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# Do Fungal Symbionts of Salt Marsh Plants Affect Interspecies Competition?

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Do Fungal Symbionts of Salt Marsh Plants Affect  
Interspecies Competition?

by

Vanessa Robertson-Rojas

A thesis submitted in partial fulfillment of the  
requirements for the degree of

Master of Science  
in  
Environmental Science and Management

Thesis Committee:  
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2020

## Abstract

The effects of arbuscular mycorrhizal fungi (AMF) as salt marsh plant symbionts may have significant effects on landscape scale distribution patterns and plant-related ecosystem functions that are important to estuarine habitats. This work investigates the effects AMF have on *Phalaris arundinacea*, *Deschampsia cespitosa*, and *Juncus balticus* when grown in a common garden experiment. Plants were grown with and without AMF inoculation in both polyculture and monoculture communities and examined for a variety of response variables that represent different competition strategies. Factorial ANOVA analysis revealed a significant three-way interaction among fungal treatment type, community type, and species for chlorophyll fluorescence, which measures plant stress. Plant stress was higher in *J. balticus* without inoculation than in inoculated conspecifics, especially when grown in the polyculture community. Conversely, plant stress was slightly lower in the invasive grass, *P. arundinacea* without inoculation when grown in a community compared to the other combinations. Posthoc tests did not detect any major differences between treatments in *Deschampsia cespitosa* or in monocultures. Graphs of the other measures of response, ones aimed at determining differences in competition, looked very similar across treatments within a species and between polyculture and monoculture, and did not warrant statistical analysis of the effect of inoculation with AMF. This experiment indicates that fungal inoculation may offer stress amelioration through photosynthetic pathway II to *Juncus balticus* and may have the opposite effect of non-native *Phalaris arundinacea*. Given that AMF may have species-specific effects, commercial inoculants, which often do not specify the origin of their soil fungi, could be

advantageous to restoration plantings in salt marsh habitats when the native species gain more advantage than locally invasive ones. With increased value placed on salt marsh habitat restoration, these findings serve as an important first step towards determining which AMF-species combinations can benefit salt marsh restoration.

## **Dedication**

This text is dedicated to the female scientists that pioneered the access of professional work in this field for my generation. All of your sacrifices, hardships, and oppressions will not be wasted. Thank you for everything you have done.

## Acknowledgements

I would like to express my gratitude to Dr. Catherine de Rivera for her role as my advisor, committee chair, and as mentor beginning in 2012 during my undergraduate degree and continuously through my master's degree completion in 2020. You have provided both insight and pragmatism, but I value your dedicated approach to collaborative and thoughtful science most. Working alongside you has changed my life and understandings about learning, teaching, and the contemplation of the natural world. Thank you also to Dr. Sarah Eppley and Dr. Jennifer Morse, my committee members who have provided deeper insight into ecological systems through their respective areas of expertise. Each of you were exceptionally enjoyable to work with, and I thank you for your critique and support. Thank you to Dr. Daniel Ballhorn, Linda Taylor, the Rae Sellings Seedbank, the de Rivera Lab, Rayna Koberstein, Olamide Alo, Elissa Connolly-Randazzo, Sara Volk, Robyn Dove for all your thoughts and helping hands. Each person or group mentioned here has generously offered their time, thoughtful pragmatism, and expertise in their respective fields that allowed me to complete this work to a profoundly improved degree. I am also grateful for the support of my family and friends who have shown unconditional support in the kindest ways. Lastly, I am indebted to the salt marsh plants who have brought the deepest joys to this work, thank you for all that you are.

## Table of Contents

Abstract.....	i
Dedication.....	iii
Acknowledgements.....	iv
List of Tables .....	vi
List of Figures.....	vii
List of Abbriviations.....	vii
Chapter 1 Study Introduction.....	1
Chapter 2 Experiment Introduction.....	6
Chapter 2 Experiment Methods.....	13
Chapter 2 Experiment Results.....	26
Chapter 2 Experiment Discussion.....	34
Chapter 3 Study Conclusions.....	42
References.....	45
Appendix A. Additional Figures.....	52

## List of Tables

Table 1: Greenhouse Conditions.....	18
Table 2: Variables and Associated UAST Strategy.....	18
Table 3: ANOVA Model.....	27
Table 4: Tukey HSD Summary.....	27
Table 5: Data Summary of All Variables.....	29
Table 6: Eigen Analysis Summary.....	32
Table 7: Root Colonization by AMF Summary.....	33
Table 8: Shapiro-Wilks Results.....	57



## List of Figures

Figures 1: The Study System.....	12
Figures 2: Experimental Design.....	18
Figures 3: Fungal Slide Images.....	22
Figures 4: Histograms of All Chlorophyll Fluorescence Treatments.....	24
Figures 5: Chlorophyll Fluorescence Across All Treatments.....	28
Figures 6: Correlation Matrix.....	31
Figures 7: QQ Plots of All Measured Variables.....	52
Figures 8: Histograms of All Measured Variables.....	55
Figures 9: Biomass Across All Treatments.....	57
Figures 10: PCA Analysis of All Measured Variables.....	58
Figures 11: Broken Stick Model.....	59

**List of Abbreviations**

AMF	Arbuscular Mycorrhizal Fungi
C°	Degrees Celsius
C3	In reference to plant fixation method, Carbon fixation pathway 3
(Fv/Fm)	Maximal possible fluorescence value over the measured fluorescence value
h	Hour(s)
IPCC	International Panel for Climate Change
N	Number of samples
PCA	Principle Component Analysis
PSII	Photosynthetic pathway two
PNW	Pacific Northwest
RCI	Relative Competition Index
SD	Standard Deviation
UAST	Universal Adaptive Strategy Theory

## Chapter 1: Study Introduction

The ecosystems services that salt marshes provide on the west coast in concurrence with their limited range compounds the need for understanding underlying function and habitat conservation. Salt marsh conservation should entail both the maintenance or restoration of physical area *and* the integrity of the complex systems seen in hydrological, vegetative, and pedological processes (Heider and Sinks 2018). Maintaining the integrity of these processes would ensure continued ecological services procured from these habitats. Within North America salt marsh habitat area is less expansive in the Pacific Northwest (PNW), but these rarer habitats remain functionally important in a variety of ways by providing rearing habitat, storm buffering, nutrient cycling, and carbon storage (Zedler and Kercher 2005). Salt marsh flora and fauna are uniquely productive in the PNW given their smaller spatial distribution (Brown and Ozretich 2009, Eilers 1987, Ewing 1986), which ultimately increases the value placed on the remaining areas of this habitat that occurs solely within estuaries.

The imminent rise of global sea levels, although variable across coastal topographies, will compound the need for thoughtful land management to secure the continued existence of salt marshes and their associated benefits. Rising ocean elevations will flood current marshes that occur directly above the intertidal area, and their persistence as coastal habitat will depend on whether or not salt marshes will accrete vertical elevation through particle settling and vegetative entrapment of seaborne soil particles and organic particles, or lateral migration to upland areas via seed transport or vegetative expansion (Borchert et al. 2018). Coastal areas surrounded by roads, levees,

converted lands (such as agriculture or residential land use) or other anthropogenic structures will be constricted by lateral migration, and thus will only persist if vertical accretion matches sea level rise (Cosby et al. 2016, Raposa et al. 2013). This limitation of upland migration trajectories within PNW coastal marshes will exacerbate the fragility of these habitats' ability to persist under climate change conditions, further landscape conversion and development, and natural evolution over time, all of which can alter the functionality and persistence of salt marshes.

Both scenarios that describe how salt marsh elevation could rise to keep pace with SLR depend on the performance of vegetative processes that have historically occurred (Raposa et al. 2013). These processes occur across a small range of elevation that typically span a relatively wide spatial area due to the flat topography that occurs over the majority of the marsh platform, with exceptions around small incised channel systems, sandy dropout locations around points of slowed water movement, and where debris is deposited within the marsh (Seliskar and Gallagher 1983). Despite occurring within a narrow elevational band, salt marshes contain several distinct bands of vegetative zones that form in response to the level of flooding that differs significantly within mere inches of elevational gain across the marsh platform. The marsh area that occurs the lowest in the elevational profile typically experiences the most tidal action, dropout and deposition of sand particles, the highest saline concentration of brackish water, and the most exchange of debris transport (Seliskar and Gallagher 1983). This area is submerged during diurnal flooding, contains un-oxygenated soils (anoxia), remains sparsely vegetated by algae/seaweeds and is commonly referred to as the mudflat. Above this area is the low

marsh, which is also flooded daily but receives moderate tidal action that is often seasonally dependent. This marsh area also contains anoxic soils and is dominated by unique vascular plants that tolerate these conditions through a variety of stress tolerance adaptations. Species of *Salicornia* in both the PNW and throughout the world are common and characteristic of low marsh vegetation that dominate spatial area in 5-30 ppt salinity. These plants can actively prevent salt entrance into their roots, sequester intracellular salts, and/or secrete salts through glands (Katschnig et al. 2013). The high marsh persists along the uppermost boundary of tidal influence and receives the least amount of flooding, tidal action, and slight oxygenation of soils during the summer season (Bertness and Ellison 1987). Due to the slight relief of flooding, salinity, and tidal action stress the high marsh plant community is more productive with vegetative material both above ground and below. It is important to note that throughout the marsh oxygenation can occur locally when burrows are created by soil infauna, crustaceans, or other animals. This occurrence creates a slightly ameliorated condition for both flora and faunal nearby and leads to increased metabolic production.

Within these zones plant diversity remains low because few plants are adapted to survive in stressful marsh conditions, but vegetative production can be prolific amongst plants that do survive in the marsh because they are released from intense competition pressure. Each zone may contain only a few dominant plant species that proliferate under this release from competition, and the remaining interactions amongst these plants are known to benefit from inter-taxa facilitation (Bertness 1994). Within a zone, plants often form bands of vegetative cover within their narrow elevational bands of dominance. It can

be posited that stress adaptations and strategies for persisting in the salt marsh determine vegetative success, and therefor determine the zonation patterns of a given marsh (Bertness and Ellison 1987, Veldhuis et al. 2019).

Fungi that persist in the soil often form relationships with the flora and fauna of the salt marsh and can impact plant communities in a variety of ways (Peay et al. 2008). Their involvement with plant physiological processes can alter plant competition (Burke et al. 2002), facilitation, reproduction, and the community structure within the marsh. Arbuscular mycorrhizal fungi (AMF) alter the plant interactions and life processes by forming connections with plant roots below ground and altering nutrient uptake and stress mitigation centralized around these below ground interfaces (Hoeksema et al. 2010). The composition and abundance of AMF communities can vary across space and time within the salt marsh and are known to respond to inundation levels, salinity (Aggarwal et al. 2012), and nutrient availability or limitation within the soil profile (Carvalho and Caçador, 2001, Carvalho 2003). While it is known that even minimal presence of fungi can alter plant interactions in the marsh (Wang et al. 2016), specific competition or facilitation interactions contextual to the salt marsh habitat require further investigation.

In particular, the degree of effect that AMF can have on plant competition within PNW salt marshes that are nutrient limited, frequently inundated, and incur year-round salinity exposure remains unstudied. This work investigates three strategies plants may express in communities under these conditions as proposed by Grimes (1977). Grimes proposed the Universal Adaptive Strategy Theory (UAST) which describes how, in the presence of physiological stress and disturbance, three adaptive strategies emerge in

plants. In low disturbance/low physiological stress situations, competition becomes imperative for persistence on the landscape. Plants that proliferate under low disturbance but high physiological stress employ stress tolerance to persist. Lastly, high disturbance and low stress results in the proliferation of ruderal or pioneer life strategies to persist on the landscape. High disturbance paired with high physiological stress prevents any plant recovery and is not considered in this framework. It is important to note that all three of these strategies are proposed as extremes, and most plants employ one to three of these strategies in variable proportions, and that their allocation balance may differ by plant life stage and habitat seral stage (Grimes 1977). Lastly, it is important to note that this framework differs from the examination of how plants interact more broadly, such as when considering competition, facilitation, and mutualism more broadly in that it specifically explores the type of competition taking place between individual plants and/or broader plant groupings. This work poses that where interspecies competition occurs in the salt marsh, AMF may have different impacts on plant competition as measured by competition, stress tolerance, and ruderal strategies as described by the UAST framework.

