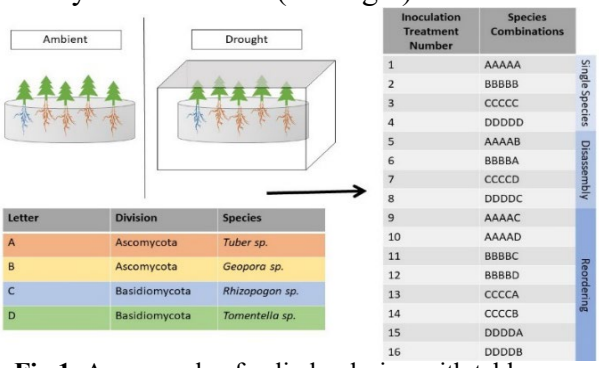
In low diversity systems such as (PJ) woodlands, prolonged drought has led to community disassembly of EMF communities, favoring communities dominated by Ascomycota species in the genus *Geopora* with a concomitant reduction in the more typical Basidiomycota EMF species[2,6,7]. While Ascomycota communities appear to benefit their host trees under stressful conditions, it remains unclear if or how these fungi provide drought tolerance to their

hosts, how they impact their hosts in more favorable conditions, or what mechanisms lead to the apparent stress tolerance[2,8]. For both community reordering and disassembly scenarios, it remains unclear how those shifts impact ecosystem function.

The proposed study will investigate how reordering or disassembly of ectomycorrhizal fungal communities in Piñon-Juniper woodlands impacts ecosystem function by asking **Q1: How do individual species of EMF vary in benefit to piñon seedlings? And Q2: What impact do community reordering versus community disassembly of piñon associated EMF have on seedlings and ecosystem function?** Previous research has shown that colonization by Ascomycota EMF (specifically members of the genus *Geopora*) can mediate the negative effects of drought on piñon pine[6,7]. However, all previous work with drought, piñon, and EMF has been observational, has only investigated communities of EMF, and has not investigated how colonization influences tree physiology [2,6,7,]. **Assessing how piñon respond to changes in EMF communities will aid in our ability to predict forest responses, tie responses to ecosystem function and may provide a strategy for successful management and regeneration of arid land forests. As a third year PhD student, this project will make up my third dissertation chapter.**

Methods. To address these questions I will initiate an experiment this winter featuring piñon seedling outplants and small-scale drought treatments in the PJ woodlands at the Sevilleta National Wildlife Refuge. Piñon seeds will be surface sterilized and germinated in trays of sterile vermiculite. Groups of five seedlings will be weighed and transferred into 0.45µm nylon mesh cylinders that prevent roots and hyphae from entering. To manipulate fungal dominance hierarchies, either all five seedlings in the cylinder will be inoculated with the same fungus or four will be inoculated with the same species and one will be different. Due to the difficulty of

culturing EMF, fungal inoculations will be done using piñon roots colonized with one of the following EMF species: *Geopora sp., Tuber sp., Rhizopogon sp., or Tomentella sp.* Each possible combination will be included resulting in 16 fungal treatments: (1-4) individual species, (5-6) disassembly-ascomycete only, (7-8) disassembly- basidiomycete only, (9-12) reordering-ascomycete dominant, and (13-16) reordering- basidiomycete dominant (see Fig 1).



Letter	Division	Species
A	Ascomycota	*Tuber sp.*
B	Ascomycota	*Geopora sp.*
C	Basidiomycota	*Rhizopogon sp.*
D	Basidiomycota	*Tomentella sp.*

Inoculation Treatment Number	Species Combinations	
1	AAAAA	Single Species
2	BBBBB	
3	CCCCC	
4	DDDDD	
5	AAAAB	Disassembly
6	BBBBA	
7	CCCCD	
8	DDDDC	
9	AAAAC	Reordering
10	AAAAD	
11	BBBBC	
12	BBBBD	
13	CCCCA	
14	CCCCB	
15	DDDDA	
16	DDDDB	

Fig 1. An example of cylinder design with tables describing the four species and all 16 inoculation treatments.

