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Using Bacterial and Fungal Inoculation Strategies to aid in the Wetland Restoration of Native Plants in Southern California

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Abstract

Restoration projects are becoming increasingly important to degraded ecosystems. The use of Plant Growth Promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) has been shown to aid the progress of certain restoration projects. In this study, the effect of different inoculation treatments on different plants native to Southern California was studied at two sites in the Ballona Wetlands Ecological Reserve (BWER). Before planting, both sites were cleared of invasive species and raked. Seeds from the species *Frankenia salina* and *Cressa truxillensis* were separated into four treatments: control, PGPR inoculated, AMF inoculated, and co-inoculated, then planted and monitored for germination over the course of two months. The plant *Eriogonum parvifolium* was grown in AMF inoculated soil then transplanted into field conditions and separated into two treatments: AMF inoculated and co-inoculated, and monitored for longest stem length (cm) over two months. Two months after planting, there was no evidence to support a difference in average longest stem length in *E. parvifolium*, nor a difference in average percent germination of *F. salina* or *C. truxillensis* across the different treatments.

Introduction

In recent years, more and more emphasis has been placed on attempts to restore degraded habitats in order to benefit from the ecosystem services and habitat importance of those degraded areas. Coastal ecosystems, such as dunes and marshes, have been disproportionately degraded due to high levels of human settlement along coastlines (Lotze et al. 2006). Coastal habitats are very interconnected, as coastal dunes often run adjacent to wetland habitats. Due to this, vegetation can be shared across the ecosystem shift from wetland to coastal dune. However, these habitats have tangible value to human and animal communities (Table 1).

Table 1. This table shows a collection of ecosystem services provided by select coastal habitats. Sourced from Barbier et al. 2011.

Habitat type	Ecosystem Services
Coastal habitats (general)	Resist biological invasion Flood mitigation Water purification
Salt Marsh	Sediment stabilization Uptake of pollutants and nutrients Nursery habitat for fish Carbon sequestration Dissipation of waves (coastal protection)
Coastal Dune	Prevent erosion Raise water tables Shorebird habitat Reduce Flooding

In the context of sea level rise caused by climate change, healthy coastal habitats can mitigate floodwaters and protect coastal communities (Barbier et al. 2011).

California specifically has lost 90% of its wetland ecosystems due to development (Zedler 1996). In the Los Angeles region alone, over 96% of vegetated coastal estuarine wetlands have been lost. This loss has been due to the effects of urbanization, both by development and by conversion of wetland habitat to upland habitat through fill deposition (Johnston et al. 2015).

The Ballona Wetlands Ecological Reserve (BWER) is one of the largest remaining wetlands in LA County, making it an important target for restoration. Before major development and urbanization, this wetland ecosystem spanned 2000 acres and a variety of habitat types (Grossinger et al. 2010). Now an almost 600-acre degraded salt marsh ecosystem in Los Angeles, CA, BWER contains habitats such as coastal dune, salt pan, upland scrub, and upland grassland. The reserve is located within the California chaparral Mediterranean ecosystem. Though the reserve extends over 600 acres, only 153 acres are considered delineated wetland due to floodgates limiting the amount of habitat exposed to tidal influence (Johnston et al. 2015). Both the size and degradation of this site make it a good candidate for restoration efforts.

The Ballona Creek watershed includes much of the city of LA, and thus carries urban runoff from the highly urbanized Los Angeles area. The creek consistently shows levels of toxic metals and fecal

indicator bacteria (FIB) higher than the EPA and City of LA recommended levels respectively (Johnston et al. 2012). Wetland restoration in urban areas is especially significant because restored wetlands have been shown to decrease the nutrient and pollutant load of urban runoff (Ehrenfeld 2000).

The Ballona Wetlands Ecological Reserve has populations of important native plants whose survival is threatened by non-native invasion. In the lower marsh areas, native vegetative species are dominant. This reserve has populations of the El Segundo Blue Butterfly, a federally endangered species that lives its entire life cycle on the native plant *Eriogonum parvifolium,* commonly named seacliff buckwheat. Other important native species include saltgrass (*Distichlis spicata*), alkali weed (*Cresssa truxillensis*), common pickleweed (*Salicornia pacifica*), and alkali heath (*Frankenia salina*). From 2007 to 2013, net ground cover of these native species has either declined or increased a maximum of 0.24 acres (Johnston et al. 2015). As much of BWER is not connected to tidal influence, these degraded areas were more easily invaded by non-native species. Over five years of monitoring, 14 acres of native non-tidal salt marsh were lost to non-native invasion (Johnston et al. 2015).

Invasive plants can disturb many natural processes including fire regimes, nutrient cycling, and hydrology budgets in ways that make it much harder for native plants to survive (Mack et al. 2000). Invasive plants have also been shown to decrease the ability of microbes to fix nitrogen (Carey et al., 2017). In the dune, upland grassland, and upland scrub habitats, non-native species are predominant. Some of the most common non-native species established in the reserve are ice plant (*Carpobrutus edulis*), crown daisy (*Glebonius coronaria*), and black mustard (*Brassica nigra)* (Johnston et al. 2012). Ice plant is the most widespread invasive plant in BWER, and forms monocultures that exclude the growth of native plants. Even after Ice plant is removed, it has lasting effects on the chemistry of the soil. Ice plant has been shown to alter the soil's chemical characteristics, lowering the pH and detrimentally effecting the germination rate of some native species (Novoa et al. 2013, Conser & Conner 2009).