## Chapter 2: Experiment Introduction

Both plants and soil microbes are crucial components of salt marsh habitats that respectively perform primary production and decomposition in marshes and connect the biotic and abiotic parts of the marsh system (Odum 1988). Primary production and decomposition processes support a wide variety of uniquely valuable habitat functions in estuarine habitats but can vary across elevation gradients (Seliskar and Gallagher 1983, Ewing 1986, Bernard et al. 1988). These functions include juvenile rearing habitat for fish (Gray et al. 2002) and birds (Weller 1994), while sequestering carbon, buffering storms, mitigating floods, and nutrient retention (Costanza et al. 1997, Nelson and Zavaleta 2012). They persist sparsely across the landscape in the PNW due to a narrow coastal shelf (Torio et al. 2013) and decades of draining and diking salt marsh habitats. The International Panel for Climate Change (IPCC) highlights the relative importance of these heterogeneous ecosystems, and how they are disproportionately impactful despite their small spatial areas (Weis et al. 2016), which engenders great interest in conservation and restoration of marsh habitat. A small variety of stress tolerant plants, sometimes with the help of mycorrhizal fungi, compete as they serve as the foundation of this stressful yet productive and important ecosystem.

Salt marshes present a uniquely stressful environment for plants to persist due to exposure to estuarine water and soil, water-logged, anoxic soils, nutrient limitation, and daily changes in water levels within an estuary, all of which contribute to plant stress (Bertness and Hacker 1994). Specialized physiological adaptations allow a limited assemblage of plants to persist in these areas (Bertness and Hacker 1987, Canepuccia et al.



2013). Some salt marsh plants have mycorrhizal associations with soil fungi that result in mutualism and/or parasitism (McHugh et al. 2004, Zhang et al. 2014). Zhang et al. found that these relationships have been shown to impact plant competition differently in a removal experiment including 5 marshland species, and that results were species specific and based on dominance/sub-dominance relationships between the study species.

Mycorrhizal fungi benefit their host plants with nutrient acquisition in exchange for carbon while a nearby non-symbiotic plant may struggle to acquire nutrients (Lin et al. 2015). In addition, AMF can benefit a subdominant host in a dominant/subdominant plant relationship where the fungal symbiont parasitizes a dominant plant by acquiring carbon from it and supplying those metabolic units to a neighboring subdominant plant (Lin et al. 2015). AMF effects on plant growth can vary in response to environmental factors such as salinity or anoxic soils (Fraser et al. 2005, Heeksema et al. 2010).

Mycorrhizal effects on plant nutrient acquisition and allocation is an emerging field of ecophysiology that has explored pathways from soil to internal cellular structures, macronutrient ratios and volumes, allocation of nutrients to various plant structures, and nutrient effects on reproductive success. Cumulatively, these various fungal symbiont effects on plants may alter competition strategy and success in a myriad of ways. Grimes' Universal Adaptive Strategy Theory (UAST) emphasizes the use of specific adaptive strategies by plants to persist on the landscape (Grime 1979). These strategies include ruderal, stress tolerance, and competition and are used here as a framework for examining competition in the high salt marsh. Ruderal plants are particularly resilient to disturbance such as browsing, burning, or other events that damage the plant, while stress tolerant

plants are able to persist in the presence of photosynthetically restrictive phenomena such as drought, shade, or high salt concentrations. Lastly, the UAST competitor strategy (not to be used interchangeably with more general primary competition strategies) occurs in plants that most effectively exploit available resources. This last UAST strategy can occur under stressful conditions or in the presence of disturbance and is specifically observed when plants exploit resources exceptionally well under any conditions (Grime 1979, Grime 1987).

Banded distributions of plant species in marshes occur when plant functional types respond to environmental conditions (Lin 2015) that are present at different elevational zones (Bertness and Sally 1994). These bands often differentiate between the low and high marsh, where conditions can differ due to elevational differences. For example, the high marsh during spring freshet in the PNW presents lower stress pressure than the low salt marsh due to rainfall and subsequent reduction in the salinity of surface water and soil pore water. Thus, the high marsh becomes a more hospitable place for plants to establish, and many plants have specially adapted to compete well for resources and dominate this elevational band (Bertness & Hacker 1987). These more benign circumstances increase the instances of interspecies competition (Grimes 1979), of which the most dominant plants are of interest due to their increased biological presence and impacts. The outcomes of interspecies competition in the high marshes, and therefore high marsh diversity and function, can be altered by plant interactions with fungal symbionts (Burke et al. 2002, Fraser and Feinstein 2005, Hoeksema et al. 2010). We do not know of any research currently that has observed the effect of mycorrhizal fungi on high marsh plant species

and their competition interactions in the current study region, despite the known value of salt marsh habitat functions in the PNW. Current practices in plant nurseries that use commercial inoculants to improve belowground plant health may be introducing locally non-native fungal communities to highly managed landscapes and habitat restoration sites alike. Halophytic and hydrophytic graminoids (grasses, sedges, and rushes) occupy much of the herbaceous layer in the high marsh, and thus are of great concern when planning for aerial cover of native plant species (Keammerer et al 2001, Lavergne et al. 2004).

Add paragraph about species within the marsh, review some salmon river lit, introduce plant community, briefly describe three graminoids examined here

#### Research Questions and Hypotheses:

We conducted an experiment examining the effect of AMF on the adaptive strategies of salt marsh plants, especially its role on competition, using three common salt marsh species, *Phalaris arundinacea*, *Deschampsia cespitosa* and *Juncus balticus*. The three species included in this experiment dominate patches they occur in within the marsh, as documented by several monitoring studies at different timelines in addition to pre-project data collected for this work (Frenkel et al. 1993, Gray et al. 2002). The overall plant community's diversity is somewhat limited by the environmental stressors described previously, but this work may offer insight to the boundaries where patches and bands of a singular species may meet another. A competition study would specifically investigate the way these three species may interact at the patch or boundary in the presence of AMF, which ultimately may impact the distribution of each species within the marsh. Thus, it is

important to consider more than two plants in order to investigate a community, with or without fungi present, when grown with the same species or in a mixed community. We predict that AMF will impact these plants differently, due to their varying life histories and competition strategies, and will allow each plant to express one or multiple strategies differently when grown in the presence or absence of AMF.

We hypothesized that each species would respond differently to AMF inoculation when grown in a community of three plants as compared to being grown in a monoculture of three individuals of the same species. It is predicted that *Phalaris arundinacea* will have a significant response to inoculation. Up to 90% of roots were inoculated by AMF in freshwater wetland habitats in a study by Fraser and Feinstein (2005) that summarized the inoculation range in freshwater wetlands (such documentation in saline systems is currently unavailable). High levels of inoculation suggest that this invasive plant benefits from efficient nutrient acquisition across many different habitat ranges, including in the PNW (Annen et al. 2008, Barnes 1999). We also predicted that *Deschampsia cespitosa*, a dominant native to the PNW that is known to associate with AMF (Ingham and Wilson 1999, Olsson et al. 2008, Seliskar 2019) would also benefit from a fungal symbiont. *Juncus balticus*, another native plant that occurs commonly in freshwater systems, has received minimal study with regards to association with AMF; however, showed comparatively low fungal association of 15-18%, but received substantial growth benefits from this relationship (Tadych and Blaszkowski 2014). Thus, it was predicted *J. balticus* would be positively affected by AMF inoculation if colonized. This slower growing plant does not compete well for space in short periods of time and given the shorter duration of

this study compared to its longer lifespan, *J. balticus* was predicted to have low colonization but marginal benefits from AMF. Cumulatively, we predict that these differing effects of fungi on unique plant species will all be more demonstrable in polyculture treatments where interspecies competition for space and resources will occur more intensely. Additionally, we predicted that each of the three species examined will demonstrate different levels of AMF inoculation/colonization in their roots after inoculation treatments in the greenhouse, based on documented cases of AMF colonization seen in multiple studies (Ingham and Wilson 1999, Olsson et al. 2008, Seliskar 2019, Fraser and Feinstein 2005). Each plant also demonstrates different competition strategies when documented in freshwater systems which will be subsequently described (Grimes, 1979).

To investigate the listed research questions a common garden experiment was conducted in a research greenhouse. Three plants found commonly in an Oregon salt marsh were grown in polycultures and in conditions replicating PNW coastal marsh conditions with and without fungal symbionts. Each of the three species was grown in a corresponding monoculture which functioned as a control for comparison to the polyculture plant responses. Each plant in each treatment was measured for various responses to the fungal treatment to detect differences in plant competition in the presence of AMF.

**Figure 1. The Study System.** A conceptual model of the estuarine system components as relevant to the study design. Plants can be seen growing across a shallow elevational gradient and forming patches and zones of monoculture stands. Below ground plant roots can be seen to grow among a thick organic layer (seen in dark black) that contain higher salt concentrations, anoxic soil, and fungal symbionts around the plant root. Each plant has different adaptive traits and strategies seen in intra and inter-species competition, as well as different relationships with fungal symbionts found in the marsh soil.

## Chapter 2: Experiment Methods

### Field Site

The marsh in the Salmon River Estuary located in Otis, Oregon (N 45.034157, E - 123.982552) was used to inform the greenhouse conditions. Environmental conditions including pH, temperature, and salinity were documented in February of 2019, and are summarized in **Table 1**. Thirty 1x1 m plots with 100 percent cover of each species were located within the high marsh. A soil core was taken from each plot at the base of 5 different plants within each plot to obtain the roots of each plant. A slurry was created from soil from each core mixed with deionized water, using 50 g of soil from each core (Sparks 2009, p.417-422) and a separate 30 g of soil to measure in site *in situ* pH (Sparks 2009, p.485-489).

### Study Species

The study species examined here include Turtle hair grass (*Deschampsia cespitosa* (L.) Beauv), Baltic rush (*Juncus balticus*), and Reed canary grass (*Phalaris arundinacea*). All three species considered occur in the Salmon River salt marshes, utilize C3 carbon assimilation pathways, are perennial, and dominate patches of open area at various elevations above mean high water. *P. arundinacea* is found in the uppermost regions of the marsh, atop a vegetative mat that does not experience frequent inundation from brackish water, while *D. cespitosa* is commonly found along marsh drainage channels that drain upland area, and thus contain slightly less saline water (1-2.5 ppt, unpublished field

data). *Juncus balticus* occurs in patches throughout the low and high marsh that are occasionally mixed with smaller patches of graminoids and forbs.

*Deschampsia cespitosa* is globally distributed bunchgrass that is found in freshwater wetlands as well as salt marshes along the west coast, across North America, and on other continents containing temperate climates and wetland habitats (Keammerer 2011, Grimes 2007 p.220). Most study on this species has occurred in freshwater systems and indicates that *D. cespitosa* uses all three competition strategies, but most often employs stress tolerance strategies to persist and even dominate in landscapes. Stress tolerance may be a particularly successful strategy to this plant that can be particularly long lived, with an individual bunch aging >30 years (Grimes 2007 p.220). In freshwater wetlands, root inoculation by AMF can range widely (7-54%), which is likely related to the expansive habitat range of the grass (Ingham and Wilson 1999, Olsson et al. 2008, Seliskar 2019). AMF colonization of *D. cespitosa* and impacts on competition success have not yet been studied.

*Juncus balticus* is a globally persistent bunch forming rush seen across variable habitats that include saline influenced and freshwater wetland habitats (Hurd et al. 1992) and is native to the PNW. In a study replicating coastal dune habitats, AMF association with *Juncus spp.* was documented, and suggested that increased root colonization occurs as soil flooding is reduced (Tadych and Blaszkowski 2014). A field study conducted across broad genera of coastal plants demonstrated that of *Juncus spp.* had a low relative competition index (RCI) when compared to 40 other species, including *P. arundinacea* in Nova Scotia, Quebec, and Ontario, Canada (Gaudet and Keddy 1995). While this species



of *Juncus*'s competition strategy has not yet been documented, a field study focusing on nitrogen uptake between *Typha spp.* and hybrids and *J. balticus* showed a low N uptake by *J. balticus*, indicating that it may employ ruderal or stress tolerance strategies instead of competition to establish and persist on the landscape (Larkin et al. 2012). There are no additional works specifically focusing on *J. balticus* ecology in salt marshes, or in relation to AMF's ecological impacts.

