

ABSTRACT

Title of Thesis: MEGAPOOLS: VEGETATION DIEBACK AND RESTORATION POTENTIAL OF A DITCHED COASTAL SALT MARSH

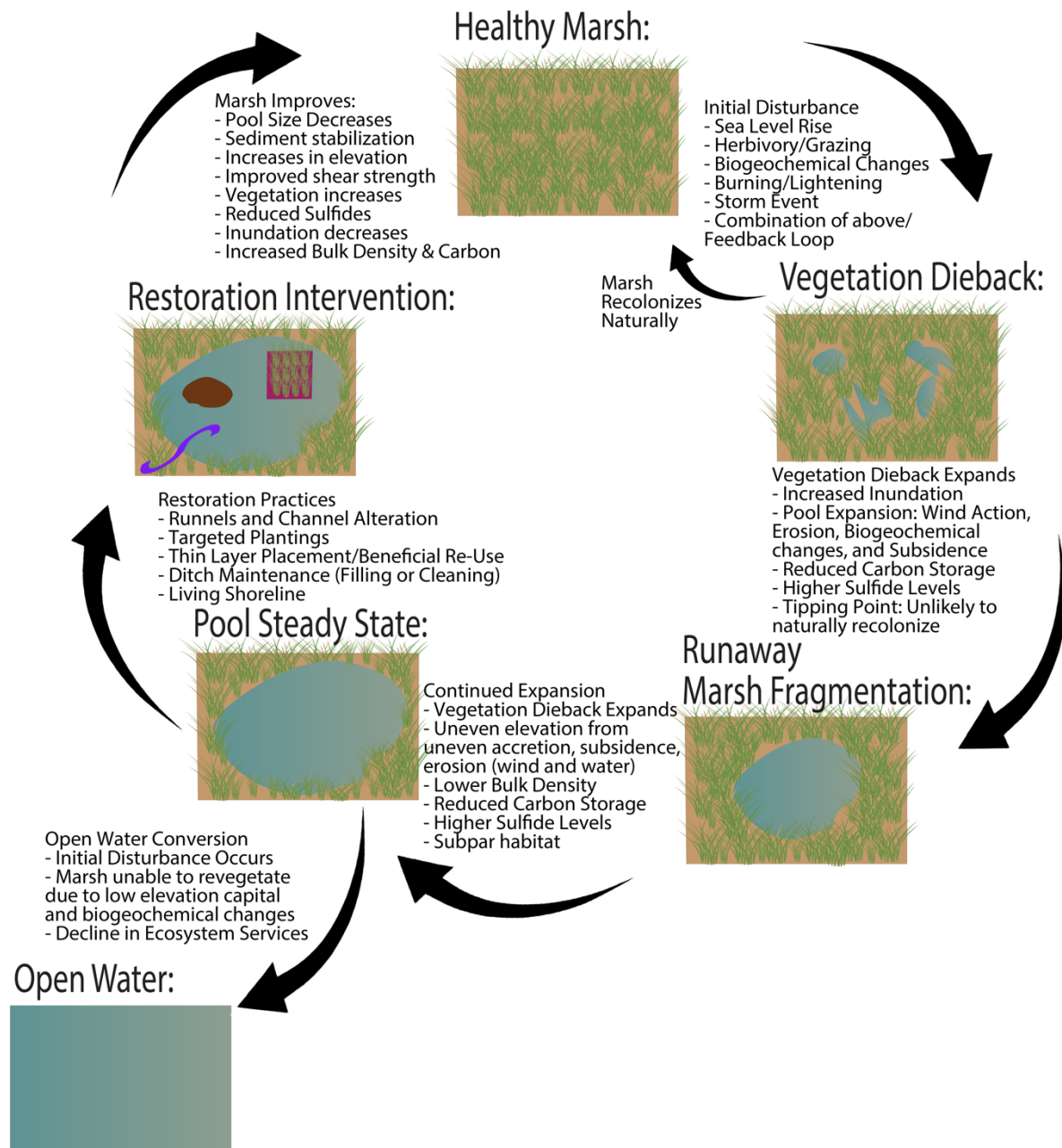
By: Katherine Anne Stahl, Master of Science, 2023

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In many ditched coastal salt marshes, megapools, or large ponded areas of vegetation dieback, have formed. In combination with sea level rise, this interior marsh loss can decrease wildlife habitat suitability, resilience to storms, and other ecosystem services. However, mechanisms of megapool formation are poorly understood, hampering restoration efforts. Here, we explored differences in environmental characteristics between megapools in different stages of formation (Fully Formed, Partially Formed and Nonformed/Control) and between Elevations within megapools (High, Medium, Low). Using IRIS Films (Indicator of Reduction in Soil), we found that Fully Formed megapools had higher sulfide concentrations than Partially formed, which in turn were greater than Nonformed megapools. We additionally found that lower elevations correlated with higher sulfides, lower plant coverage, lower belowground biomass, lower Carbon Density, and predicted megapool type. We noted that in terms of elevation, vegetative cover, and

biomass, Nonformed and Partially formed were more similar as were High and Medium elevations. Whereas in terms of soil characteristics, Fully Formed and Partially formed were more similar as were Medium and Low Elevations. To combat megapools and dieback, we will assess the effectiveness of two restoration techniques, the first of which is assessing the survival and growth of plantings at different spacings, elevations, and megapool formation levels. We found survival and growth was higher in Partially formed megapools than Fully formed, and no impact by spacing or elevation. Our second restoration technique is runnels, or 15" channels that reconnect megapools to ditches, which were installed in January of 2023. The data collected above will act as baseline data, repeated again. These baseline results support a close relationship between pool stages of formation, carbon storage, elevation, vegetation health, biomass production, and sulfide levels (Graphical Abstract).

GRAPHICAL ABSTRACT



Graphical Abstract:

This graphical abstract is a visual representation of the abstract above, depicting the process of megapool formation and vegetation dieback as found in this paper and synthesizing relevant literature (Day et al., 2011; Mariotti & Fagherazzi, 2013; Vinent et al., 2021).

MEGAPOOLS: VEGETATION DIEBACK AND RESTORATION POTENTIAL OF A
DITCHED COASTAL SALT MARSH

By: Katherine Anne Stahl, Master of Science, 2023

Thesis submitted to the Faculty of the Graduate School of the
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CHAPTER ONE: MARSHES, DITCHES, MEGAPOOLS AND MORE: BACKGROUND ON CURRENT CHALLENGES IN SALT MARSHES AND POTENTIAL RESTORATION SOLUTIONS

INTRODUCTION:

Ecosystem Services:

Salt marshes act as a unique ecosystem providing benefits to people and organisms alike. Even while growing in anoxic conditions, salt marsh plants are still productive providing nutrient accumulation and carbon sequestration (Craft et al., 2009). These distinct vegetative conditions create habitat for invertebrates and make conditions hospitable for other plants, in turn attracting birds and small mammals (Bertness & Ewanchuk, 2002; Hughes, 2004). Water quality is also improved, benefiting recreational and economic opportunities for local residents. During times of disturbance such as storms, salt marshes can provide wave attenuation, protecting infrastructure (Kennish, 2001). Anthropogenic impacts such as ditches and sea level rise threaten the health and existence of salt marshes (Kearney & Stevenson, 1991).