Though there are many clear benefits to restoration, projects are expensive, labor-intensive, and require long-term maintenance in order to be successful. As these projects are difficult, ecologists are constantly looking for new strategies that can aid in the successful reestablishment of a healthy ecosystem for the least amount of cost. A massive restoration project for BWER was recently approved involving the removal of 9,800 ft of Ballona Creek levees, restoring 200 acres of coastal wetlands, and reshaping the now-channelized Ballona Creek to an unchannelized riverine shape. Along with the more wildlife focused restoration, pedestrian trails will be created, allowing people from the nearby community access to the greenspace (CDFW 2021). An example of a successful large-scale wetland restoration can be seen with the 2013 restoration of Malibu Lagoon. Six years after the initial restoration, native vegetative cover improved to 96%-100%, fish and bird species richness had improved, and previously existing hypoxic zones were eliminated (Johnston et al. 2019).

Focusing specifically on the revegetation process in restoration, recent studies have begun to question whether the microbiome of the soil could be used to aid the establishment of native vegetation and allow it to compete with invasive species. These studies concern the rhizosphere, which is the area directly at the surface of the soil containing plant roots interacting with microorganisms. This area of the soil is full of complex interconnected relationships between plants, bacteria, fungi, nematodes, and other organisms. The rhizosphere is characterized by symbiosis that can form between plants and microorganisms. Certain bacteria, commonly called plant-growth promoting bacteria (PGPB) provide functions for a plant in exchange for the secretion of sugars from the plant that the bacteria will

be able to ingest. Some of these mechanisms include nitrogen fixation, phosphorous solubilization, auxin production, plant immune system stimulus, and others (de-Bashan et al. 2012). Fungi also form symbiosis with plants, the most studied of these being arbuscular mycorrhizal fungi (AMF). These species form hyphae which extend out from plant roots and aid in nutrient uptake (Asmelash et al. 2016). Degraded soils also have degraded microbiomes, yet restoration projects have not made the addition of bacterial or fungal inoculants a general practice (Wagg et al. 2014).

Though incorporating inoculants is not a general restoration practice, some studies have shown the potential benefit of doing so. One study regarding the restoration of a desertified Mediterranean habitat in Spain showed the inoculation of native plants with native AMF species and N-fixing PGPB species leading to more successful reestablishment of those species in the long term as well as an increase in soil fertility (Requena et al. 2000). Other studies have used either bacterial or fungal inoculants and found similar results. During restoration of soil in the Nanmangalam Reserve Forest by planting native tree species, Ramachandran & Radhapriya (2016) found that the trees inoculated with native PGPB grew more biomass than in control areas. This process of inoculation shows promising ability to aid in the restoration of degraded habitats not only through aiding revegetation efforts, but also through soil restorative properties.

In this paper, the result of *in situ* experiments in the BWER on how various microbial inoculant treatments affect the growth of several native plant species used for restoration will be presented. The plants chosen in this experiment were alkali heath (*Frankenia salina*), alkali weed (*Cressa truxillensis*), and seacliff buckwheat (*Eriogonum parvifolium*). These plants were selected since all native to the Ballona Wetlands and are used in a neighboring revegetation effort. Seacliff buckwheat is the host plant for the federally endangered El Segundo blue butterfly that grows in the dune ecosystem. Alkali weed and alkali heath are both halophytes that thrive in salt marshes. Divided into three experiments, the following questions are addressed:

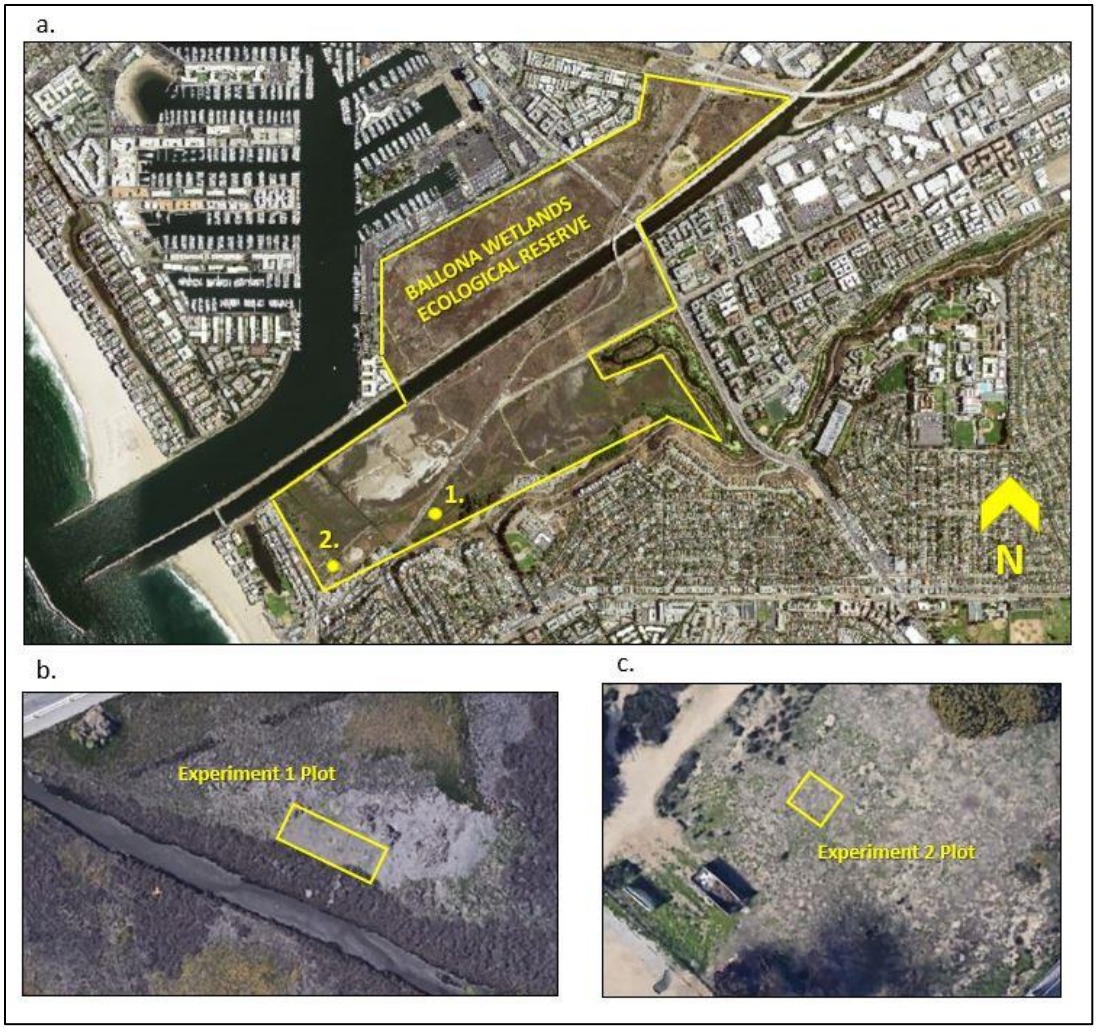
a. What is the effect of a PGPR inoculant on the percent germination of two native plant species: *F. salina*, and *C. truxillensis?*
b. What is the effect of an Arbuscular Mycorrhizal Fungi (AMF) inoculant on the percent germination of two native plant species: *F. salina*, and *C. truxillensis?*
c. What is the effect of the co-inoculation of the bacterial inoculant and the AMF inoculant on the percent germination and growth of three native plant species: *E. parvifolium*, *F. salina*, and *C. truxillensis?*

Methods

Experimental locations and habitat

Two field experiments were conducted in different locations within the Ballona Wetlands (Figure 1). Experiment 1 was located in an area at the cross of three habitat types: Iceplant stand, Non-tidal salt marsh, and ruderal marsh. The soil in this area has high salinity levels. Experiment 2 is located in a non-native dune habitat where soils are very sandy.

Figure 1. (a) A view of Ballona Wetlands Ecological Reserve as a whole with the locations of Experiment 1 and 2 marked. (b) The size and location of the plot for Experiment 1 is shown in greater detail. (c) The size and location of the plot for Experiment 2 is shown close up.



Experiment 1. *Frankenia salina* and *Cressa truxillensis*

Seed acquisition and pretreatment

Seeds were acquired from the native plant nursery S&S. *F. salina* seeds were collected from Temecula, CA and *C. truxillensis* seeds were collected from Escondido, CA. In a previous study *C. truxillensis* seeds showed increased percent germination when scarified with light grit sandpaper (Lyford, unpublished data). *C. truxillensis* seeds were mechanically scarified by dragging each seed 0.5 inch across P220 sandpaper, using a smaller piece of P220 sandpaper to grip the seed. *F. salina* seeds required no pretreatment. Approximately 50 seeds were used per treatment.

Inoculation and placement of seeds

Paenibacillus polymyxa and another *Paenibacillus spp.* isolated from *C. truxillensis* and having plant growth-promoting characteristics were grown in lab (Lyford, unpublished data). The species were mixed and suspended in carboxymethyl cellulose (CMC) to create a liquid inoculant. The sterile inoculant was 1% CMC. After pretreatment *F. salina* and *C. truxillensis* seeds were sorted into containers of approximately 50 seeds each to prepare for placement in the field. One mL of inoculant was pipetted into each container of *C. truxillensis* seeds, and 3 mL were pipetted into each container of *F. salina* seeds 15 minutes prior to being placed in the field. The increase in inoculant volume for *F. salina* was due to the presence of seed chaff along with the seeds for that species. Sterile inoculant was applied to the seeds not treated with bacterial inoculant. The batches of seeds were emptied into the corner of a 0.25m x 0.25m plot using sterilized tweezers, and a sterilized metal spatula was used to distribute the seeds as evenly as possible throughout the plot. The instruments were sterilized using 70% ethanol.