Cylinders will then be planted into ten split blocks. Each block will include two sets of all 16 fungal treatment combinations, separated by flashing. Each split block will be enclosed in a 2m x 2m shelter that completely excludes rainfall. Shelters will be constructed using metal conduit, clear acrylic panels, and PVC gutters. Rainfall will be collected and dumped off plot. To mimic the shade produced by nurse trees, shelters will be covered by a shade cloth that reduces PAR. For the first 3 months following transplant, I will evenly water all cylinders to ensure establishment before imposing the drought treatment. After 3 months, I will begin to hand water cylinders weekly to ensure a drought and rainfall addition treatment. Each fungal inoculation x moisture treatments will be replicated 10 times (16 fungal treatments x 2 moisture treatments x 10 replicates = 320 cylinders). I will install devices to measure soil moisture into each split block to ensure moisture conditions. After 12 months, seedlings and cylinders will be harvested. Shoot and root dry biomass, mortality, EMF colonization, and EMF community composition will be measured. To investigate possible mechanisms responsible for drought tolerance I will also measure photosynthetic rate, water potential and stomatal conductance bi-monthly**. Funding from the BGSA GRAC will support travel in my personal vehicle to and from my field site.**

Literature Cited

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Budget

Item	Cost per unit	Quantity	Total Cost	Funding Source	Status
Piñon seeds	$25 per bag of 500	4	$100	Sevilleta Graduate Fellowship	Accepted
Nylon mesh for cylinders	$2.25/sq. ft	218	$491	Sevilleta Graduate Fellowship	Accepted
Metal conduit & Soil moisture probes	N/A	N/A		Provided by applicant's lab	Accepted
Screws	$0.25/screw	250	$62.5	John W. Marr Ecology Fund	Accepted
Metal washers	$3.08/bag of 25	10	$30.8	Sevilleta Graduate Fellowship	Accepted
PVC for drought shelter gutters	$10.76	4	$43.04	John W. Marr Ecology Fund	Accepted
Acrylic panels	$23/panel (26" x 8')	3	$69	John W. Marr Ecology Fund	Accepted
Data loggers	$220/logger	4	$880	Sevilleta Graduate Fellowship	Accepted
Tree Labeling Tags	$97.25	2	$194.5	John W. Marr Ecology Fund	Accepted
Molecular Supplies	~$100/sample	~300	$3000	Sevilleta Graduate Fellowship	Accepted
Gas Mileage	**$0.18/mile**	**2222 miles**	**$400**	**BGSA GRAC**	**Pending**
Remaining Gas Mileage	$0.18/mile	5058 miles	$910	GPSA SRG ($500) John Marr Ecology Fund ($100) Personal Funds ($310)	Pending/ Accepted
				Total Requested from BGSA GRAC= $400	

Budget Justification

Because total gas mileage expenses are greater than the funding amount, I have split up the gas expenses into two items. Total mileage: 140 miles per trip x 52 weeks x $0.18 per mile = $1310. In addition to applying for the BGSA GRAC I have applied to the UNM Graduate Professional Student Association Student Research Grant ($500, Submitted September 18th). I have already received funding from the Sevilleta Graduate Fellowship and the John W. Marr Ecology Fund.

Previous use of GRAC Funds

I was previously awarded GRAC research funds in Fall 2018 to collect soil cores for the first two chapters of my dissertation. Funds were used for gas for multiple trips to my field site outside of Las Vegas, NM. I also used funds for necessary supplies such as sharpies and Ziplock bags. I collected soil cores from all nine of my plots in 2018 and had remaining funds to collect soil cores from all plots again in 2019. Last spring, I collected roots from the soil cores and identified the mycorrhizal fungi on them using molecular techniques. In July 2019 I was able to present these results at the International Conference on Mycorrhiza in Merida, Mexico. My poster was titled "**Mycorrhizas and Host Tree Mortality in the Piñon-Juniper Woodland"**. These funds allowed me to complete all sampling needed for my first two dissertation chapters. I am currently in the process of finishing up molecular work and data analyses and will begin writing these chapters shortly.