In a typical coastal marsh setting, ecological systems have evolved to rely heavily on high and low tides to bring in nutrients, sediment, oxygen, seeds, and even small scale disturbances. The presence of ditches alters these interactions, creating uneven sediment allocation (Wigand et al., 2017). In a non-ditched marsh, high tides bring in sediment allowing for accretion of a marsh to keep pace with sea level rise accumulating on average 2mm annually. With this sediment, comes nutrients and minerals that enhance plant growth. Equally important is low tide, allowing for soil aeration and improving conditions for plant growth, which increases organic matter accumulation (Mendelssohn & Seneca, 1980).

Ditching:

Salt marshes across the east coast were ditched in the 1930's and 40's under the civilian conservation corps in an effort to control mosquitoes by decreasing habitat. Some estimates say that up to 90% of salt marshes were ditched in grids (Leisnham & Sandoval-Mohapatra, 2011). The consequences of ditching include altered salt marsh hydrology and ecology impacting natural processes such as tidal fluctuations and sediment accretion. Sea level rise has exacerbated consequences of ditches (Corman et al., 2012). In ditched marshes, an influx of pools have appeared creating a so-called "waffle maple syrup effect" where water is trapped internally on the marsh (U.S. Fish & Wildlife Service, 2020a, 2020b). These pools differ in biogeochemistry from natural pools and adjacent marsh decreasing ecosystem services like habitat provided in a natural marsh or pool (Vincent et al., 2013). Additionally, marshes with pools are less resilient to sea level rise and climate change with an increased risk to permanent conversion of open water (Himmelstein et al., 2021). While temporary vegetation dieback and pool formation is a known feature of salt marsh ecosystems, sea level rise and impacts of ditching have created a tipping point coined "Runaway marsh fragmentation" where pools expand until permanent conversion (Vincent et al., 2021). As pools expand, wind driven erosion increases as larger tidal action occurs within pools. The power of waves and wind velocity increases with greater pool size as fetch increases (Himmelstein et al 2021). At smaller pool sizes, there is not enough fetch for tidal flats to experience erosion, however after a threshold size marsh erosion accelerates rapidly with positive feedback loops of sediment loss (Mariotti & Fagherazzi, 2013).

Sea levels are expected to rise by up to 100 cm within the next 100 years, no match for the 0.2cm of annual accretion in salt marshes (Craft et al., 2009; Mendelssohn & Seneca, 1980). In combination with subsidence, primarily due to the anthropogenic impacts of groundwater

depletion, and lack of migration capacity, salt marshes are in dire need of assistance (Kearney & Stevenson, 1991). In New Jersey, 30–100% of all marsh interior vegetation loss is from megapools, and megapools with lower tidal range are more vulnerable to megapools (Smith et al., 2021).

Restoration Techniques:

A number of restoration techniques have been developed and applied, with mixed success on a national scale due to different tidal ranges and site factors. For instance, beneficial re-use of dredged material, also referred to as thin layer placement, is a promising technique to improve elevation capital and the lifespan of a salt marsh (Raposa et al., 2022). However, the reality is that a majority of marshes are privately owned, making landowner preference, permitting, and funding availability a decisive factor for the ability to restore marshes (Castagno et al., 2021). For instance, restoration using thin layer placement of sediment is costly, requires adequate sediment availability, extensive in advance permitting, and sufficient adjacent channel depth or infrastructure to transport sediment (Raposa et al., 2022). Importantly, low technology techniques like runnels come at a much lower cost and with lower regulatory and implementation barriers than some other techniques (Besterman et al., 2022).

Ditch plugs once acted as a promising restoration technique, placing sediment or other earthen material in a ditch to block interior water flow and increase lateral water flow. The purported benefits of ditch plugs were to restore tidal fluctuations and create more open water systems for waterfowl (Riepe, 2010). While successful in forested freshwater wetlands, ditch plugs led to further degradation of marshes in coastal salt marsh systems as they did not function like natural pools and the consequences spread across the marsh (Vincent et al., 2013). These ditch plugs caused such extensive harm to marshes that today the U.S. Fish and Wildlife Service

is involved in an ongoing effort to remove ditch plugs from sites where they were implemented (Pers. Comm. Dan Murphy). The lessons learned with this technique have led to more extensive monitoring and pilot projects before wide scale implementation of new restoration techniques.

Runnels:

In marsh areas where ponds/pools are present, runneling may present a feasible option to restore vegetation and marsh hydrology (Perry et al., 2021). Runnels are an up-and-coming restoration technique with the goal of lessening internal pools and increasing vegetation coverage and vertical marsh growth through accretion of organic matter and sediment trapping (Maher et al., 2022). Ponds caused by ditching may not provide the same ecosystem services that natural ponds provide (Vincent et al., 2013). In talking with practitioners, one common word choice to distinguish natural ponding and pools from anthropogenically formed pools is “megapools” (U.S. Fish & Wildlife Service, 2020a, 2020b). It seems that megapools typically form in close proximity to grid ditching due to sediment buildup on the edges creating levees of sediment from ditch construction, maintenance, and natural accretion over time (Corman et al., 2012). In combination with subsidence and sea level rise, water can become trapped behind the sediment levees and is unable to exit. Overtime, data suggests that these pools can create conditions harming vegetation (Wigand et al., 2017). Once these megapools form, they are less likely to revegetate than other pool formation types (Smith et al., 2021).

In order to combat the impacts of the sediment levees, runneling can restore hydrology and growing conditions by creating a flow path through the blockage. Runnels are shallow channels no deeper than 15” and wider than 20” that reconnect megapools to nearby ditches or riverine channels (Perry et al., 2021). Runnels differ from ditches in purpose and dimensions, and that they are sinuous in nature and have been found to improve hydrology, decrease

inundation, and promote plant recolonization decreasing bare ground which can increase habitat for nesting marsh birds (Watson et al., 2022). In combination with runnels, plantings of the dominant native vegetation can occur to speed up revegetation. Runnel projects typically take 3-5 growing cycles for improved vegetation coverage, and this process can be delayed without runnel maintenance (McKown et al., 2023). Runnels were initially used in conjunction with other techniques to decrease the presence of mosquitoes. However, in the early 2000s when an increasing number of megapools were present they began to be used as restoration techniques for combatting megapools. Pilot projects for runnels have predominantly occurred in New England, and have only recently started to show up southward. There is little peer reviewed published data on emerging technique, though anecdotal evidence and gray literature exist. Initial data suggests that runnels improve plant colonization, increase percent cover, stem height, and stem count, and decrease open water and bare ground coverage where control plots maintain the same or worse in pool formation (Perry et al., 2021). Areas without sufficient elevation capital may not be good candidates for runnels (Watson et al., 2022). Additionally, these techniques are known to not be a long term solution but rather buy time and are more cost effective in preventing expansion of pools (Besterman et al., 2022). .