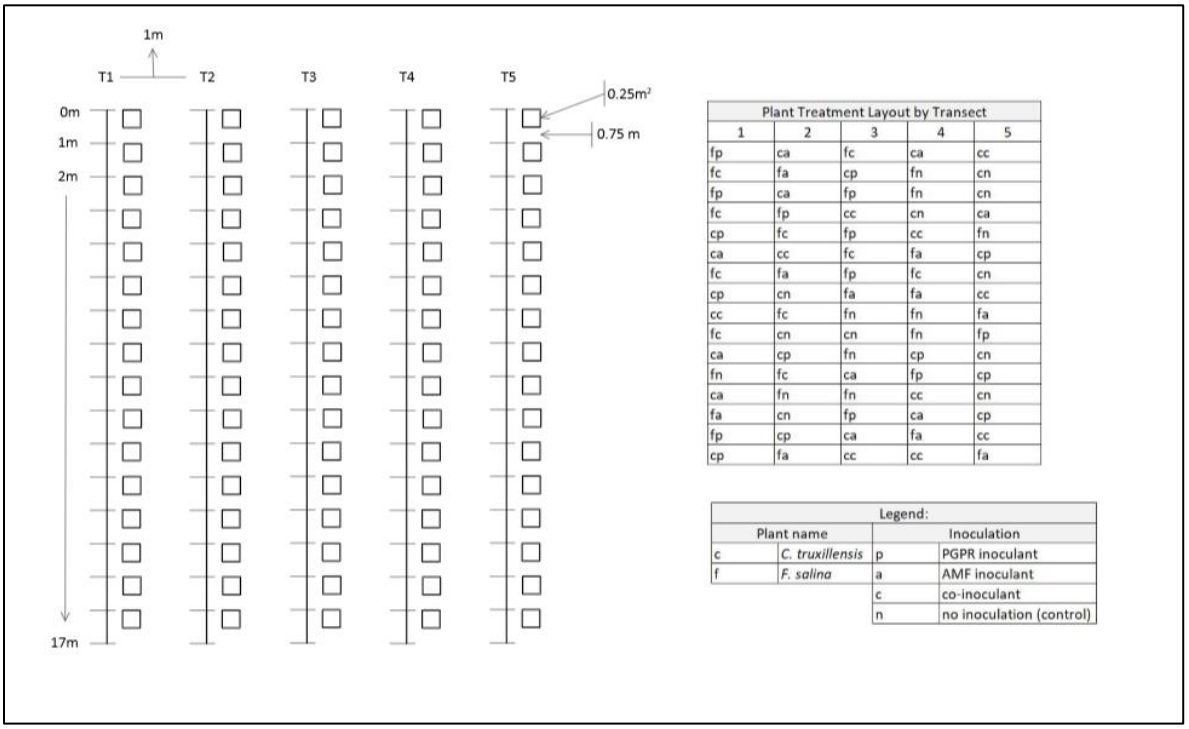
The fungal inoculant was sourced from S&S seeds containing a consortia of native arbuscular mycorrhizal fungi (AMF) species able to form symbiosis with native plants. One sixth cup of dry inoculant was scooped from a large bag using a measuring cup, then shaken from the cup as evenly as possible to cover selected plots after the seeds were applied.

All plots were then irrigated thoroughly using a backpack sprayer filled with brackish water from the nearby wetland channel. Both plants are halophytic and the brackish water may give them an advantage over the invasive seed bank present in the soil. The plots were watered using the same method twice a week for the first two months after planting. Two months after planting, the percent germination will be recorded.

Experimental Design

Four treatments were applied to each of the two plant species with ten replicates per treatment. The treatments were: inoculation with PGPR species, inoculation with AMF species, co-inoculation, and no inoculation (control). The treatments were distributed along 5 transects, with 16 per transect (Figure 2). The distribution of treatments within the plot was chosen randomly by writing all possibilities on pieces of paper, putting them in a shoebox, and picking out a piece, until the layout was filled.

Figure 2. Experiment 1 layout



Plant Treatment Layout by Transect				
1	2	3	4	5
fp	ca	fc	ca	cc
fc	fa	cp	fn	cn
fp	ca	fp	fn	cn
fc	fp	cc	cn	ca
cp	fc	fp	cc	fn
ca	cc	fc	fa	cp
fc	fa	fp	fc	cn
cp	cn	fa	fa	cc
cc	fc	fn	fn	fa
fc	cn	cn	fn	fp
ca	cp	fn	cp	cn
fn	fc	ca	fp	cp
ca	fn	fn	cc	cn
fa	cn	fp	ca	cp
fp	cp	ca	fa	cc
cp	fa	cc	cc	fa

Legend:			
Plant name		Inoculation	
c	*C. truxillensis*	p	PGPR inoculant
f	*F. salina*	a	AMF inoculant
		c	co-inoculant
		n	no inoculation (control)

Figure 3. Cleared plot for Experiment 1 showing the 5 transects and 16 plots per transect marked by orange flags

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Experiment 2. *Eriogonum parvifolium*

Nursery growth

Seeds from *E. parvifolium* were collected from the Ballona Wetlands Ecological Reserve. Seed chaff was spread onto a shallow and wide pot using soil that consisted of 60-70% sphagnum peat moss inoculated with 7 AMF species (Table 1). After 2 months, emergent seedlings were repotted carefully one per pot into 1-gallon pots. They were watered with freshwater every 3-4 days for 3 weeks until they reached a larger size. The nursery pots were kept in the Ballona Wetlands Ecological Reserve under a chicken wire cage to prevent herbivory.