*Phalaris arundinacea*, a non-native mat forming grass, has become dominant in many freshwater wetlands worldwide due to its competition success (Grimes 2007 p.430). Recent literature has documented this species expansion into estuarine habitats along upper marsh fringes (Annen et al. 2008, Barnes 1999). *Phalaris arundinacea* is known to associate with AMF in variable freshwater habitat types and have shown 3-90% root colonization, 50% on average, across different habitat types (Fraser and Feinstein 2005, Bauer et al. 2003). In the native European range of *P. arundinacea*, this plant is known to use the competition strategy through its strong rhizomatic reproduction that creates large clonal patches to persist in wetland and wetland transitional habitats, which is likely its competition strategy in invaded ranges (Martina and von Ende 2013, Grimes 2007 p.430). There is currently no work pertaining to *P. arundinacea* competition specifically in the presence of AMF in salt marshes.

### Greenhouse Experiment

A greenhouse experiment was conducted to examine the effect of AMF inoculation on three high marsh species, grown both individually and together in a community.

Monocultures of *D. cespitosa*, *J. balticus*, and *P. arundinacea* and corresponding polycultures of all three species each had an inoculated and a sterile treatment (Figure 2). Each of the resulting eight treatment groups initially had 30 replicate pots (N=240 pots) and were used in a 3-factor fully crossed design (species, culture, fungal inoculant factors). The 240 pots were spread out evenly across four 1.2x20m benches in a random block design. Environmental conditions in the greenhouse included controlled temperature at a range of 18-30° C, humidity at >80%, with natural light subsidized by artificial light when needed for 16 h daily throughout the entire greenhouse room.

Plants were grown for a 70-day period with most conditions replicating reference marsh conditions (Table 1). Plants were germinated under spring/early summer lighting conditions (18 h lights on, 6 h lights off), and between 24 and 29 °C), weighed, and seedlings of the same weight (within 0.01 g) were transplanted into each pot. Plants were placed equidistant from each other in a triangle shape, 3 cm apart. An 80:20 ratio of sterile sand to vermiculite was used as a growth medium. All sand was washed with a 10:1 water to bleach ratio, rinsed with a 10% Benomyl fungicide solution and allowed to rest for 36 hours before transplanting. Each pot was filled with the growth medium to within 2.5-3.5 cm of the top of the pot then placed in a 9.46L reservoir. The water in the reservoir was filled as needed to maintain a water level within 7 cm of the top of the substrate profile. A layer of landscape fabric was placed at the bottom of each pot to prevent sand from draining out of each pot, while maintaining connection with the water reservoir around the pot after Burke et al. (2002). With the goal of replicating reference conditions of limited nutrients, plants were not fertilized. Any nutrients present came from the initial

sand/vermiculite mix or the inoculation treatments that occurred every two weeks (see below). Salinity, pH, and nutrient levels were monitored throughout to maintain similar conditions to the reference marsh.

Soil was collected from the reference marsh and applied to all treatments (sterilized or left inoculated) in order to introduce AMF from the restored marsh to the appropriate treatments. Seventy grams of soil from the reference marsh was collected 48 hours prior to soil inoculation treatments via collection of soil cores within monocultures of plant species. This soil was collected by coring at the base (within 5cm of each plant base) of all three species in the marsh, each with 30 replicates that were combined, homogenized, and mixed with a 1:3 soil: water ratio to create a slurry. Cores were collected within the top 10cm of the marsh soil profile to capture the most active area in the soil profile for AMF. The total volume of soil was homogenized, then divided in half. One half was sterilized with 10% Benomyl fungicide solution to reduce unintentional AMF colonization, and then applied to non-fungal treatment pots, 70mL of slurry was applied to each pot. The second half of the soil slurry was applied directly to the fungal treatment plots as in-tact, unsterilized soil to provide inoculation exposure to the fungal treatment pots which also received 70mL of slurry in each pot. The application of 70mL of an inoculant or a sterilized soil slurry was replicated every 14 days after initial transplanting.



**Table 2. Variables and Associated UAST Strategy.** The table below lists all plant variables measured and their associated adaptive strategy as theorized by Grimes (1979).

<i>Variable</i>	<i>Associated UAST Strategy</i>
Canopy Cover	Competition (horizontal space)
Plant Height	Competition (vertical space)
Number of Leaves	Competition (vertical and horizontal space)
Number of Dead Leaves	Ruderal (ground mat material)
Chlorophyll Fluorescence	Stress (photosynthetic production)
Relative Competition Intensity	All (biomass between communities)
Root Weight	All (biomass allocation to roots)
Shoot weight	All (biomass allocation to shoots)
Total Biomass	All (biomass allocation of each plant)
Root: Shoot Ratio	All (biomass allocation between above and below ground material)

Plant height, canopy cover, and number of leaves were measured every 17 days during the experiment. A photograph of each pot was taken during each sampling period. Environmental conditions were monitored continuously, and plants were watered as needed to maintain pot reservoir fullness throughout the experiment. The experiment ended at the pre-determined cut-off, when the average canopy cover exceeded 75% and roots began to grow out of the bottom of pots, which occurred at 70 days.

Additional variables such as number of dead leaves, chlorophyll fluorescence (Equation 1), biomass, root weight, shoot weight, root/shoot ratios, and an RCI Index (relative competition intensity) were measured or calculated at the completion of the study due to the destructive or impractical nature of their respective sampling techniques during the experiment.

Equation 1: *Chlorophyll Fluorescence* =  $F_v/F_m$

Where  $F_v$  = variable fluorescence measured, and  $F_m$  = maximum fluorescence yield. This ratio results in a unitless ratio that is always lower than 1.00, with healthy plants averaging 0.83 (Cendrero-Mateo et al. 2015)

Each pot's plants then were washed free of growth medium, and each plant's root mass was separated. The fresh weight of roots and shoots of each plant were then weighed separately. A root to shoot ratio was calculated (root: shoot). The relative competition intensity (RCI) was calculated to create a relative competition index comparing biomass between communities.

(Equation 2, after Weigelt and Jolliffe 2003) seen below.

Equation 2:  $RCI = [(P_{mono} - P_{poly}) / P_{mono}] * 100$

Where  $P$  = Population metric (here seen as Final Biomass Weight (g)),

*mono* = Monoculture populations, and

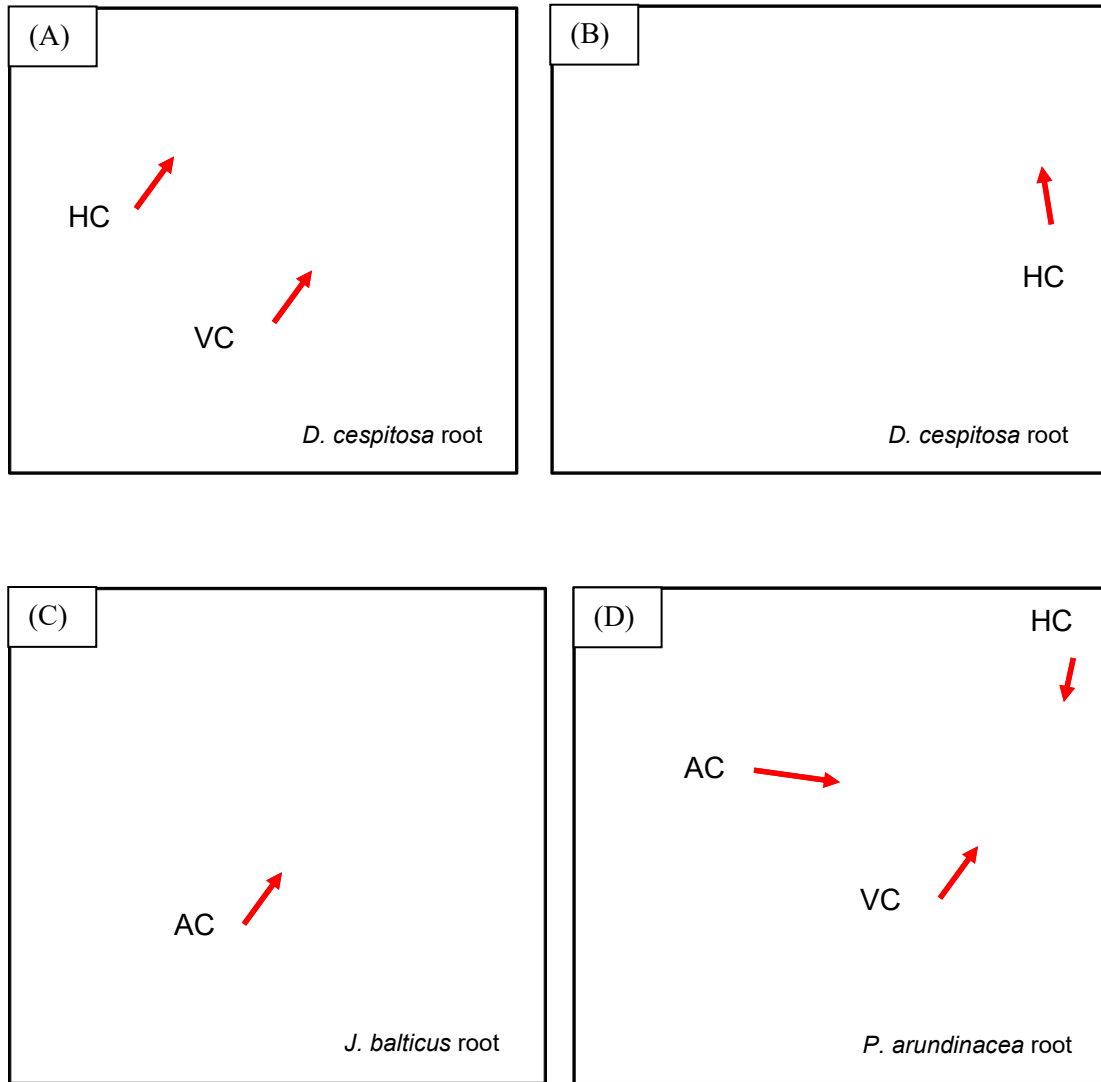
*poly* = Polyculture populations.

A subset of pot soils was sampled for salinity and pH. After washing and weighing each plant, five pots were selected from each treatment using a random number generator for root colonization by AMF analysis. Final measurements of plant height, canopy cover, number of live, number of dead leaves, final weight, and plant stress as seen by

chlorophyll fluorescence ( $F_v/F_m$ ) were recorded. See Table 2 for UAST association of all measured variables.

#### Determination of Percent Root Colonization by AMF

To confirm the inoculation of targeted +AMF treatments, a subset of plant roots from all treatments were assessed for percent colonization by AMF after McGonigle (1989). Plant roots from treatments given 10% Benomyl fungicide were also assessed in this way to observe any colonization of AMF structures. Root samples were separated from their respective aboveground biomass and washed clean of the growth substrate. The upper 20cm of the root system (after Carvalho and Caçador 2001), was separated from the root mass and placed in a labelled histology cassette. Roots were cleared in a 10% KOH solution for 15-20 minutes using a hotplate. Samples were washed free of KOH using Deionized (DI) water three times, soaked in a Trypan Blue Lacto-Glycerin Solution for 60 minutes, and then soaked in a 2% HCl solution for 30 minutes. The resulting stained root samples were then washed and stored by refrigeration at 0-1.6°C in Lactoglycerol until used for slide creation. The stained roots were cut into 5cm lengths and were laid on a glass slide (each slide contained 5 rows of 5cm lengths). Slides were viewed at 200x magnification over an 89-cell grid. All 89-cells in each slide grid was observed for presence of the fungal structures shown below. Arbuscules, vesicles, and/or hyphae, examples of which can be seen below in Figure 3, were counted individually per grid cell to determine the proportion of each root in each cell colonized by different AMF structures (McGonigle 1989).