OBJECTIVES:

There are distinct but related objectives of this study. First and foremost, a field study to understand how ditches contribute to vegetation dieback by comparing megapools in various stages of formation and assessing their characteristics. The next objectives focus on assessing the efficacy of two restoration techniques, the first of which is plantings at different elevations and spacings, and the second is collecting baseline data to assess the ability of runnels to restore hydrology and vegetation. While assessing runnel effectiveness will not be a part of this project, as runnel restoration can take up to 5 growing cycles for vegetative response, the data collected for the field study of megapools will act as the baseline data. Other secondary objectives include understanding if mosquitoes utilize megapools and how restoration will impact them, to appease concerns from regulators and community members, and also to collect baseline data that can be used by another lab to assess differences to remote sensing drone work. To explore these hypotheses, we selected 6 Partially formed megapools, 6 Fully formed megapools, and 3 Nonformed to be assessed. A subset of 2 Partially formed and 2 Fully formed will be used for the planting experiment. To address these goals, I have developed several specific primary and secondary objectives:

Primary Objectives:

- 1) Explore Differences in characteristics such as vegetation, hydrology, elevation, soil, and sulfides at varying pool formation levels and elevations by measuring characteristics
- 2) Assess survival and growth of plantings at different elevations (High and Low) and spacings (Clumped and Dispersed) as a potential restoration technique for areas with pool formation

Secondary Objectives:

- 3) Collect Baseline data for future work in assessing the effect of runnel installation on parameters measured in Obj. 1 & 2 that will be repeated after construction
- 4) Understand if megapools provide habitat for mosquitoes, and how runnels may impact this to help with community perception of marsh restoration projects
- 5) Provide baseline data from Obj. 1 for use in comparing to remote sensing drone work

STUDY SITE:

This project was conducted at a 40-acre privately owned marsh 15 miles south of Ocean City, Maryland referred to as Rum Pointe. Other names colloquially include “Green Point, Green Pointe, and Rum Point.” Like many nearby marshes, it was ditched in the 1930’s and is experiencing vegetation die-off in distinct shallow ponded areas. These shallow ponded areas lack vegetation and have been coined “megapools”. They are prevalent; however vegetation dieback is not uniform with other nearby areas still Partially or even entirely vegetated.

Historically, Rum Pointe acted as an important nesting habitat for birds of concern such as Salt Marsh Sparrow (Petitioned for listing under the Endangered Species Act in 2023) and Black Rail (listed as threatened under Endangered Species Act 2020). Black Rail are still found on nearby Assateague State Park lands, however they have not been found on Rum Pointe Marsh in the last 30 years, most likely due to degraded habitat. This marsh is also located near many private residences, several roads, and the 611 Verrazano bridge. This community was particularly impacted by Hurricane Sandy due to degraded marshes. Restoration of this marsh will provide habitat for these key species, and improve coastal resiliency for people and infrastructure alike.

Additionally, this site is representative of what is occurring on other nearby marshes and acts as a great case study to better understand vegetation die off and potential restoration techniques. Our partners, U.S. Fish and Wildlife Service and Maryland Coastal Bays Program, plan to introduce this restoration effort on a larger scale should it be successful. Across the 40 acres, approximately 15 acres of it is on the southernmost portion with challenging site access. In the northern 25 acres, there is approximately 5 acres of upper marsh with trees and high elevation plants that do not have megapool formation, though tree die off from salt water intrusion is occurring. In the remaining 20 acres, 15 locations have been selected randomly from a list of 30 sites across the marsh. Of that selection, 6 areas have complete megapool formation,

6 areas have partial formation, and 3 Nonformed sites have no pool formation. The word Nonformed was chosen over control as these Nonformed sites have also undergone marsh management such as mosquito ditches, where as a true control site would denote an unditched marsh. However, unfortunately there are no unditched marshes in this area. While restoration and drone capture will occur on the entire of the 35 acres (minus the 5 acres of upper marsh), more intense data collection will only occur at these 15 sites.

PARTNERSHIPS:

This project started in winter of 2020 as part of an interdisciplinary workgroup. The workgroup SMART-TEAM (Salt Marsh Assessment and Restoration Team) focuses on restoring salt marshes in the Maryland Coastal Bays. The team is made up of partners from local, state, federal, academic, community, non-profit, and more. Workgroups of this type are crucial for successful marsh restoration, and restoration projects that include community members and regulators have better success ([Maher et al., 2022](#)). This study site was selected because of its location in priority habitat for salt marsh sparrow and black rail, it is representative of adjacent areas, size, ease of access, and because of the existing relationship with landowner. Current partners include: National Park Service, Towson University, Audubon Mid-Atlantic, Maryland Department of Natural Resources, U. S. Fish & Wildlife Service, Maryland Coastal Bays Program, University of Maryland-College Park, University of Maryland Center for Environmental Studies, Lower Shore Land Trust, Maryland Department of Agriculture Mosquito Control Division, National Marine Fisheries Service, U.S. Army Corps of Engineers, Western Maryland Resource Conservation and Development Council, and Maryland Department of the Environment. Funding for this project came from the U.S. Fish & Wildlife Service: Partner's for Fish and Wildlife and the Regional Headquarters Office.

THESIS ORGANIZATION:

Chapter 1, this chapter, explores background information in the literature and outlays objectives for the following chapters. Chapter 2 explores Primary Objective 1, differences in sulfide characteristics at varying pool formation and elevation levels. Chapter 3 explores differences from Primary Objective 1 more broadly in vegetation, hydrology, elevation, soil, and more at varying pool formation and elevation levels as well as the Secondary Objective 4 of mosquito presence. Similarly, Secondary Objective 5 of remote sensing baseline data will utilize data collected in Chapter 3. Chapter 4 explores survival and growth of plantings at different elevations and spacings as a potential restoration technique for areas with pool formation from Primary Objective 2. Secondary Objective 3 of Baseline data for effect of runnel installation on parameters will utilize data from Chapter 2, 3, and 4 for future monitoring.

CHAPTER TWO: ASSESSING PORE WATER SULFIDE USING IRIS IN VARYING STAGES OF POOL FORMATION

INTRODUCTION:

In recent years, mid- and north-Atlantic coastal salt marshes have had an increasing number of internal ponds arise due to vegetation dieback (Himmelstein et al., 2021). This internal ponding seems to be connected to the presence of mosquito ditches, as placed in the 1930s by the civilian conservation corps and modified and maintained by local mosquito divisions as well as landowners (Besterman et al., 2022). Pools develop in interior areas of the marsh because they have decreased flushing and lower sediment input. Sidecasting from ditch construction can create uneven elevations with high elevations along the edge of ditches and lower internally on the marsh and can lead to uneven natural sediment accretion (Temmerman et al., 2012). This uneven elevation can lead to declines in flushing and increases in stagnant water-logging increasing salinity and sulfides which decreases oxygen and redox potential and decreases vegetation growth, vegetation health, increases sediment loss, and creates a negative feedback loop in inundated lower elevational locations (McKown et al., 2023).

Internal ponds that appear to have formed in response to ditching have been coined “megapools,” which distinguishes these anthropogenically influenced pools from naturally occurring pools or salt pannes due to their difference in biogeochemical characteristics and non-transitory nature (Burdick et al., 2020). There is ongoing concern about the increasing presence of megapools due to the rapid expansion of their presence as well as that they do not provide the same habitat benefits or other ecosystem services as natural marsh areas. For instance, inundated areas store less new carbon, have more subsidence, lower bulk density which can decrease sediment stability, and increased sulfides (Himmelstein et al., 2021; Vincent et al., 2013).