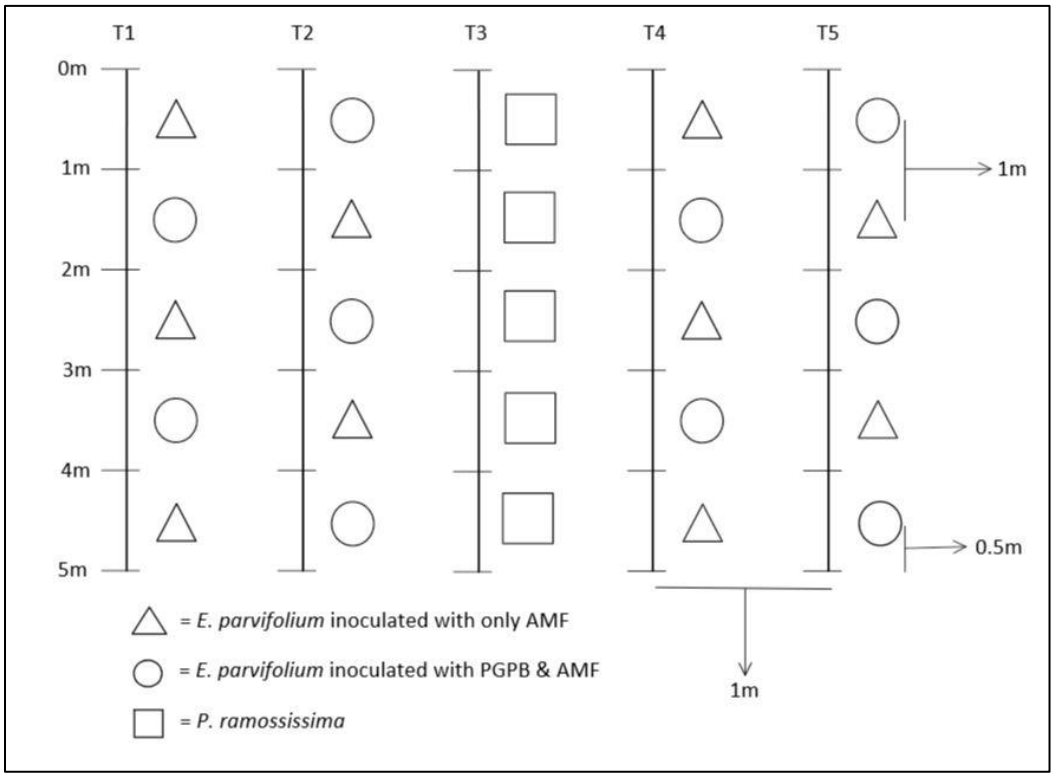
Table 2. AMF species included in the soil used to pot E. parvifolium seedlings

Species name	Concentration
Glomus intraradices	0.05 propagules/gram
Glomus clarum	0.05 propagules/gram
Glomus deserticola	0.05 propagules/gram
Glomus monosporum	0.05 propagules/gram
Glomus etunicatum	0.05 propagules/gram
Glomus mosseae	0.05 propagules/gram
Gigaspora margarita	0.05 propagules/gram

Inoculation and experimental design

Twenty *E. parvifolium* seedlings were planted along 4 transects, separated by a transect of 5 branched phacelia (*Phacelia ramosissima)* seedlings incorporated as part of general habitat restoration efforts (Figure 4). All twenty seedlings were previously inoculated with the AMF species mentioned in Table 1. Ten *E. parvifolium* plants were co-inoculated with 2 mL of the same liquid inoculant mixture as detailed in Experiment 1. Ten *E. parvifolium* plants were inoculated with the same amount of the sterile mixture. The inoculant was applied directly to the soil surrounding the plant after transplant from the 1-gallon pot to the study site. Each plant was covered with a chicken wire cage to prevent herbivory. Each plant was watered thoroughly with freshwater.

Figure 4. A diagram of the layout of the experiment involving E. parvifolium plants



Data collection and analysis

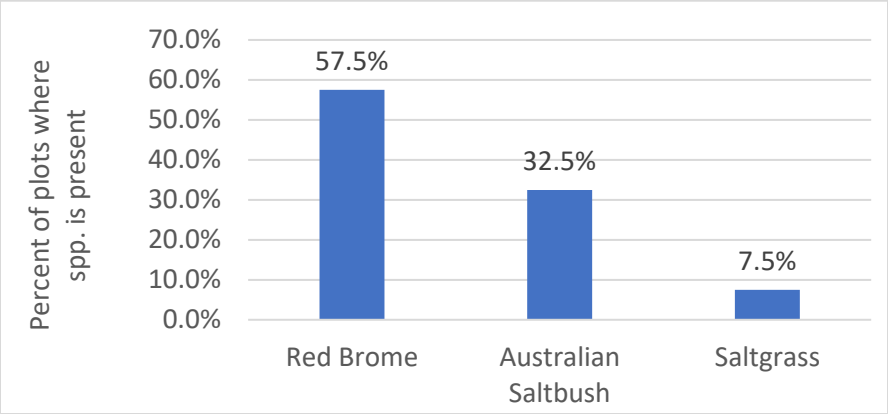
Both experiments were run for two months. For Experiment 1, the percent germination was recorded, and data was analyzed for difference using a one-way ANOVA. For Experiment 2, length of the longest shoot in centimeters was recorded. Data was analyzed for difference using a T-test. Both datasets were analyzed in Microsoft Excel using the Data Analysis ToolPak.

Results

Experiment 1. *F. salina* and *C. truxillensis*

Two months after planting, data were collected on the number of seedlings that grew in each plot. While there were seedlings of other plants present in the plots, 0 seedlings of the studied plants were observed. The three plants most present in the plot were red brome (*Bromus madritensis ssp. rubens*), Australian saltbush (*Atriplex semibaccata*), and saltgrass (*Distichlis spicata*) (Figure 1).