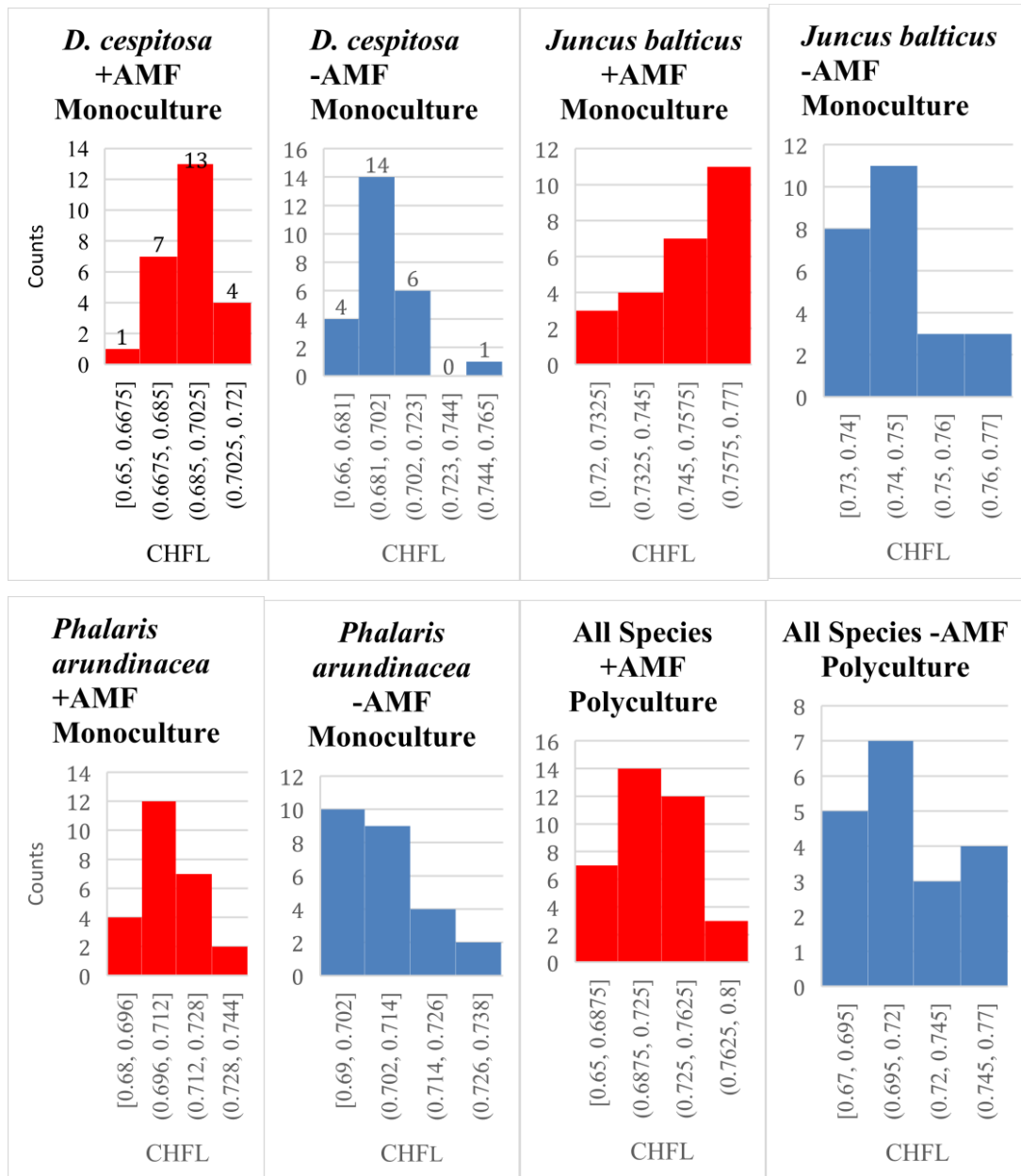
**Figure 3. Fungal Slides.** Different roots with AMF structures are shown in digital imagery taken through a dissecting microscope at 40X magnification. AC= Arbuscular Colonization, VC =Vesicular Colonization, and HC =Hyphal Colonization. (A) *Deschampsia cespitosa* root with HC and VC, (B) *Deschampsia cespitosa* root with HC and unknown structure, (C) *Phalaris arundinacea* root with AC, HC, and VC, and (D) *Juncus balticus* with AC in the bottom left.

#### Data Analysis

Data were analyzed in R Studio Version 1.2.1335. Records with missing values (NAs) were removed. Pots with plant mortality were removed from the study, which



reduced replication from 30 to 25. Variables that required post-experiment calculation included root: shoot ratio, RCI, and total biomass. The values for plants grown in monoculture were averaged per pot, creating a 1:1 comparison between each species in monoculture and polyculture. The data distribution of quartiles was summarized with QQ Plots, distributions were visualized with histograms, and a correlation matrix was created to examine the nature of collinearity between all variables (Figure 4). Shapiro-Wilks and Bartlett tests for normal distribution of data and equal variance (respectively) were performed on all variables to meet ANOVA test assumptions, using  $\alpha = 0.05$  for all tests.



**Figure 4. Histograms of All Treatments.** Histograms of each treatment as seen by chlorophyll fluorescence (CHFL), which shows somewhat of a normal data distribution with exceptions in the *P. arundinacea* AMF Monoculture, *J. balticus* +AMF Monoculture, and *J. balticus* -AMF Monoculture treatments, which show a right skew distribution. Log, square root, and arcsine transformations were attempted to correct this skewness but did not yield results that pass test for normality. The last plot, QQ plots of chlorophyll fluorescence, shows that the data matches the theoretical model to a reasonable extent.

A principal component analysis (PCA) that uses orthogonal transformations was performed to identify potentially correlated variables to reduce data dimensionality, and to develop models that best explain the variance in the dataset. A PCA was performed (Appendix 1) and included a broken stick analysis to determine which principal components (PC's), and the variables they contained, to consider for statistical testing. The broken stick model showed that only PC1 and PC2 should be considered. An eigen analysis was conducted to observe which variables accounted for the most variance within PC's, which would suggest use of specific, singular variables for ANOVA analysis instead of analyzing all variables together in a MANOVA analysis. Additionally, histograms and boxplot representations of these variables were considered, from which only chlorophyll fluorescence, root weight, and canopy cover were considered for ANOVA analysis. Variables that were not weighted heavily (Table 6) in PC1 or PC2 were not included in ANOVA analysis. Chlorophyll fluorescence contained the most variance within PC1, while root weight and canopy both showed negative relationships.

PCA showed that chlorophyll fluorescence explained a larger amount of variance across all response variables, and thus a 3-way ANOVA test was conducted on this singular variable. A full ANOVA model was created that included community type, species identity, and +AMF or -AMF treatment type and all the interactions. A post-hoc Tukey HSD test was conducted to examine pairwise comparisons between all grouping variables created in this experiment. Bar charts, which demonstrate highly similar means among the treatments for the remaining variables, were made and can be seen in Appendix A.

## Chapter 2: Experiment Results

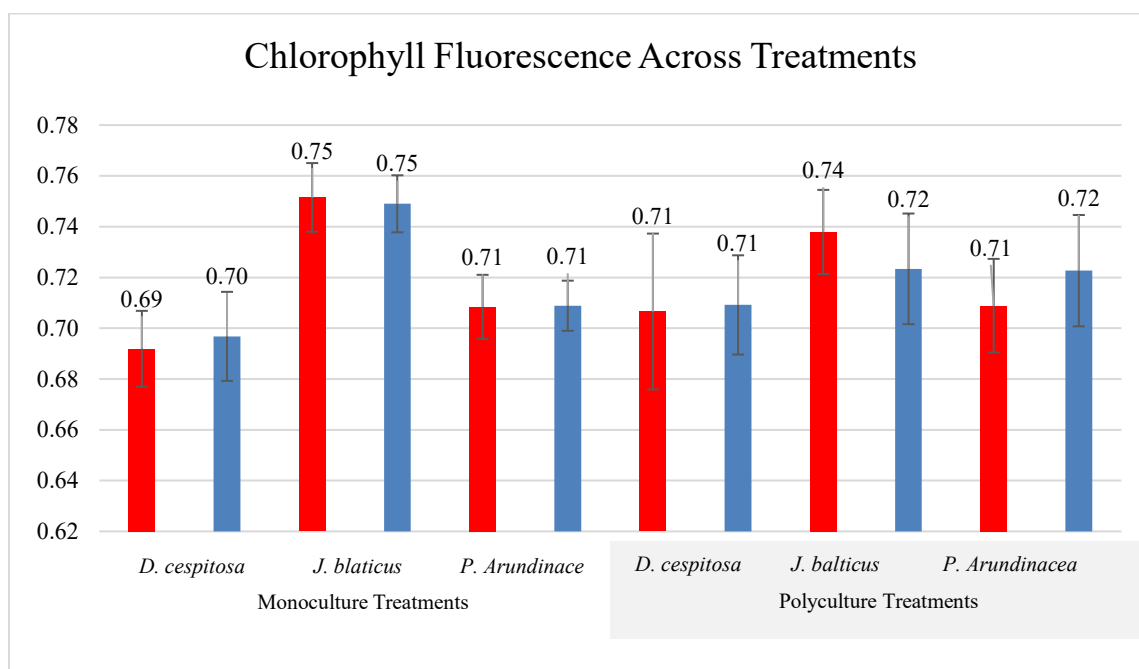
Chlorophyll fluorescence was significantly affected by AMF treatment (with or without mycorrhizal fungi), plant species (*D. cespitosa*, *J. balticus*, or *P. arundinacea*), and community (monoculture or polyculture), suggesting AMF has species-specific effects on chlorophyll fluorescence, or plant stress, across culture settings (Table 3, p-value of 3-way interaction: 0.03). Chlorophyll fluorescence was shown to have differences between inoculation treatments in *J. balticus* in the polyculture communities (Table 4 and Figure 4). Indeed, Figure 4 shows that in polyculture communities *J. balticus* had a higher chlorophyll fluorescence reading (meaning lower plant stress) with AMF than without, and *P. arundinacea* exhibited the inverse, and both species exhibited a greater difference between the AMF treatments in polyculture than in monoculture. These interactions suggest that *J. balticus* experienced the most stress without AMF compared to with AMF when growing among other plant species; in contrast, *P. arundinacea* may have experienced the most stress in polyculture with AMF. The effect of AMF differed among species so these results were only apparent when examining the interactions among the three factors and the AMF treatment by species interactions (Table 3,  $p = 0.018$  for species \* AMF treatment).

**Table 3. ANOVA Model.** Three-way ANOVA full model for chlorophyll fluorescence where  $p \leq 0.05 = *$ ,  $p \leq 0.01 = **$ ,  $p \leq 0.001 = ***$ , and  $p \leq 0.0001 = ****$ . The test included all three grouping variables and shows significant three-way interactions where  $p=0.03^*$ .

<i>ANOVA Model</i>	<i>Df</i>	<i>Sum Sq</i>	<i>Mean Sq</i>	<i>F-value</i>	<i>Pr(&gt;F)</i>
<b>AMF Treatment</b>	1	0.00001	0.000011	0.063	0.80
<b>Species</b>	2	0.02716	0.013580	78.924	< 2e-16 ***
<b>Community</b>	1	0.00001	0.000010	0.057	0.81
<b>Treatment x Species</b>	2	0.00141	0.000703	4.086	0.018 *
<b>Treatment x Community</b>	1	0.00004	0.000040	0.235	0.63
<b>Species x Community</b>	2	0.00318	0.001590	9.243	0.0001 ****
<b>Treatment x Species x Community</b>	2	0.00126	0.000630	3.661	0.03 *

**Table 4. Tukey HSD Summary.** Pairwise comparisons between alike species in the same community, each with different inoculation treatments (+AMF or -AMF). (Species codes used for test summary DECE= *D. cespitosa*, JUBA= *J. Balticus*, PHAR= *P. Arundinacea*). The comparison in bold draws attention to near differences of *J. balticus* between fungal treatments (-AMF or +AMF), while *P. arundinacea* differences between fungal treatments (-AMF or +AMF) are not significant but remain of interest.