Megapools also may not provide habitat for nekton as natural ponds do due to their increased sulfide concentration (Baird et al., 2013; Himmelstein et al., 2021). Additionally, once present these megapools do not convert back to vegetated marsh without intervention and can therefore accelerate conversion of marsh to open water (Temmerman et al., 2012). As megapools increase in size, feedback loops lead to continued expansion as wind driven erosion, subsidence, and sediment loss accelerates Megapool growth (Mariotti & Fagherazzi, 2013). Megapools can appear in varying stages of formation converting from Nonformed Fully vegetated areas, to several small Partially Formed megapools, and then converting eventually to a stable state Fully Formed megapool, referred to as runaway pool fragmentation (McKown et al., 2023).

Sea level rise can decrease plant production through an increase of porewater hydrogen sulfide, which is toxic to plants (Morris et al., 2002). Research has also shown that areas with lower vegetation health such as megapools have varying biogeochemical features in soil and water such as lower stability, redox levels, shear strength, and can have higher sulfide concentrations (Berkowitz et al., 2018; Bradley & Morris, 1990; Vincent et al., 2013). However, in order to understand what is causing the conversion of vegetation to open water and develop restoration solutions may prevent this expansion, there is a need for research on sulfide concentrations in Fully Formed megapools, Partially Formed megapools, and areas without formation (McKown et al., 2023). Understanding the role of sulfides is important to understand how anthropogenic influences such as development, runoff, management, and restoration as well as sea level rise can impact marshes and nutrient cycling (Zhou et al., 2007). For instance, Puchoff & Lawrence 2022 showed that thin layer placement, at varying thicknesses in a microtidal *Spartina alterniflora* salt marsh, had lower sulfide concentrations and increased plant growth (Puchkoff & Lawrence, 2022).

Sulfate from seawater is a terminal electron acceptor during microbial respiration that is energetically favorable under the anaerobic and strongly reducing conditions in salt marsh soils (Howarth, 1984; Sapkota et al., 2022). Tides bring in ocean water containing sulfate (SO_4^{2-}) anions, which can then be reduced to intermediates SO_3^{2-} , $\text{S}_4\text{O}_6^{2-}$, $\text{S}_2\text{O}_3^{2-}$, and S during methanogenesis, providing energy for microorganisms (Jørgensen et al., 2019). A final byproduct of this cycle is S^{2-} , known for its negative impacts to vegetation (King et al., 1982).

In small doses, sulfidic conditions can increase *Spartina alterniflora* growth as resources are directed into aboveground biomass production rather than belowground biomass (Rolando et al., 2022). However in higher amounts, sulfides harm plant growth by reducing nitrogen uptake and preventing any biomass production (DeLaune & Pezeshki, 1994). In general, sulfides typically increase with depth (Rabenhorst et al., 2010). Presence of below ground biomass such as roots and rhizomes, and increasing production can help decrease sulfides (Alldred et al., 2020). Presence of sulfides can also impact plant community composition and health of plants. Gradients of *Spartina alterniflora* productivity occur in relation to frequency of flushing, where interior marsh areas may have higher sulfides and lower growth while areas closer to tidal creeks can have lower sulfides and higher growth (Rolando et al., 2022).

Sulfidic conditions can also impact nekton as sulfides typically can indicate declines in dissolved oxygen content in the water column which can lead to fish die offs; fish are typically resilient to changes in salinity and temperature (Grimalt et al., 1999). These impacts on nekton can in turn have ramifications for birds, mammals, amphibians, and reptiles that utilize nekton as a food source (Luther et al., 2004). In rare circumstances, high concentrations of sulfides can also have public health ramifications when in gaseous form of hydrogen sulfide. One case study of Rhode Island where a sewage effluent led to eutrophication, algal blooms, and decomposition

of biomass creating noxious hydrogen sulfide gas (Fulton et al., 2003). At our study site Rum Pointe, anecdotal observations showed several fish die off events of sheepshead minnows in pools. Sheepshead minnows, *Cyprinodon variegatus*, among other common marsh species, have been shown to be vulnerable to high levels of sulfide compounds (Baird et al., 2013; Tabak et al., 2003).

Indicator of Reduction in Soils (IRIS) is a technique where iron (Fe) oxide paint is applied onto polyvinyl chloride (PVC) films. Once painted, these films can be inserted into the ground where microorganisms through respiration utilize the Fe Oxides present, which strips the paint off of the film. The discoloration present can be used to assess reduction present. IRIS technique has also been modified for manganese where manganese oxides can be used, which may be useful when temperatures are too low for biological activity of iron oxides. The IRIS method is officially recognized by the National Technical Committee and used commonly for practitioners to identify hydric soils and note reducing conditions for wetland delineation (Rabenhorst, 2008).

While the main purpose of IRIS is to document reducing conditions, it can also be used to document concentration of soluble sulfide in porewater (Rabenhorst et al., 2010). The use of IRIS Fe Oxide films for sulfide determination was first noted when black discoloration was observed immediately after films were pulled from reducing conditions with sulfides present. This black pigment coating is FeS, and is present when Fe(III) reduces to Fe(II), and forms the solid phase FeS. This pigmentation disappears when oxidized in air, requiring speedy processing times (Sapkota et al., 2022). This black discoloration present can be used to assess porewater sulfide concentrations thanks to the model of standards developed by (Rabenhorst et al., 2022) where known concentrations have been correlated with coloration present. Other existing

methods for pore water sulfide both involve lab testing of water samples and include 1) extractors or “sippers” that collect porewater out but have poor resolution of specific depths and 2) equilibrium dialysis which place water in but require long time periods for equilibration. The benefits of using IRIS for pore water sulfides are that depth-specific concentrations are available in short time periods (5 minutes, 60 minutes, and 360 minutes) and require no additional post-sampling laboratory analysis (Rabenhorst et al., 2010). Additionally, they are easily replicable, and have standardized interpretation (Sapkota et al., 2022). While the IRIS sulfide method is a well-documented- technique (Rabenhorst et al., 2010), it has rarely been used to address variation in sulfide across environmental gradients at the ecosystem scale, particularly in coastal wetlands inundated with ocean-strength seawater like true salt marshes.

The amount of sulfide present can vary by frequency of tidal flushing, amount of sulfate in adjacent water column, geography, land use, vegetation, and more. For instance, freshwater wetlands typically have fairly low sulfide concentrations close to 0 while coastal salt marshes may have more (Lamers et al., 2013). In a brackish marsh in Edgewater, Maryland using IRIS films, sulfide concentrations were found to vary between 0 and 245 mg/L of sulfide (Rabenhorst et al., 2010). A brackish river system in New Orleans, Louisiana was found to have sulfide concentrations between 0 to 70 ppm using electrodes (Day et al., 2011). In North Carolina, a coastal salt marsh had much lower sulfide concentrations, closer to 10 ppm, varying based on amount of vegetation present and restoration status (Currin et al., 1995).

The objective of this study was to assess differences in sulfide concentrations in the marsh sediment pores between megapools at different stages of formation and between elevations levels within megapools, as well as to assess the feasibility of using IRIS to document sulfides at an ecosystem scale. We hypothesized that sulfide concentrations would increase with

decreasing relative elevations due to greater inundation and soil waterlogging. We also hypothesized that sulfide concentrations would be higher at greater soil depths than at shallower depths. Additionally, we hypothesized that sulfide concentrations would be higher in Fully Formed megapools than in Partially Formed or Nonformed locations, because of lower elevation and higher sulfides that caused plant dieback .