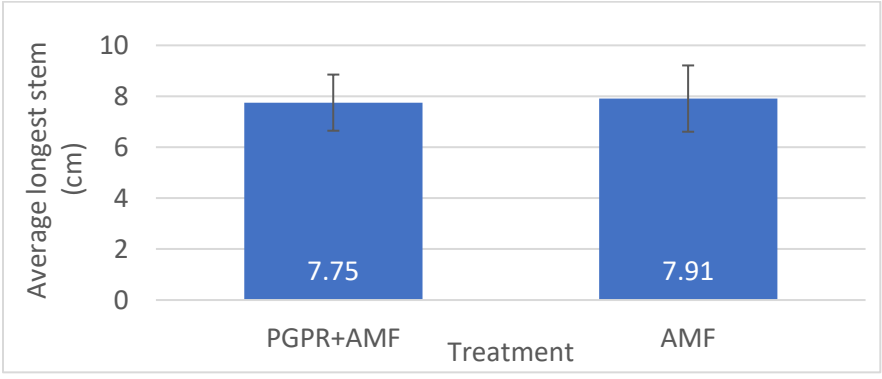
Figure 5. Figure 5 shows the three plants present in the highest percentages on the plot of Experiment 1 and the percentage of cover within the plot.



Experiment 2. *E. parvifolium*

Two months after transplant, the difference in longest stem length of *E. parvifolium* seedlings between treatments was not statistically significant (Figure 2). The mean stem length for the treatment containing PGPR and AMF inoculants was 7.75 cm and the mean stem length for the treatment with only AMF inoculation was 7.91 cm. The difference between means has a calculated p-value of 0.64 and is not significant. The p-value was calculated using a two-tailed two sample t-test assuming unequal variance. Qualitatively there was no difference in the average visual health of the seedlings in different treatments.

Figure 6. Figure 6 shows the average longest stem length for two different treatments of E. parvifolium plants (n=10). The error bars show standard error values of 1.10 and 1.30 for PGPR+AMF and AMF respectively.



Discussion

These experiments addressed the question of whether or not microbial inoculants were a viable addition to restoration planning in the Ballona Wetlands Ecological Reserve both through seed inoculation and liquid inoculation of seedlings. Both of these experiments did not show statistically significant results due to a variety of factors.

Experiment 1. *Frankenia salina* and *Cressa truxillensis*

The research question that was attempted to be answered in this experiment was whether or not the combination of bacterial and fungal inoculants or inoculants on their own had a significant effect in aiding the emergence of *C. truxillensis* and *F. salina* seedlings. The comparison of seed pre-treatments in this experiment was not successful because of the lack of success of seedling emergence both across *F. salina* and *C. truxillensis* treatments. There are a variety of factors that most likely influenced the lack of seedling emergence including competition from invasive species, water erosion of seeds, seed storage, unusually dry weather, and the short time period of experimental monitoring. In a laboratory experiment, *C. truxillensis* scarified by light grit sandpaper showed 92.3% germination, which is incredibly high (Lyford 2020). In previous literature, *F. salina* was shown to have an average of 50.6% dormant seeds, however the non-dormant portion germinate readily without pretreatment (CSU Stanislaus). In a literature review of 30 experiments that studied the use of direct seeding in restoration, the average germination was 23.9%, and the average success (measured as the proportion of individuals still alive at the end of the experiment) was only 11.4% (Ceccon et al. 2015). This study also found that the factors of climate, pre-germinative treatments, and successional group had no significant impact on germination rates (Ceccon et al. 2015).

When setting up the experiment, lower germination rates were expected and accounted for by the distribution of 50 seeds in a small ($0.25m^2$) plot. While germination was expected to be lower than in more controlled conditions, zero seedling emergence was observed in two months of monitoring which implies the impact of other factors affecting the germination rates.

Water erosion was a likely factor where seeds may have been washed from the plots during irrigation. Smaller seeds have been shown to be more likely to be washed away by rain or water erosion (Wang et al. 2012). *F. salina* seeds in particular are small, averaging 1-1.5mm in size. It's likely that some *F. salina* seeds were washed away from the plot site through either precipitation or when manually being watered. The seedlings were watered manually directly after seeding and three weeks after seeding. They were watered using a backpack sprayer which expelled water at high pressure, making it likely to have washed away some seed.

Seed storage is another possible factor in the low germination. Due to changing policies during the COVID-19 pandemic, the seeds were purchased and sorted into plastic containers in November 2020 and planted in the field early February 2021. Extended periods of time in storage have been shown to decrease the viability of seeds, though the rate at which this happens is very dependent on the individual species (Bortey et al. 2016). The storage period is unlikely to have affected the viability of *C. truxillensis* as seeds of that species can be stored for up to 2 years (Barton et al. 2016). Competition is also a likely factor in the decrease in germination.

The plot area was covered with mainly *Atriplex baccata* (Australian saltbush) before it was hand pulled out. The area was then raked in an attempt to remove invasive species seed bank. However, *A. baccata* seedlings have been observed in many places throughout the plot. Large invasive species seed banks have been shown to negatively impact the recruitment of native species in a Mediterranean environment, specifically causing an obstacle in the seedling stage (DiVittorio et al. 2007). Considering the presence of *A. baccata* seedlings and the absence of *F. salina* and *C. truxillensis* seedlings, it is likely that competition was a factor in the lack of recruitment.