<i>Tukey HSD Summary</i>	<i>Pr(&gt;F)</i>
-AMF:DECE:MONO * +AMF:DECE:MONO	1.00
-AMF:JUBA:MONO * +AMF:JUBA:MONO	0.99
-AMF:PHAR:MONO * +AMF:PHAR:MONO	1.00
-AMF:DECE:POLY * +AMF:DECE:POLY	1.00
<b>-AMF:JUBA:POLY * +AMF:JUBA:POLY</b>	<b>0.12</b>
-AMF:PHAR:POLY * +AMF:PHAR:POLY	0.35



**Figure 5. Chlorophyll Fluorescence Across all Treatments.** Bar chart showing mean chlorophyll fluorescence (SD above bars) across the two community types (monoculture versus polyculture), three species, and AMF treatments. +AMF or -AMF treatments are indicated in red and blue, respectively. The averages of *J. balticus* seen in the polyculture treatment indicate differences between fungal treatments, and show decreased photosynthetic stress as measured in the PSII pathway when AMF inoculation is present.

### Summary Statistics Results

Twenty-five replicates of each treatment survived through the entirety of the experiment, 10 variables were recorded after measurements and calculations, and there were 8 treatments across species, community, and fungi treatments (Table 5 below). Exploratory statistics (discussed below) demonstrated that all other 9 variables besides chlorophyll fluorescence were impacted or differentiated by the presence or absence of Fungi (+AMF or -AMF). The variables show potential differences between species (*D. cespitosa*, *J. balticus*, and *P. arundinacea*) and communities (Monoculture or Polyculture) while showing little difference between the fungal treatments (+AMF or -AMF). For

example, canopy cover of *P. arundinacea* has the highest %, is 4-5% greater in the monoculture, but differs by less than 0.5% between fungi treatments.

**Table 5.** Averages of all response variable across all treatments. M= Monoculture treatment, P= Polyculture Treatment. DECE= *D. cespitosa*, JUBA= *J. Balticus*, PHAR= *P. Arundinacea*. CHFL= Chlorophyll fluorescence. RCI= Relative Competition Index, and represents the biomass as relative between culture treatments. No values can be used for the monoculture lines for the RCI: they are used to create the relative values to the polyculture lines.

DATA SUMMARY BY AVERAGES					
TREATMENT	Canopy Cover (%)	CHFL	# Leaves	# Dead Leaves	Plant Height (cm)
<b>M-DECE+AMF</b>	<b>25.24</b>	<b>0.69</b>	<b>10.69</b>	<b>3.12</b>	<b>24.10</b>
M-DECE-AMF	24.97	0.70	10.41	3.00	23.09
<b>M-JUBA+AMF</b>	<b>18.26</b>	<b>0.73</b>	<b>4.48</b>	<b>1.14</b>	<b>35.10</b>
M-JUBA-AMF	16.83	0.71	4.20	0.86	34.14
<b>M-PHAR+AMF</b>	<b>28.04</b>	<b>0.66</b>	<b>5.73</b>	<b>3.11</b>	<b>33.70</b>
M-PHAR-AMF	26.48	0.65	6.02	3.09	30.75
<b>P-DECE+AMF</b>	<b>23.55</b>	<b>0.71</b>	<b>9.14</b>	<b>3.55</b>	<b>28.66</b>
P-DECE-AMF	21.93	0.65	8.59	2.61	29.98
<b>P-JUBA+AMF</b>	<b>20.14</b>	<b>0.70</b>	<b>6.55</b>	<b>2.45</b>	<b>27.01</b>
P-JUBA-AMF	21.86	0.63	7.52	2.66	27.49
<b>P-PHAR+AMF</b>	<b>24.91</b>	<b>0.67</b>	<b>9.03</b>	<b>2.82</b>	<b>28.26</b>
P-PHAR-AMF	21.00	0.59	6.34	2.81	27.68

TREATMENT	BIOMASS	ROOT	SHOOT	ROOT: SHOOT	RCI
<b>M-DECE+AMF</b>	<b>1.35</b>	<b>0.75</b>	<b>0.60</b>	<b>1.30</b>	<b>NA</b>
M-DECE-AMF	1.25	0.71	0.54	1.38	NA
<b>M-JUBA+AMF</b>	<b>1.25</b>	<b>0.85</b>	<b>0.41</b>	<b>2.22</b>	<b>NA</b>
M-JUBA-AMF	1.48	1.06	0.42	3.13	NA
<b>M-PHAR+AMF</b>	<b>2.57</b>	<b>1.88</b>	<b>0.69</b>	<b>2.60</b>	<b>NA</b>

M-PHAR-AMF	2.25	1.63	0.62	2.68	NA
<b>P-DECE+AMF</b>	<b>1.54</b>	<b>0.95</b>	<b>0.59</b>	<b>1.72</b>	<b>-1.49</b>
P-DECE-AMF	1.74	1.12	0.62	2.06	-2.24
<b>P-JUBA+AMF</b>	<b>1.71</b>	<b>1.21</b>	<b>0.50</b>	<b>2.27</b>	<b>1.79</b>
P-JUBA-AMF	1.16	0.75	0.41	1.85	3.37
<b>P-PHAR+AMF</b>	<b>3.24</b>	<b>2.34</b>	<b>0.90</b>	<b>3.58</b>	<b>-0.03</b>
P-PHAR-AMF	2.77	1.97	0.80	2.43	-2.11

A correlation matrix that included all variables showed high correlations among the variables related to biomass: roots, shoots, root:shoot ratios, and total biomass (Figure 5). All other variables had correlation values below 0.80 (after Feldman 185) and were considered non-covariate. Root weight was selected from this group of related variables to singularly represent biomass response variables to avoid the multicollinearity effect that using all four variables could cause. This variable was selected of the four due to its eigen value discussed in the subsequent PCA analysis results.



Correlation Matrix							
<b>Chlorophyll Fluorescence</b>							
-0.50	<b>Number of Dead Leaves</b>						
0.31	0.52	<b>Canopy Cover (%)</b>					
-0.62	0.57	0.53	<b>Number of Live Leaves</b>				
0.42	0.37	0.09	0.10	<b>Plant Height (cm)</b>			
0.14	0.10	0.56	0.13	0.41	<b>Root Weight (g)</b>		
0.16	0.11	0.66	0.58	0.25	0.69	<b>Shoot Weight (g)</b>	
0.05	0.16	0.63	0.18	0.38	<b>0.96</b>	<b>0.83</b>	<b>Total Biomass (g)</b>

**Figure 6. Correlation Matrix** The correlation matrix demonstrates collinearity with a Spearman's rank correlation greater than 0.80 between root weight and biomass, and between shoot weight and biomass. Both Root weight and shoot weight are combined to measure total biomass, and thus their correlation is logical. All other variables have low correlations.

Results from the PCA analysis revealed that all variables considered together did not account for variance within the dataset but that perhaps chlorophyll fluorescence, root weight, and canopy cover accounted for the most variance within the dataset. These results show that multivariate statistical tests would not be appropriate for this dataset. Eigen analysis showed that PC1 and PC2 were comprised of all variables, and each response variable accounted for a considerable proportion of the variance within their respective PC (Table 6).

**Table 6.** The eigen analysis quantified the loading of each relevant Principal Component (PC1 and PC2). The only positive eigenvector of PC1 was chlorophyll fluorescence. Canopy cover, and the related plant mass variables: root to shoot ratio, root weight, shoot weight, and biomass all showed the strongest negative accounting for variance within this dataset. This indicates that chlorophyll fluorescence is the largest positive predictors of that axis. PC2 is explained negatively by the root to shoot ratio, and positively by number of live leaves and chlorophyll fluorescence. These values were used to further explore this data, but the results yielded indication that no further analysis was appropriate.

**Eigen Analysis Summary:**

<b>Variable</b>	<b>PC1</b>	<b>PC2</b>
<b>Chlorophyll Fluorescence</b>	0.25	0.37
<b>Number of Dead Leaves</b>	-0.29	-0.34
<b>Canopy Cover</b>	-0.42	-0.11
<b>Number of Live Leaves</b>	-0.33	-0.38
<b>Height</b>	-0.11	0.37
<b>Shoot Weight</b>	-0.40	0.37
<b>Root Weight</b>	-0.45	0.0
<b>Biomass</b>	-0.44	0.29
<b>Root: Shoot Ratio</b>	0.48	-0.61

### Root Colonization by Arbuscular Mycorrhizal Fungi Results:

It was not possible to achieve complete soil sterilization through 10% benomyl applications, but control treatments (-AMF) generally experience low levels of inoculation due to fungicide treatment. This demonstrates the vigorous nature of fungal inoculation despite sterilization techniques in greenhouse experiments. Percent root colonization assessment (N = 120 slide samples) confirmed that +AMF treatments were colonized, and -AMF treatments had low levels of colonization (Table 7).

**Table 7.** Percent of root colonization (%) by AMF, arbuscules (%), vesicles (%), and hyphae (%) seen in each treatment group. In culture, MONO= monoculture, POLY= polyculture. For Plant Species: *D. cespitosa*= *Deschampsia cespitosa*, *J. balticus*= *Juncus balticus*, and *P. arundinacea*= *Phalaris arundinacea*. The total % colonization does not necessarily equal the sum of all structures given that multiple structures may be found at each tally point.

#### Root Colonization by Arbuscular Mycorrhizal Fungi Summary

Culture	Plant Species	Fungal Treatment	%			
			% Colonization (STDV)	Arbuscules (STDV)	% Vesicles (STDV)	% Hyphae (STDV)
Mono	<i>D. cespitosa</i>	-AMF	12.6 (3.1)	5.5 (2.8)	5.2 (2.2)	11.6 (3.1)
Mono	<i>D. cespitosa</i>	+AMF	<b>45.8 (8.7)</b>	<b>17 (5.5)</b>	<b>13.8 (4.9)</b>	<b>34.3 (6.9)</b>
Mono	<i>J. balticus</i>	-AMF	7.1 (1.6)	2.4 (1.2)	3.6 (2.9)	6.7 (1.7)
Mono	<i>J. balticus</i>	+AMF	<b>33.9 (3.7)</b>	<b>33 (3.7)</b>	<b>9.7 (2.5)</b>	<b>10.8 (3.3)</b>
Mono	<i>P. arundinacea</i>	-AMF	5.9 (2.7)	5.3 (2.4)	2.9 (1.7)	2.2 (1.8)
Mono	<i>P. arundinacea</i>	+AMF	<b>46.5 (13)</b>	<b>43.2 (12.5)</b>	<b>15.6 (5.4)</b>	<b>29.1 (9.5)</b>
Poly	<i>D. cespitosa</i>	-AMF	8.3 (3.5)	6.9 (3.1)	4.1 (2.7)	3.4 (3.5)
Poly	<i>D. cespitosa</i>	+AMF	<b>33.3 (3.3)</b>	<b>6.7 (3.6)</b>	<b>13.2 (4.1)</b>	<b>30.3 (4.1)</b>
Poly	<i>J. balticus</i>	-AMF	6 (2.1)	1.5 (.9)	2.3(1.4)	5.6 (1.9)
Poly	<i>J. balticus</i>	+AMF	<b>12.2 (3.9)</b>	<b>3.9 (2)</b>	<b>6.2 (2.3)</b>	<b>11 (4.1)</b>
Poly	<i>P. arundinacea</i>	-AMF	5.7 (2.6)	4.8 (1.9)	2.8 (1.7)	0.9 (0.8)
Poly	<i>P. arundinacea</i>	+AMF	<b>30.2 (6.7)</b>	<b>8.1 (4.6)</b>	<b>14.6 (5.9)</b>	<b>27.5 (6)</b>

## Chapter 2: Experiment Discussion

AMF impacts to plant communities can be highly context dependent, as is demonstrated by the interaction among fungal treatment type, community type, and plant species. Additionally, differences in chlorophyll fluorescence between fungal treatments of *J. balticus* roots with or without fungi show the potential for AMF to alter individual plant health. In this experiment a species-specific result showed that AMF collected from native marsh soil provided more stress relief for the native *J. balticus* than *P. arundinacea*, a known problematic invasive grass. These species-specific effects of AMF may in turn impact the make-up of plant community structure in salt marshes, particularly the high marsh.