METHODS:

Study Site:

This project occurred at a 40-acre site known as Rum Pointe, approximately 24 km south of Ocean City, MD (Figure 2-1). This site is part of a larger research project assessing megapool stages as well as restoration solutions to improve vegetation health. It is a heavily ditched marsh that contains megapool stages representative of marshes in the surrounding watershed. In vegetated low marsh areas, the dominant vegetation is *Spartina alterniflora* with only two other species present *Spartina patens* and *Salicornia virginica* making up less than 5% of the total cover. High marsh is made up of *Spartina patens*, *Distichlis spicata*, *Iva frutescens* and *Phragmites australis* as the dominant species with other species making up less than 5% cover. Three soil profile descriptions were collected, using standard description methodology found in Schoeneberger et al., 2012, one at each of the three elevation positions for one Fully formed Megapool to be used as a representative understanding (Appendix A2-1) (Schoeneberger et al., 2012). The soil pedions showed that high and medium had a mineral layer Oi followed by an organic layer Oe, whereas low had no mineral layer only organic layer Oe. Similarly, looking at the Von Post assessment of decomposition, low had greater decomposition with higher Von Post numbers at 7 and 8 than medium at 5 and 8 and high at 5 and 7. While generally these soil profile

descriptions point to potential loss of mineral layer in lower elevations and greater decomposition, more replicates should occur before firm conclusions can be drawn.

The salinity of the adjacent water bodies was measured intermittently (n=7) around once every 2 months and fluctuated seasonally, with lower salinities observed in winter than summer. Ranges of salinities for ditches and nearby open water were 18.2-31.1 ppt, with Nonformed megapool sites (n=3) next ranging from 23.19-34.59 ppt. Partially formed megapools (n=6) were higher with 28.8-38.6 ppt, and Fully Formed megapools (n=6) were 29.3-39.7 ppt.

The marsh was divided up into 62 units as defined by areas contiguous between ditches, Sinepuxent bay, or high marsh (see definition in paragraph above), and each unit was given a number (Figure 2-1). High marsh areas were excluded and Units 37-62 were excluded for this study because their location made access for sampling impractical. In the field, each of the remaining 36 marsh units was then classified as one of three different megapool stages of formations: Partially, Fully, and Nonformed (Figure 2-2). Fully formed megapools were defined as marsh units with a singular large contiguous circular open water pool with less than 10% of vegetation cover or hummocks (small raised areas) within the open water. Nonformed marsh units were defined as vegetated areas with less than 10% of open water within the marsh unit vegetation. Marsh units with several fragmented irregularly shaped open water areas with more than 25% open water and vegetation cover separating fragments within the marsh unit were categorized as Partially Formed. Any marsh units with high marsh or in-between classifications were excluded. A random number generator was used to select 6 Fully, 6 Partially, and 3 Nonformed locations from the set of 36 marsh units (Figure 2-1).

Two transects were established in each marsh unit (for methodology see Chapter 3). Between each set of transects and centered, three study plots (High, Medium, Low) for IRIS

sampling were established per marsh unit (Figure 2-2). Using RTK survey equipment, elevations were taken across each plot, and the elevations at each position recorded in each marsh unit (Table 2-1.). In the Fully and Partially formed megapools, these plots correspond to points along an elevation gradient, with High Plot located near the edge of the marsh unit, Low Plot in the deepest part of the pool, and Medium Plot at an intermediate elevation. In the Nonformed megapools all three plots were at a similar elevation with High only slightly larger than Medium or Low which were identical. Based on presence and absence and percent cover of vegetation (see Chapter 3), High elevation was vegetated in all three megapool stages of formation. Low was unvegetated in Fully and Partially formed megapools but vegetated in Nonformed megapools. Medium, located in the transitional area from higher to lower elevations, was the divide between vegetated to non-vegetated in Fully and Partially formed megapools and always vegetated in Nonformed megapools. While Low plots in the Nonformed pool are hereby referred to as low, they were technically not statistically different from medium plots, both low and medium were different from high plots though.

IRIS Film construction, deployment, and analysis

Paint for IRIS films was prepared using the formulation described by (Rabenhorst & Burch, 2006), and was applied to 10 mil PVC film as described by Rabenhorst (2018). After drying, PVC sheets were cut to the appropriate size of 60 cm by 7.5cm. At this site, 15 interior marsh study areas were chosen to represent megapools at 3 stages of formation: 6 Fully Formed megapools, 6 Partially Formed megapools, and 3 Nonformed megapools. Study areas are distinct in that they are separated by ditches from other marsh areas and have 3 plots each (Figure 2-2). At each of the 45 plots, five replicate IRIS films were deployed at each location across three days in August of 2022, resulting in a total of 225 films deployed. Within each plot, 5 films were

randomly deployed in the ground for 5 minutes. Films were inserted into the ground using the approach described by Rabenhorst (2018) where, after first making a pilot hole with a 1” push probe, the IRIS films were inserted (Figure 2-3). After exactly 5 minutes, films were removed and rinsed in a basin where they remained submerged while removing any adhering sediment, and were quickly dried, and immediately scanned using a Fujitsu iX100 portable scanner (Figure 2-4). The total time from extraction of the film to completion of the scan was generally less than 30 seconds. Images were collected and stored as 300 dpi jpeg files.

These images then underwent a conversion to black and white binary image using Adobe Photoshop 2022 using Adobe Photoshop 2022 using the channel mixer function as described by (Rabenhorst et al., 2010). These converted images were then quantified using a MATLAB routine which compares the gray colors on the films to standard images prepared in the lab at specified sulfide concentrations. Some areas had such high levels of sulfides that the Fe oxides on portions of the deployed films were completely converted to FeS and stripped off the film leaving white areas, rather than black areas. Because white areas typically represent zones as having zero levels of sulfide, photos were edited using Adobe Photoshop to convert these white areas (in the midst of black zones) to also be black, for the purpose of analysis of the images (Figure 2-5). Sulfide concentrations were calculated for each image at 1 cm vertical depth increments along the length of the 50 cm film (Rabenhorst et al., 2022).

Data Analysis

Data were analyzed and plotted using R version "Spotted Wakerobin" using the R Studio 2022.07 interface. A mixed-model analysis of variance (ANOVA) was used to test the effects on sulfide of megapool stage, and elevation, depth, and their interactions using the lme4 package. Megapool stage was treated as a fixed variable, elevations a fixed variable nested within the

megapool stage, and depth as a fixed repeated measure variable. Repeated measures ANOVAs are typically associated with time, however here they are associated with depth. Post hoc means separation was performed using the Tukey test. Assumptions of normality and heterogeneity were met.

RESULTS:

Sulfide concentration increased with depth in all three megapool stages of formation regardless of transect position, megapool formation stage or initial sulfide levels (Figure 2-6). The sulfide concentrations show exponential growth from 0 to 20 cm depth, below which concentrations reach a steady state ($P < 0.0001$, Table 2-2). In Partially Formed megapools, high elevation level is similar in sulfide concentration to all Nonformed and high Fully Formed level levels for the first 20cm, however low and medium start with more sulfides overall and are similar levels, all three levels converge to high levels after 20 cm.