Figure 7. A seedling found on the plot site for experiment one identified as Australian saltbush.

Finally, the two most important factors in germination are water and temperature levels. The optimal range of temperature for germination in many Mediterranean plant species ranges from 50-68˚F. Germination can be suppressed at temperatures 77-86 ˚F (Krichen et al. 2014). In a study of *Cressa cretica* plants, germination was highest from 50-68˚F with a recorded value of 25%. At temperatures with a high of 77˚F, germination was still high with 19%. When temperatures reached a high of 86˚F, germination was 8%, much lower (Khan 1991). From 02/05/2021 to 03/31/2021, the high recorded temperature exceeded the higher range only nine times (NOAA).

Table 3. The recorded maximum daily temperatures at LAX airport from 2/5/2021 to 3/31/2021 with temperatures over 77˚F in bold (NOAA).

February 2021 Day	*Max Temperature (˚F)*	*March 2021 Day*	*Max Temperature (˚F)*
5	72	1	75
6	**79**	2	75
7	72	3	56
8	65	4	67
9	61	5	**78**
10	62	6	69
11	68	7	63
12	69	8	65
13	64	9	63
14	68	10	54
15	64	11	53
16	70	12	58
17	68	13	63
18	69	14	63
19	71	15	55
20	65	16	59
21	73	17	67
22	**80**	18	70
23	**78**	19	72
24	75	20	68
25	72	21	70
26	75	22	68
27	73	23	70
28	72	24	76
		25	59
		26	66
		27	**77**
		28	**87**
		29	**82**
		30	**81**
		31	**86**

While temperatures were often higher than the optimal range likely for these species, they were rarely in the higher range that corresponds to inhibited or much lower germination rates. It is possible that the high temperatures inhibited germination on a small scale, but unlikely that the temperature inhibited germination to a high extent. A more likely explanation for the lack of germination is the abnormally low precipitation levels during the experiment. There were only 5 days with precipitation during the two months of monitoring.

Table 4. A table showing the precipitation in inches and the date for all the instances of precipitation within the time frame of 2/5/2021 to 4/5/2021 (NWS).

Date	Precipitation (in)
02/12/2021	0.02
03/03/2021	0.05
03/10/2021	0.66
03/11/2021	0.01
03/15/2021	0.21

This is an abnormally dry winter for Los Angeles as normal precipitation levels for February are 4.48 inches and for March are 2.97 inches (NOAA). Even with the supplemental watering, it is likely that the seeds suffered water stress during this dry winter.

Table 5. The monthly precipitations for 2021 in the duration of this experiment compared to the normal monthly precipitations which are based off climate records from 1981-2021 (NOAA).

2021 Monthly Precipitation (in)	Normal Monthly Precipitation (in)	Change from Normal (in)
February		
0.02	4.48	-4.46
March		
0.93	2.97	-2.04

The last factor to discuss is the experimental timeline. The seeds were only able to be monitored for a short time-period of two months due to unforeseen scheduling conflicts and the COVID-19 pandemic. It is possible that in a field context both species require longer periods of time before germinating and emerging.

Ultimately, the most likely explanation for the low germination of both *C. truxillensis* and *F. salina* is a combination of drought stress, competition with invasive species *A. baccata*, longer than expected storage time, a short experimental monitoring timeline, and seed being washed away with rainfall or watering.

Experiment 2. *Eriogonum parvifolium*

Experiment two focused on the effect of co-inoculation, as all 20 seedlings were grown in soil containing AMF species. As of two months after transplant, the native PGPR inoculation produced no significant difference in longest stem length of *E. parvifolium* seedlings. Out of the 20 seedlings transplanted, 17 qualitatively are green and larger than they were when planted. Three are yellow and less likely to succeed. It is likely that if an effect were to be produced on the seedlings, more time would be required to monitor them. A study of a restoration of Mediterranean dunes in Spain showed that co-inoculating a native plant with a native microbial and fungal consortium had no significant effect on plant height or diameter after 1 year. After 5 years, the plants inoculated with the native fungal and

native PGPR consortium were almost twice as large as the other two treatments (Requena et al. 2000). As these seedlings have only been monitored and inoculated for 2 months, it is unlikely to see any difference at this point.

Conclusions

- The experimental timeline on both experiments was shortened due to unforeseen scheduling conflicts and the COVID-19 pandemic.
- In order to see long-term effect, experimental timelines of at least 6 months should be allocated for field experiments involving microbial inoculants.
- Field-based experiments involve a variety of uncontrollable variables that make isolating an effect difficult.
- Microbial inoculants have the potential to be a cost-effective tool in restoration and should be explored further under more controlled conditions.