It was hypothesized that plants with AMF would express their competition strategies differently when grown in a community because this context would exemplify interspecific competition, whereas the monocultures exemplify intraspecific competition. To examine the effect of AMF on the competition strategies of three salt marsh plant species, ten response variables were measured in both monocultures and polycultures, with and without AMF inoculation (+AMF or -AMF, respectively). Results for chlorophyll fluorescence, which measures plant stress through its photosynthetic pathway II, when concerning *J. balticus* with or without fungus in the polyculture treatment, showed differences in the way plants expressed their competitive strategy with a relative difference in plant stress tolerance. The other 9 variables that are associated with the way plants assimilate and allocate physical mass (root: shoot ratios and plant biomass), demonstrated similar means between the two AMF treatments and so were not evaluated

with factorial ANOVA. The data showing high similarity between the +AMF and -AMF treatments for the 9 other response variables (Table 2, Appendix 1) did not directly support the hypothesis that competition strategy expression would be exacerbated in the polyculture.

The differing nature of each study species' competition strategies, association with AMF, and persistence across landscapes globally makes for uniquely interesting comparisons amongst the family of graminoid plants, Poaceae, that typically dominate salt marsh systems. It was also hypothesized that each of the three species examined will demonstrate different levels of AMF inoculation/colonization in their plant roots. These levels of colonization also varied by species, which indicates that each species does indeed have their own level of symbiosis and that these growth levels were demonstrated during the early stages of their life histories.

*Juncus balticus*, a native plant that occurs commonly in freshwater systems, is not known to frequently associate with AMF, and thus was not expected to be greatly affected by AMF inoculation, or lack thereof. This slower growing plant does not immediately compete well for space, and instead invests energy into producing high volumes of small seeds that locally disperse. This plant also has a rapid regrowth response to browsing and physical disturbance in freshwater systems (Mårtensson 2017). In this study *J. balticus* was predicted to show a stress tolerant or ruderal response given its lack of competition for resource success shown by Larkin et al. 2012, which would be supported by actual confirmation that this species experiences reduced stress when inoculated by AMF when grown in a polyculture community. This study does indeed observe this and shows that

differences between +AMF and -AMF in the polyculture during the shorter duration of this study (Figure 5, Table 4) were noticeable. This finding might be expanded upon by future experiments with longer timelines that may be fruitful in demonstrating the nature of this phenomenon. Field study in salt marshes that examines *J. balticus* chlorophyll fluorescence, with +AMF and -AMF treatments in may also support the idea that AMF reduces physiological stress of this species when grown in a stressful environment.

*Phalaris arundinacea* was expected to have a significant response to inoculation as it is known to associate with AMF and frequently demonstrates high levels of inoculation in freshwater wetlands (Boutton 2019). This plant's success in invading wetlands around the world has been widely documented, and is attributed to its prolific acquisition of nitrogen, and rapid root development that dominates surrounding soil area (Lavergne and Molosky 2004). While there was confirmed inoculation, +AMF in monocultures did not show differences in any of the response variables, although differences in chlorophyll fluorescence can be seen in *P. arundinacea* grown in polycultures between +AMF and -AMF can be seen (Figure 5). This trend, however, was not statistically supported by the Tukey HSD test. If *P. arundinacea* demonstrated differences akin to the trends seen here, it would mean that AMF inoculation decreased the plant's abilities to mitigate stress from the environment, which is shown by the +AMF treatment having the lower average chlorophyll fluorescence compared to -AMF treatments.

*Deschampsia cespitosa*, a widespread native bunchgrass in Oregon wetlands and around the world, is known to associate with AMF and is known to benefit from a fungal symbiont upon successful inoculation in freshwater systems contaminated with heavy

metals (Davy et al. 2019). While this species experienced inoculation in the greenhouse, it did not demonstrate differences in competition when measurements were compared between +AMF and -AMF treatments across all 10 measured response variables. The chlorophyll fluorescence measurements visually look slightly higher in polyculture compared to monoculture communities but did not significantly differ (posthoc  $p > 0.05$ ). These results show that AMF does not impact this species in this context as seen by any of the three independent grouping variables. This plant may ultimately not compete differently in the presence of fungal symbionts when growing in salt marshes and may instead associate with fungi due to evolution of symbiosis which in this context forms neither a helpful symbiont nor parasitic symbiont.

Across all three species, there were no visual trends or statistically significant differences between AMF treatments in monocultures. The only near differences, previously discussed in relation to *J. balticus* and *P. arundinacea*, presented themselves in the polyculture community type. Future work that explored this possible difference may provide interesting insight about whether AMF mediate interactions differently between intra- versus interspecific species within a salt marsh community. A neighbor removal study that examined 5 freshwater wetland species revealed several species-specific neighboring effects that were driven by AMF and varied between facultative and competitive interactions based on plant identity and dominant or subdominant establishment (Zhang et al. 2013). Additionally, another study in a freshwater system demonstrated the effects of AMF on size inequities on intraspecific communities (Ayres et al. 2006). In environments containing brackish water the differences in habitat type may

parallel freshwater systems but may also be altered by the presence of salt stress that is mitigated differently by plant species (Liancourt et al. 2005). In salt marshes AMF effects may impact both intra- and interspecific interactions, but this study suggests the interspecies interactions may be more pronounced in the specific interactions seen here between *D. cespitose*, *J. balticus*, and *P. arundinacea*.

Chlorophyll fluorescence measures document the photosynthetic response of a plant as seen in the PSII pathway, more specifically a “photosynthetic quenching” response (Maxwell and Johnson 2000). Differing chlorophyll fluorescence values in *P. arundinacea* with and without AMF inoculation could indicate that this species may be responding to environmental stresses in the presence or absence of AMF. The greenhouse conditions were modelled after the reference marsh in Salmon River, OR, and thus the argument can be made that stress responses of *P. arundinacea* with AMF may differ in natural conditions as well. Results from the Tukey HSD test ( $p=0.35$ ), and visuals depicted in Figure 3 show that *P. arundinacea* may have higher stress in its PSII pathway system in the presence of AMF, which may show that AMF parasitizes the non-native species in this context. If this invasive plant experiences higher stress with fungal symbionts in natural conditions, it can be expected to decline under continued stressful marsh conditions, and potentially under the additional stressors predicted to occur under climate change conditions. This decline could benefit mixed native plant communities that ultimately do not outcompete this highly invasive plant in competition for nutrient resources. This species has been seen to alter wetland and salt marsh function greatly, with differing effects across plants, animals, and abiotic processes (Annen et al 2008, Barnes



1999, Spyreas et al. 2010) and remain of interest to land managers in all PNW wetland habitats.

It is interesting however, that none of the other growth metrics measured appeared to have differences between inoculated and non-inoculated treatments and were eliminated from further testing during preliminary data exploration. Consideration of the short timeline under which this experiment occurred may give insight to this observation. In order to circumvent pot edge effects on root systems, the experiment was ended 70 days after the first measurement. This represents the juvenile life stage of the study species. Each of these species may benefit from fungal symbionts at different times in their lives. For example, a plant with rapid biomass expansion due to resource exploitation as an adaptive strategy (Competitor strategy) may benefit from early colonization from AMF whereas a plant that responds rapidly to browsing and disturbance events may benefit from long-term sustained colonization by AMF (Ruderal strategy) as theorized by Grimes 1979. At these times various growth variables and additional measurement of reproductive material assimilation may be more impacted by AMF treatments.

It may well be that the variables measuring competition did not vary strongly with AMF treatment or culture setting because the experiment ended too soon for these variables to be strongly affected by those factors. Chlorophyll fluorescence may be a metric that shows early or throughout a plant's life, whereas differences in biomass distribution may occur later in the life history of long-lived grasses such as those involved in this experiment. Hence, future work could include experiments over a longer duration, which would require larger pots, and perhaps transplanting of older plants instead of

transplanting seedlings 1-2 weeks after germination. This would better observe the effects of AMF on these species during juvenile and reproductive life stages. Additional field experiments that apply 10% Benomyl solution to eliminate fungi within plots across different populations of graminoids, which include both native and non-native species, would better study competition expression directly in the study system, the salt marsh. These field studies could occur at different elevations within a study marsh and could include water regime alteration treatments to mimic sea level rise under different predicted climate change conditions. Future work could also explore AMF effects of high marsh plant stock used for restoration. It is becoming easier for native plant nurseries to apply commercial inoculants containing AMF and additional rhizobia across many soil microbe taxa to their plant stock in hopes of bolstering plant health through symbiont benefits. These additions could aid *J. balticus* more than natives in some situations and is worthy of consideration by applied scientists responsible for land management of native plant communities.

Overall AMF was shown to change alter stress tolerance as a competition strategy between the three salt marsh plants examined here. The data suggest that AMF may impact *J. balticus* and *P. arundinacea* inversely in their stress response, but only when grown in a mixed community. AMF may decrease the physiological stress of *J. balticus* plants, and increase stress seen in *P. arundinacea*, which may overall aid in the growth and success of one native plant over a highly invasive non-native plant. This may occur due to a variety of mechanisms, each of which would require further study, but ultimately suggest that the AMF native to the reference marsh may evoke proliferation of native

species. It is unknown how intentional fungal inoculation affects plant competition in salt marshes, which are dominated by herbaceous plants. Introduction of nonnative AMF communities may impact regional land management efforts that recently have begun to consider inoculation of native plants when restoring salt marshes, to better ensure planting survival. These inoculation treatments currently do not target site-specific fungal communities, but instead primarily are composed of species most beneficial to agricultural crops. If native AMF are used to inoculate their respective native plant community, and show greater benefits to native plants over non-native plants, these inoculant treatments could improve the overall survival and success of native plantings, and will increase the associated functionality that these plants provide. Conversely, commercial inoculants that do not contain AMF sources from a given site, unique habitat or specific location may not benefit native plants in the same way. These globally common fungi may form unforeseen, potentially helpful relationships with globally successful invasive plants such as *P. arundinacea*. The effects of commercial inoculants on native and non-native plant communities will require further research, and current restoration efforts may do well to practice caution in their use. Blind investment in commercial inoculants seeking to bolster the survival of native plants may be counterproductive and should be approached with reserve given limited nature of restoration project capacity (both logistical and financial limitations are to be considered here). “Limited restoration funds might be more efficiently spent on diverse plant stocks, inoculating with native soil that already contains site-specific microbial communities, and monitoring.

### Chapter 3: Study Conclusions

This work sought to observe how different plants responded through secondary competition to AMF inoculation in a uniquely stressful loving environment. Much work to date has focused on plant individuals and community responses to AMF, which has led to the general understandings that in exchange for photosynthetic byproducts these fungi can benefit their symbiotic hosts when nutrients or water are limited, and can offer stress or predation amelioration. However, within salt marsh systems such as the reference marsh used in this research, it was hypothesized that the symbiotic relationship between AMF its three host plants might express itself differently due to the stronger presence of stress as an ecosystem driver. It was specifically hypothesized that any changes in this relationship would be most pronounced and impactful when examined in a small community of plants dominate the high marsh landscape, where stress amelioration would be the most impactful. AMF was specifically shown to impact plants in a three-way interaction between AMF treatment, community type, and species, with a stress relief effect seen in one of the native plants, *J. balticus*, when it was inoculated with fungi.