Sulfide concentrations were also related to megapool stage of formation ($P < 0.0001$) and transect position ($P = 0.0123$). Results show that Nonformed Megapool locations have similar sulfide levels across the three elevation levels at all 50 cm plateauing at 25cm. However, sulfide levels at positions Medium and Low were higher at shallow depths from 0-18 cm in Fully formed than in Partially formed megapools, which in turn were greater than in Nonformed megapools, resulting in a three-way interaction ($P < 0.0001$; Table 2). Fully formed megapools on the other hand have higher sulfide concentrations at the high elevation level for the first 18cm similar to all Nonformed and high Partially Formed level levelss, however are similar to the sulfide levelss occurring at the medium and low elevation levels for 18-50cm. For example, in positions Medium and Low at 10 cm depth, sulfide levels were about 500-550 ppm in Fully formed megapools, 400 ppm in Partially formed megapools, and 25 ppm in Nonformed

megapools. There was little difference in vertical sulfide profiles between positions Medium and Low in any megapool stage. Sulfide profiles at position High, the vegetated position for all marsh units, were similar in Partially and Nonformed megapools, but started higher and increased more rapidly with depth in Fully formed megapools.

DISCUSSION:

The difference between megapool stages of formation shows that elevation is closely linked to sulfide levels. This presumably occurs due to frequency of flushing, low elevations internal to the marsh have lower flushing and increased sulfides whereas high elevations on the exterior marsh have increased flushing and decreased sulfides (Rolando et al., 2022). Partially formed megapools at high elevation levels had similar sulfide concentrations to high, medium and low elevation levels of Nonformed and high Fully Formed elevation positions for the first 20 cm. This difference in sulfide levels between stages of megapools has been documented in other research, which found sulfide concentrations to be twice as high in porewater sulfide in unstable ponds than vegetated stable areas (Himmelstein et al., 2021). It is notable that the Nonformed location, where all three elevation levels had vegetation present, had similar sulfide profiles across depths and levels. On the other hand, medium and low elevation levels of Partially and Fully Formed were similar.

Belowground biomass can help to lower sulfides, however alternatively, high presence of sulfides can lower biomass. It is difficult to entangle which relationship precedes which (Berkowitz et al., 2018). The relationship of depth and elevation corresponds closely to vegetation presence. High elevation areas were Fully vegetated while medium and low were not. The sulfide concentrations present were extraordinarily high compared to measurements in other coastal wetlands. For instance, in a brackish marsh in Edgewater, Maryland, sulfide

concentrations approached 245 mg/L at their highest (Rabenhorst et al., 2010). In A New Orleans, Louisiana concentrations approached 70 ppm at their highest, and in North Carolina up to 10 ppm (Currin et al., 1995; Day et al., 2011). It is notable that the depths 0-10 and 10-20 cm where roots are present are biologically meaningful because of root and rhizome presence which can decrease sulfide levels (Aldred et al., 2020). There may be lower sulfur levels when vegetation is present and higher levels when roots are not prominent and have less variation between elevation levels showing similarity. Alternatively, high sulfides can lead to die off which could also cause decreased vegetation presence (Rolando et al., 2022). From 20-50 cm, when roots and rhizomes are not prominent, the sulfide levels increase. In areas where there is not vegetation, such as low elevation position of Partially and Fully Formed, this same pattern is not seen as the 0-20 depth sulfide concentration is much higher because of the lack of rhizomes and roots. It is also notable that the exact depth where the jump to plateau occurs differs across the elevation levels. At the Nonformed megapool levels the plateau occurs around 25cm whereas the Fully Formed megapools show the same at 18 and Partially at 20 cm.

There is a dip present in the low elevation sulfide levels seen after the 30cm mark of the Partially Formed and 45 cm mark of the Nonformed. This seems to be due to the lower sulfide of marsh units numbered 27 and 28 for Partially Formed and 26 for Nonformed (Appendix Figure A2-1, A2-2, A2-3). It is possible that these have lower sulfide levels or that the analysis is an artifact of the challenges with the model at extraordinarily high sulfide levels. An additional observation is that these three sites are in close geographic proximity and therefore could have different environmental characteristics such as less advanced degradation.

Some possible limitations of the study include: white editing technique, timing, damage during deployment, and modeling. In areas with high sulfides where films became white, photos

were edited to black for analysis which could cause some uncertainty in the exact modeled values. Current IRIS sulfide models allow for 3 different deployment times with 5 minutes as the shortest. At such high sulfide concentrations, a shorter deployment time would have been used to avoid this issue of white stripping. However, because the time required to extract and scan the films is around 30 seconds, deployments shorter than 5 minutes are not practicable. Some films had white lines from scratching during use, however, the surface area of scratches is relatively low and the scratches were prominent on all films meaning data is comparable to one another.

One area that could be explored more is seasonal and temporal variations of sulfides. During cooler temperatures, biological activity, which plays a crucial role in sulfide cycling, is lower (Stein et al., 2007). However less is known about day to day variation, and this methodology represents a snapshot in time. Two plots received 5 IRIS films replicated across two separate days and they were comparable. However, further exploration for temporal variations across days, seasons, and years could be useful.

In order to restore megapools back to vegetated areas of marsh, it is evident that sulfides must be lowered by decreasing inundation and soil water logging, increasing tidal flushing (Campbell & Wang, 2019). Additionally, this increased tidal flow will improve conditions for vegetation to grow biomass and capture sediment while also improving sediment flow during high tides which will improve elevation capital (Vincent et al., 2021). Restoration solutions, such as runnels, that improve this water flow and vegetation health should be explored (Perry et al., 2021; Puchkoff & Lawrence, 2022).

CONCLUSION:

In Maryland Coastal Salt Marshes and throughout the Mid and North Atlantic, there has been a rapid increase in areas with low vegetation coverage and increasing bare ground or open

water in between mosquito ditches coined megapools. The mechanisms for Megapool formation are an ongoing topic of research with knowledge that varying elevations and biogeochemical factors such as high sulfides (S^{2-}) can impact vegetation health and growth and vice versa. Here we sought to explore differences in sulfide concentrations to better understand the impacts of differences in stages of formation and elevation positions. Using IRIS films painted with Fe Oxide to assess precipitation of Fe when Fe(III) reacts with porewater sulfide, we tested sulfide levels at three stages of formation: Fully Formed, Partially Formed, and Nonformed. This led to testing of 15 marsh units and at three elevation levels (High: Fully Vegetated, Medium: Vegetated-Non-vegetated divide, Low: Open-water/Bare-ground) totaling 45 plots. Within each plot, 5 replicate films were used to account for heterogeneity in the soils resulting in 225 films used in total. We found that Sulfide concentrations increased with depth in all megapools and at all elevations starting between 0-400 near the surface and increasing until a plateau was reached around 20cm at concentrations of 500-700ppm. We found that Nonformed areas had similar sulfide concentrations across all three elevation levels while Fully and Partially had differing high sites from low and medium. We also found that Fully Formed pools had higher concentrations in low and medium areas than Partially Formed. These results support a close relationship between pool stages of formation, vegetation health, and sulfide levels.