References:

- Lyford, H. (2020). Developing Growth Promotion Strategies for *Cressa Truxillensis* and *Distichlis spicata* to Improve Success of Degraded Wetland Restoration in Los Angeles, CA. Unpublished senior theses.
- Barton, M. L., Medel, I. D., Johnston, K. K., & Whitcraft, C. R. (2016). Seed collection and germination strategies for common wetland and coastal sage scrub species in Southern California. *Bulletin, Southern California Academy of Sciences*, *115*(1), 41-71.
- DiVittorio, C. T., Corbin, J. D., & D'Antonio, C. M. (2007). Spatial and temporal patterns of seed dispersal: an important determinant of grassland invasion. *Ecological Applications*, *17*(2), 311-316.
- Krichen, K., Mariem, H. B., & Chaieb, M. (2014). Ecophysiological requirements on seed germination of a Mediterranean perennial grass (Stipa tenacissima L.) under controlled temperatures and water stress. *South African Journal of Botany*, *94*, 210-217.
- Bortey, H. M., Sadia, A. O., & Asibuo, J. Y. (2016). Influence of seed storage techniques on germinability and storability of cowpea (Vigna unguiculata (L) Walp). *Journal of Agricultural Science*, *8*(10), 241-248.
- Wang N, Jiao JY, Lei D, Chen Y, Wang DL. 2012. Effect of rainfall erosion: seedling damage and establishment problems. *Land Degradation & Development* **25**: 565–572. DOI:10.1002/ldr.2183.
- Ceccon, E., González, E. J., & Martorell, C. (2016). Is direct seeding a biologically viable strategy for restoring forest ecosystems? Evidences from a Meta-analysis. *Land Degradation & Development*, *27*(3), 511-520.
- Pedrini, S., Balestrazzi, A., Madsen, M. D., Bhalsing, K., Hardegree, S. P., Dixon, K. W., & Kildisheva, O. A. (2020). Seed enhancement: getting seeds restoration-ready. *Restoration Ecology*, *28*, S266-S275.
- CSU Stanislaus Valley Flora Propagation Center (2009). Species Profiles: *Frankenia salina*. http://esrp.csustan.edu/vfpc/profiles/FRSA.pdf
- Requena, N., Perez-Solis, E., Azcón-Aguilar, C., Jeffries, P., & Barea, J. M. (2001). Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. *Applied and environmental microbiology*, *67*(2), 495-498.
- Khan, M. A. (1991). Studies on germination of Cressa cretica L. seeds. *Pak. J. Weed Sci. Res*, *4*(1), 89-98.
- NOAA. National Weather Service Forecast Office Los Angeles/Oxnard. https://w2.weather.gov/climate/index.php?wfo=lox
- Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., ... & Jackson, J. B. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, *312*(5781), 1806-1809.
- Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological monographs*, *81*(2), 169-193.
- Zedler, J. B. (1996). Coastal mitigation in southern California: the need for a regional restoration strategy. *Ecological Applications*, *6*(1), 84-93.
- Ehrenfeld, J. G. (2000). Evaluating wetlands within an urban context. *Urban Ecosystems*, *4*(1), 69-85.

- Grossinger, R.M., E.D. Stein, K. Cayce, S. Dark, A.A. Whipple, and R.A. Askevold. 2010. "Historical Wetlands of the Southern California Coast: An Atlas of US Coast Survey T-sheets, 1851-1889." Prepared for the California State Coastal Conservancy. Technical Report #589.
- Johnston, K.K., Del Giudice-Tuttle, E., Medel, I.D., Piechowski, C.J., Cooper, D.S., Dorsey, J., and Anderson, S. 2012. "The Ballona Wetlands Ecological Reserve Baseline Assessment Program: Second Year Report." Santa Monica Bay Restoration Commission. Prepared for the California State Coastal Conservancy, Los Angeles, California.
- Novoa, A., González, L., Moravcová, L., & Pyšek, P. (2013). Constraints to native plant species establishment in coastal dune communities invaded by Carpobrotus edulis: implications for restoration. *Biological Conservation*, *164*, 1-9.
- Conser, C., & Connor, E. F. (2009). Assessing the residual effects of Carpobrotus edulis invasion, implications for restoration. *Biological invasions*, *11*(2), 349-358.
- Mack, R. N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological applications*, *10*(3), 689-710.
- Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences*, *111*(14), 5266-5270.
- de-Bashan, L. E., Hernandez, J. P., & Bashan, Y. (2012). The potential contribution of plant growth-promoting bacteria to reduce environmental degradation–A comprehensive evaluation. *Applied Soil Ecology*, *61*, 171-189.
- Asmelash, F., Bekele, T., & Birhane, E. (2016). The potential role of arbuscular mycorrhizal fungi in the restoration of degraded lands. *Frontiers in microbiology*, *7*, 1095.
- Carey, C. J., Blankinship, J. C., Eviner, V. T., Malmstrom, C. M., & Hart, S. C. (2017). Invasive plants decrease microbial capacity to nitrify and denitrify compared to native California grassland communities. *Biological invasions*, *19*(10), 2941-2957.
- California Department of Fish and Wildlife (2021). The Ballona Wetlands Restoration Project. https://wildlife.ca.gov/Regions/5/Ballona-EIR
- Johnston, K.K., I.D. Medel, R.C. Abbott, M.W. Grubbs, E. Del Giudice-Tuttle, C. Piechowski, M. Wong Yau, and J. Dorsey. 2015. "Ballona Wetlands Ecological Reserve: Comprehensive 5-Year Monitoring Report." Report prepared by The Bay Foundation for the California State Coastal Conservancy. 193 pp.
- Ramachandran, A., & Radhapriya, P. (2016). Restoration of degraded soil in the Nanmangalam reserve forest with native tree species: effect of indigenous plant growth-promoting bacteria. *The Scientific World Journal*, *2016*.
- Johnston, K., Grubbs, M., Enyart, C., Dagit, R., Cooper, D. (2019). Malibu Lagoon Restoration and Enhancement Project Final Comprehensive Monitoring Report (Year 6).