To examine plant responses to AMF inoculation while under physiological stress a common greenhouse experiment was conducted to closely measure 10 response variables in the absence of environmental variability. This was done by exposing all plants to homogeneous environmental conditions, and only altering whether plants were inoculated with AMF. After the experiment ended, data were first explored through multivariate statistical analyses, but it was found that most of the measured response variables contributed little to explaining variance within the dataset. The subsequent univariate

analysis revealed that being grown in a mixed community, specific plant species, and AMF inoculation or lack thereof had a three-way effect on the chlorophyll fluorescence, a stress measure, levels within plant leaves. Additionally, chlorophyll fluorescence differed significantly between *Juncus balticus* plants with or without AMF inoculation, but only when this plant was grown in a mixed community as opposed to when it was grown with other *J. Balticus* plants. This effect was *P. arundinacea* showed a near significant effect of chlorophyll fluorescence,

This work shows the importance of considering which species of plants may forms symbiotic relationships with site specific fungi, and how that relationship may affect plant interactions. The results of such interactions could alter the composition of plant communities and may be used to assess land management practices in the future. The identities of the AMF in the Salmon River estuary were not revealed in this work but future investigation of AMF species present that would require DNA sequencing or morphological identification to understand the diversity and abundance of present AMF species, could give further insight to the nature of these relationships. The use of DNA sequencing to identify fungal species would lead to better taxonomic documentation, and would release pressure from the highly specialized field of visual morphological identification, which currently creates a bottleneck around the amount of data that can be collected about various AMF species. It would be particularly important to understand whether the AMF present were locally endemic, globally common, or some mixture of the two. If AMF species were globally abundance then use of commercial inoculants would be potentially less problematic, and more research would be likely to focus on these

species and lend insight to the system. If AMF species present at the Salmon River site were locally endemic, then perhaps the native plants would have more closely evolved to form relationships with these species. This would mean that the introduction of globally abundance species, by the use commercial inoculants for instance, could benefit non-native plants that are better suited to form symbiotic relationships with fungi from afar. Additionally, using equipment from other parts of the world could introduce foreign fungal species can cause similar unintentional introductions.

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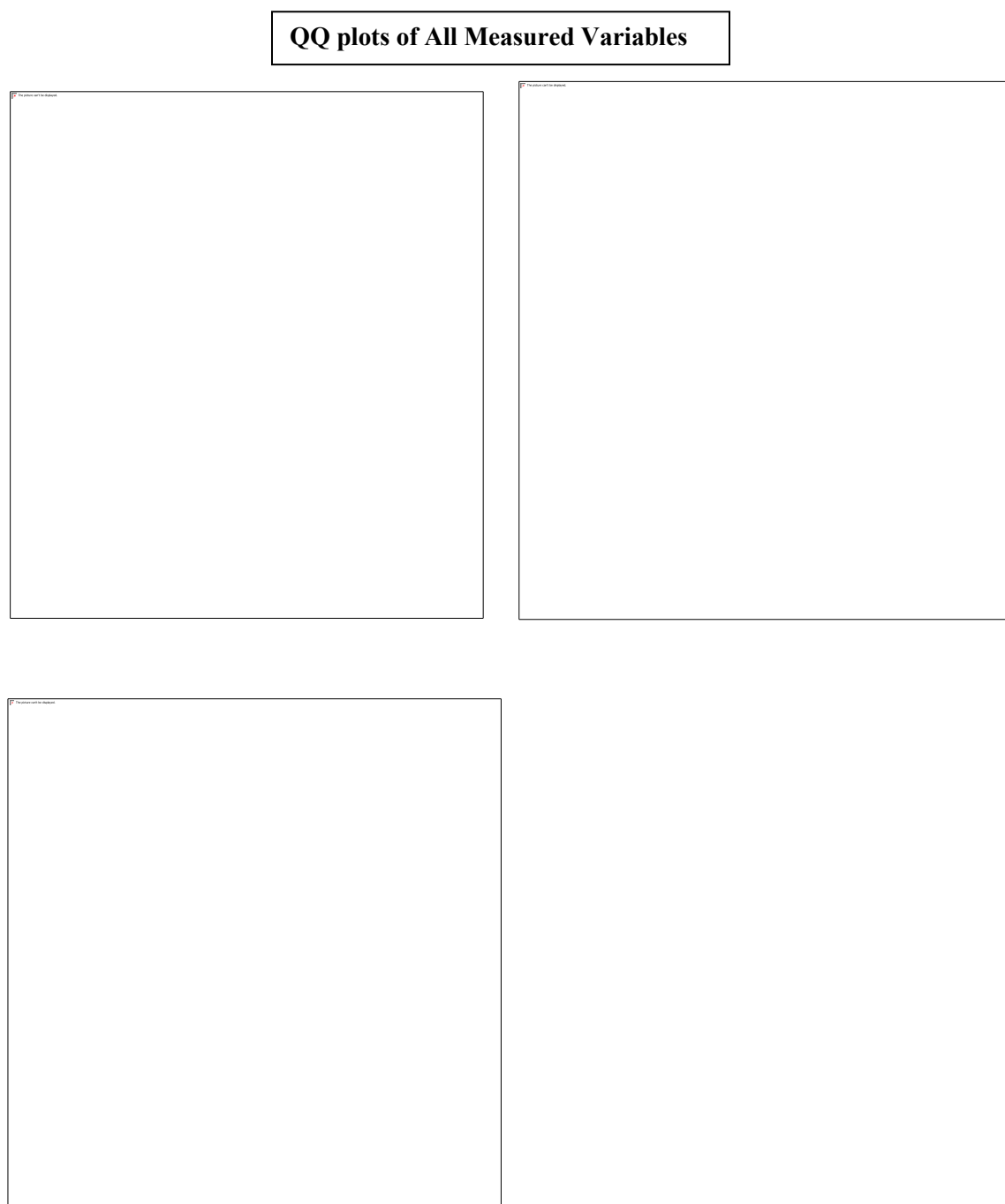
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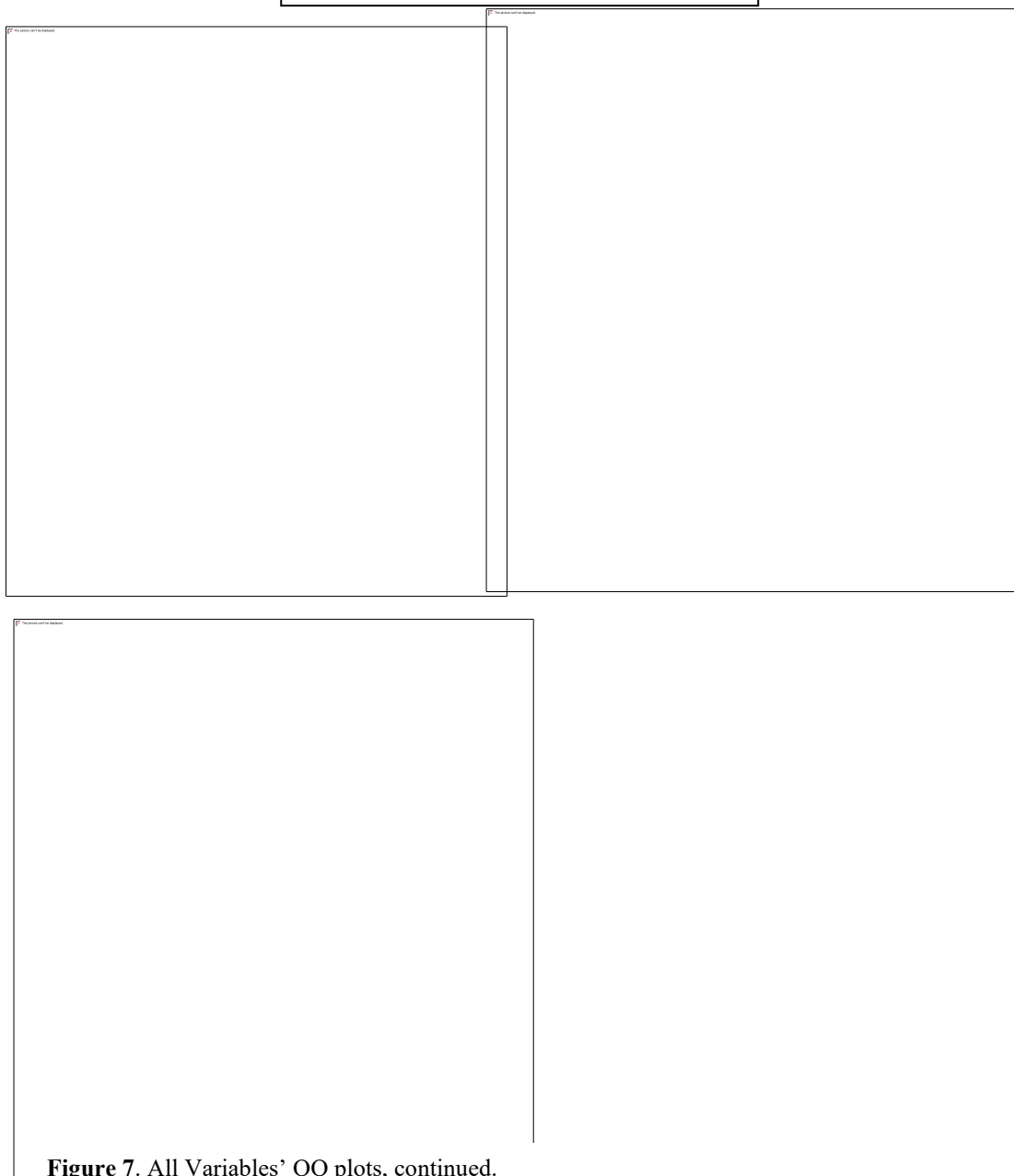
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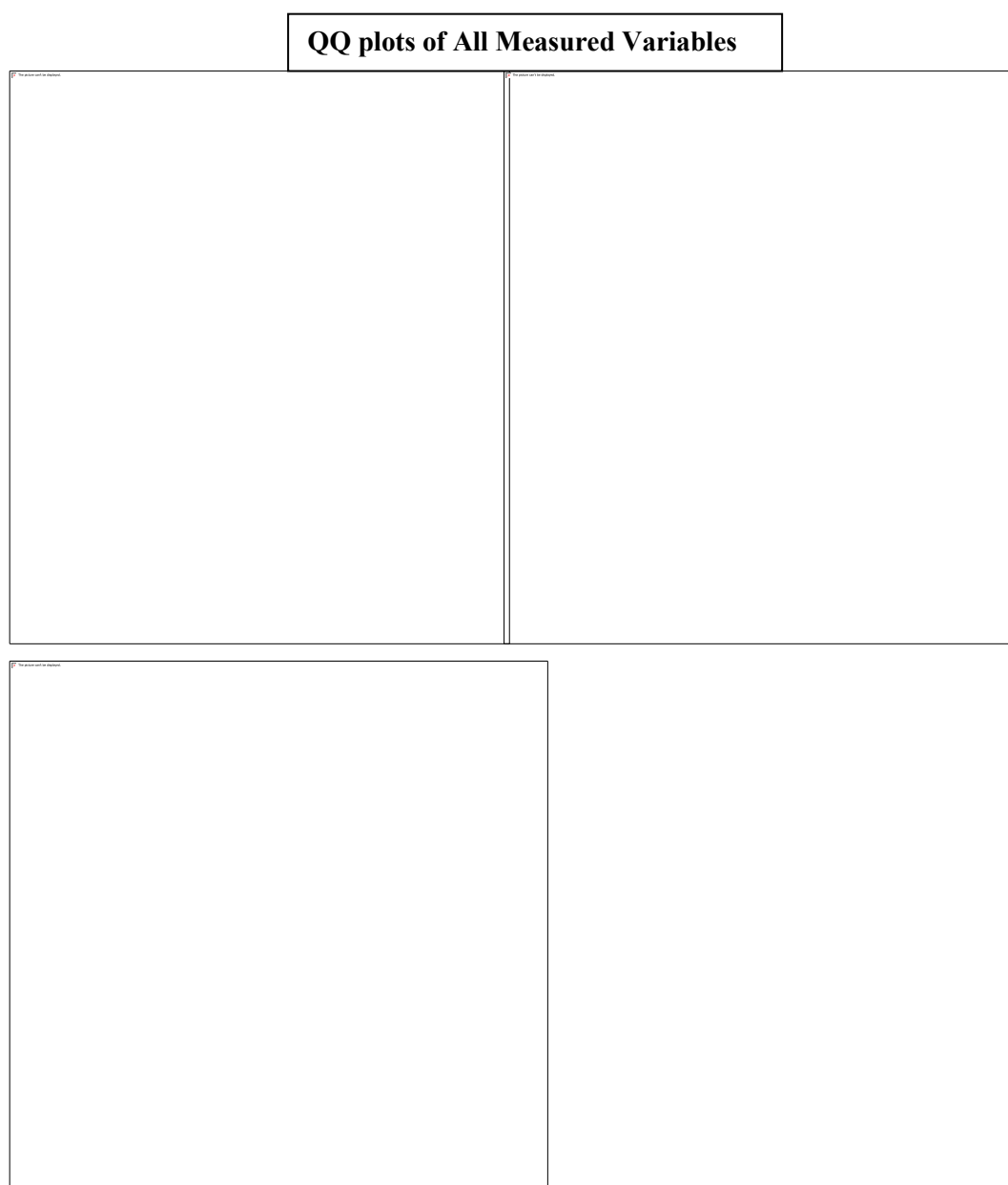
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## Appendix A. Additional Figures