FIGURES:



Figure 2-1

This map details the location of the study site as well as the marsh unit numbers. The color of the marsh unit numbers corresponds to the stages of pool formation for the 15 study locations. Presence of megapools and ditches can be seen visually on the aerial imagery.

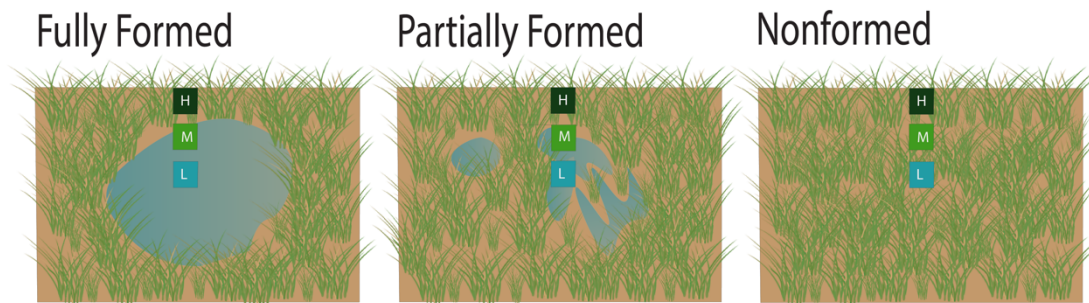


Figure 2-2

Megapools were given one of three categories (Fully, Partially, or Un Formed). Within each megapool, 3 plots were established in each marsh unit. High signifies Fully vegetated and highest elevation. Medium for Partially and Fully Formed signifies area between vegetated and non vegetated divide at medium elevation, and low signifies open water or bare ground with no vegetation at lowest elevation. For Nonformed, low and medium elevations were lower than the high areas but not different from each other in elevation and were still Fully vegetated.



Figure 2-3

Photo of Jordan Kim, University of Maryland Graduate Student, inserts an IRIS film into the ground



Figure 2-4

Katherine Stahl (Far Left Dark Blue Shirt) finishes the field scan of a recently pulled IRIS Film while Sophia Seufert (Center Lower Light Blue Shirt) of U.S. Fish and Wildlife Service washed the next IRIS Film Strip. Nuelle Johnson (Center Upper Black Shirt), a University of Maryland undergrad, acted as the timekeeper verbally calling out how much time was left. In the background, a megapool with a water logger is present. Though this protocol technically requires only 2 people, having 4 people kept stamina high for many long field days in the heat.



Figure 2-5

Process for IRIS analysis. Raw IRIS films with white sulfide removal are edited to be black, and then undergo a grayscale conversion before uploaded to sulfide calculator.

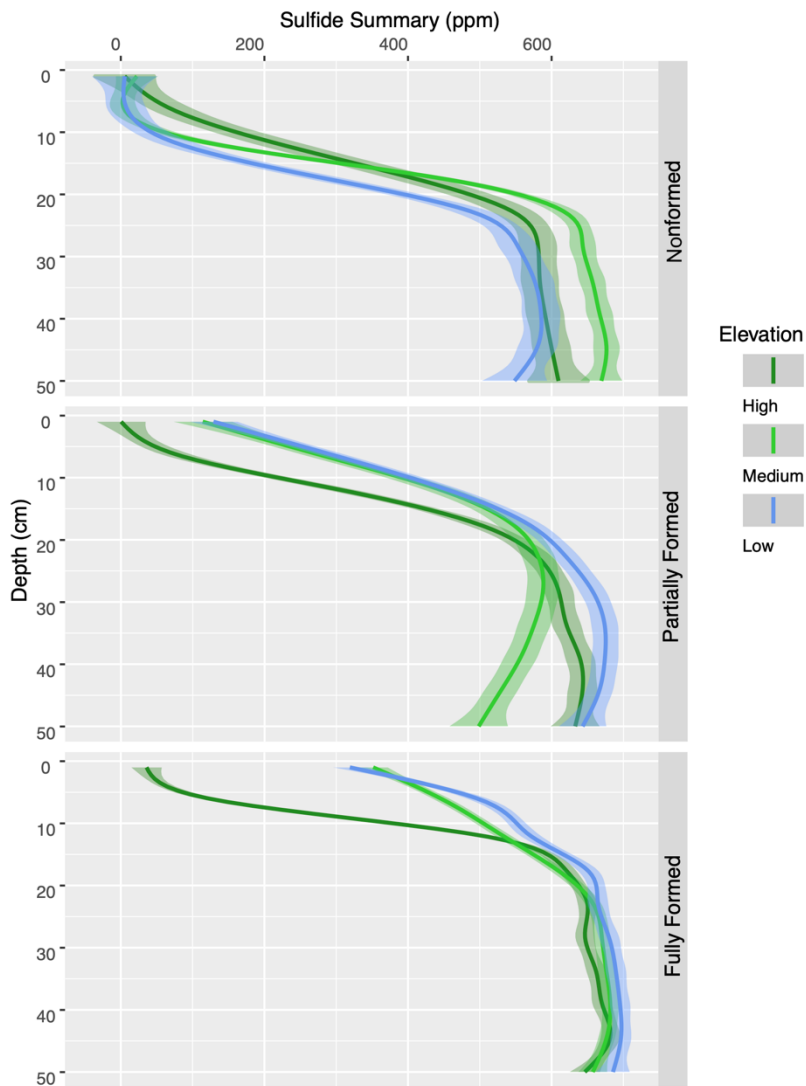


Figure 2-6
Sulfide levels in ppm at depth cm averaged across each megapool type

TABLES:

Table 2-1

Shown here is the data from elevation taken at each plot in meters averaged across each type of megapool

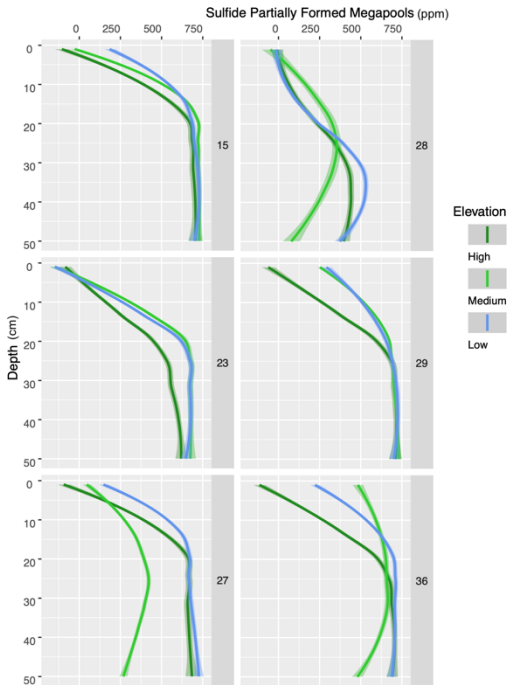
	Nonformed (n=3)	Partially Formed (n=6)	Fully Formed (n=6)
High	0.61m	0.62m	0.58m
Medium	0.58m	0.50m	0.46m
Low	0.58m	0.43m	0.30m