**Figure 7.** QQ plots of all variables initially considered in this data set.

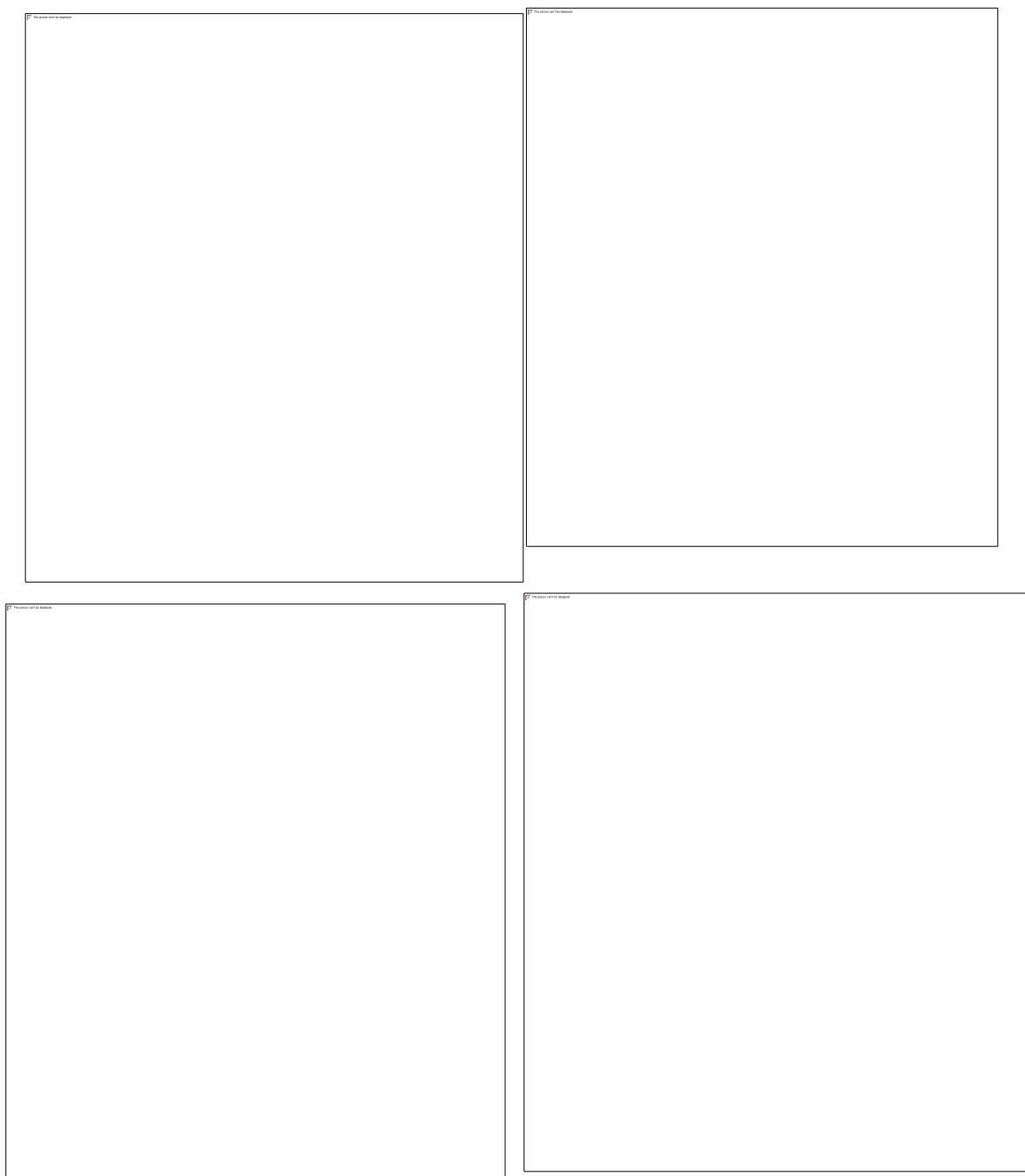
**QQ plots of All Measured Variables****Figure 7.** All Variables' QQ plots, continued.



**Figure 7.** All Variables' QQ plots, continued.



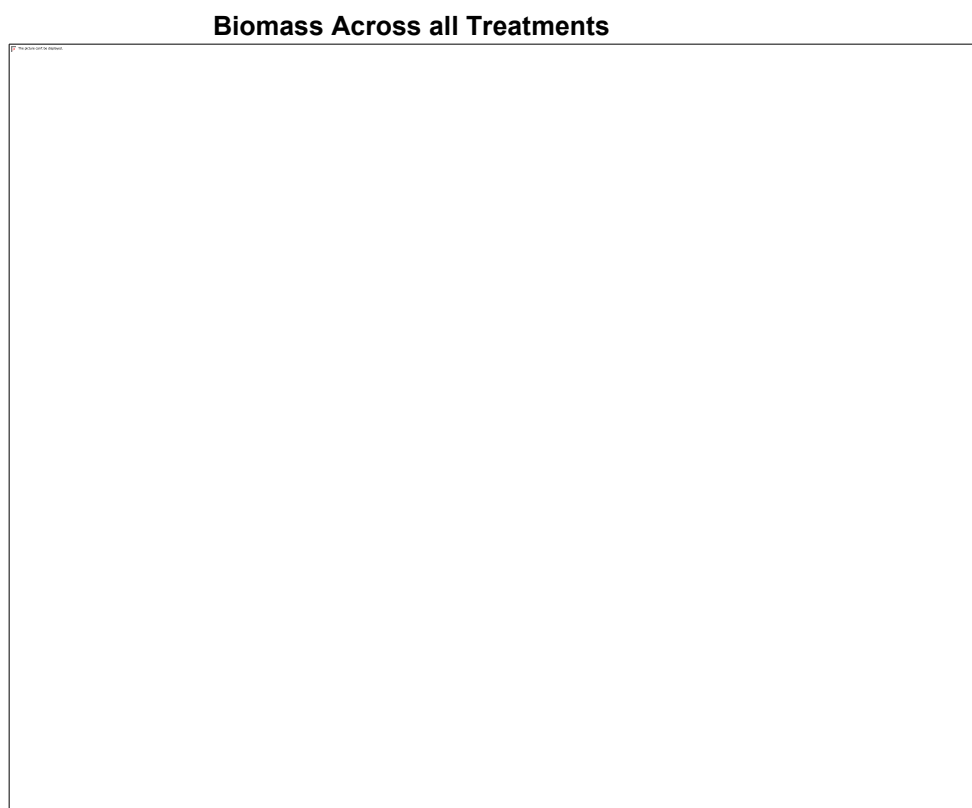
## Histograms of all Variables



**Figure 8.** Histograms of all variables



**Figure 8.** Histograms of all variables

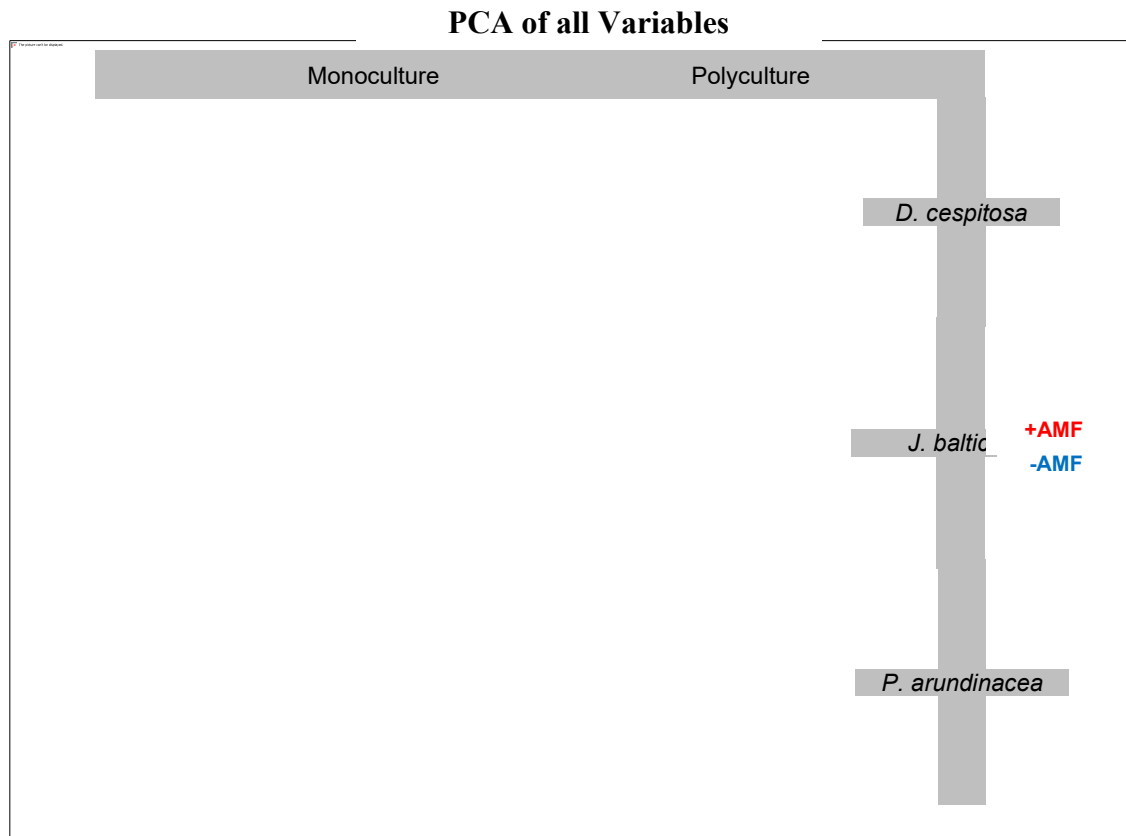


**Figure 9.** Boxplot of biomass across all treatments.

**Table 8.** Shapiro-Wilks results seen across all variables

**Shapiro-Wilkes Results:**

Variable	Test Statistic (w=)	P-value
Canopy Cover	0.98678	0.02278
Chlorophyll Fluorescence	0.93006	2.143e-09
Number of Dead Leaves	0.96299	5.583e-06
Number of Live Leaves	0.97676	0.0004642
Plant Height	0.98464	0.009435
Root Weight	0.95254	3.326e-07
Shoot Weight	0.955	6.412e-07
Biomass	0.97758	0.0006236
Root:Shoot Ratio	0.96245	4.786e-06



**Figure 10.** PCA results. The data points from each treatment are highly overlapping and are not clustered indicating that there is little difference in point values between the treatments. The columns are separated by monoculture and polyculture treatments. The rows are by species with *D. cespitosa*, *J. balticus* and *P. arundinacea*. PC1 (Comp. 1) explains 42% of the variance of the data and PC2 (Comp. 2) explains 23%. The data points represented in PCA analysis show +AMF and -AMF treatments as seen by all response variables in PC1 and 2. Treatments were highly overlapping and visually indistinguishable from one another. In total, PC1 (43%) and PC2 (23%) accounted for 65% of variance within the dataset. Eigenvectors quantified the amount of association between each of the measured variables and PC1 and PC2, respectively (Table 3). The largest eigenvectors of PC1 are percent canopy cover, and the related plant mass variables: root to shoot ratio, root weight, biomass. PC2 is associated negatively with the root to shoot ratio, and positively with number of live leaves and chlorophyll fluorescence. These values were used to further explore this data with non-parametric tests performed on singular variables that made significant contributions to accounting for variance within the dataset.

### Broken Stick Model



**Figure 11.** This scree plot shows each principal component and its eigenvalues in grey bars. The broken stick model overlaid indicates that both PC1 And PC2 explain more variance than would be randomly expected. PC1 is shown here to account for 42% of variance within the dataset, and PC2 accounts for 23%, which totals in 65% total.