Table 2-2

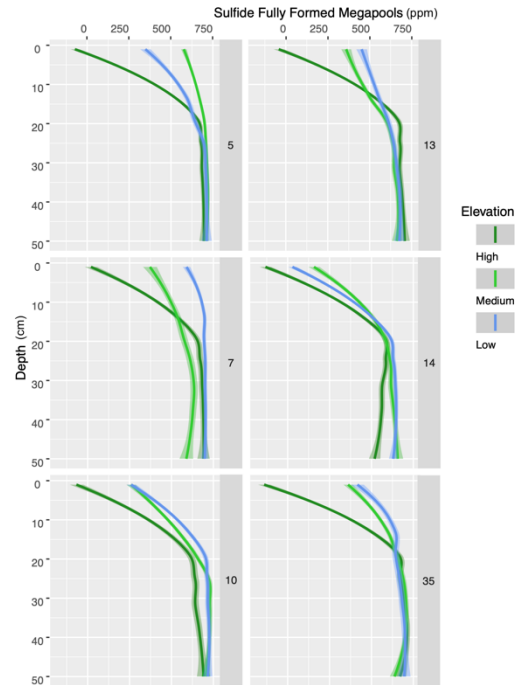
This analysis of the variance table depicts the results of a repeated measures anova assessing sulfide against depth, Megapool, elevation, and their interactions.

	Sum Sq	Mean Sq	NumDF	DenDF	F	P
Depth	236095687	236095687	1	11016.0	4887.2180	<0.0001
Megapool	1048783	524392	2	37.3	33.0660	<0.0001
Elevation	157250	78625	2	37.3	4.9578	0.0123
Depth:Megapool	16049948	8024974	2	11016.0	506.0217	<0.0001
Depth:Elevation	4030967	2015484	2	11016.0	127.0881	<0.0001
Megapool:Elevation	183368	45842	4	37.3	2.8906	0.0352
Depth:Megapool:Elevation	6888041	1722010	4	11016.0	108.5828	<0.0001

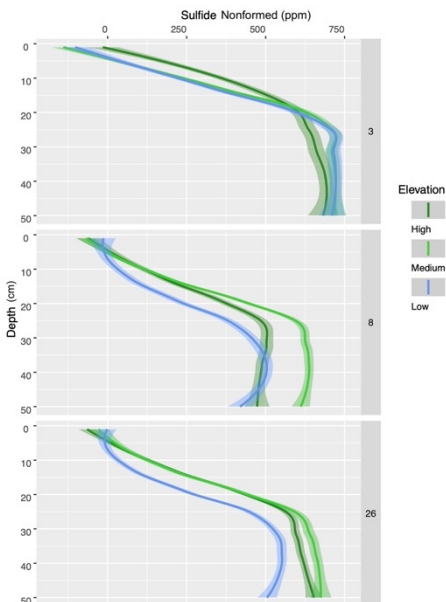
CHAPTER TWO APPENDIX:



Appendix Figure A2-1. Sulfide levels in ppm at depth cm in Partially Formed Megapools for all transects



Appendix Figure A2-2. Sulfide levels in ppm at depth cm in Fully Formed Megapools for all transects



Appendix Figure A2-3. Sulfide levels in ppm at depth cm in Nonformed Megapools for all transects

Appendix Table A2-1 of Soil Morphological Descriptions

This Table show cases the soil morphological descriptions for three soil pedons one at high, medium, and low elevations of a fully formed Megapool.

Horizon #	Method	Horizon	Boundary cm	Class	Color	Von post	Rubber fiber	Fluidity	HCL Aroma	H2O2 reaction 30%
1	50 MC	Oise	30	P	2.5Y 3/2	H5	60	-	2	VS
2	50 MC	Oese1	47	MP	2.5Y 3/1	H7	38	-	2	NE
3	50 MC	Oese2	75	MP	2.5Y 3/2	H7	35	-	2	NE
4	50 MC	Aseg	91	SiCL	5Y 3/2	-	-	VF	2	SL
5	50 MC	Oase	144	M	10YR 2/1	H8	20	-	2	NE
6	50 MC	Ase	186	SiL	2.5Y 3/1	-	-	VF	2	NE
7	50 MC	O'ase	206* +	M	10YR 2/1	H9	15	-	2	NE

Classification: Typic Sulfihemist **Elevation:** High **Completed by:** Jordan Kim **Notes:** * denotes Sand Lense at 201cm

Horizon #	Method	Horizon	Boundary cm	Class	Color	Von post	Rubber fiber	Fluidity	HCL Aroma	H2O2 reaction 30%
1	50 MC	Oise	31	P	10YR 3/2	H4	55	-	2	NE
2	50 MC	Oese	72	MP	2.5Y 3/2	H8	30	-	2	NE
3	50 MC	ACse	87	SiCL	5Y 3/1	-	-	VF	2	VS
4	50 MC	O'ese	123	MP	10YR 2/1	H6	39	-	2	NE
5	50 MC	Oase	153	M	10YR 2/1	H10	10	-	2	NE
6	50 MC	Ase1	175	SiL	2.5Y 3/1	-	-	VF	2	NE
7	50 MC	Ase2	188+*	SiL	2.5Y 3/1	-	-	VF	2	NE

Classification: Typic Sulfihemist **Elevation:** Medium **Completed by:** Jordan Kim

Horizon #	Method	Horizon	Boundary cm	Class	Color	Von post	Rubber fiber	Fluidity	HCL Aroma	H2O2 reaction 30%
1	50 MC	Oese1	37	MP	10YR 2/2	H7	35	-	2	-
2	50 MC	Oese2	65	MP	10YR 3/2	H8	30	-	2	-
3	50 MC	Aseg	72	SiCL	5Y 3/1	-	-	VF	2	ST
4	50 MC	Oise	93	P	10YR 2/1	H2	55	-	2	-
5	50 MC	O'ese	136	MP	10YR 2/1	H8	25	-	2	-
6	50 MC	Oase	149	M	2.5Y 2.5/1	H10	10	-	2	-
7	50 MC	Ase	179	SiL	2.5Y 2.5/1	-	-	-	2	-
8	50 MC	O'ese	214+	MP	10YR 2/1	H9	20	VF	2	-

Classification: Typic Sulfihemist **Elevation:** Low **Completed by:** Jordan Kim

Abbreviations Present in Table Above:

Class Abbreviations: M-Mucky, P-Peat, Si-Silt, C-Clay, L-Loam

Von Post Humification Scale: Degree of Decomposition from H1 to H10 where H1- Completely Undecomposed to H10-Completely Decomposed, and H2-H9 represents intermediate

Fluidity: VF- Very Fluid

H2O2 Reaction 30%: VS-Very Strong, ST-Strongly, SL-Slightly Effervescent, NE-Non Effervescent

CHAPTER THREE: CHARACTERISTICS OF MEGAPOOLS IN THREE DIFFERENT FORMATION STAGES AND ACROSS THREE DIFFERENT ELEVATIONS

INTRODUCTION:

While salt pannes, ponds, and pools are an important natural part of marsh ecosystems, in the last twenty years a sudden influx of rapidly expanding pools adjacent to mosquito ditches has been a cause for increasing concern, especially in the face of sea level rise (Coverdale et al., 2013). These pools have been nicknamed “Megapools” to distinguish them from naturally occurring ones (U.S. Fish & Wildlife Service, 2020a). Most notably, these megapools do not have the same biogeochemistry, for example higher in salinity, as naturally occurring ones and therefore may not provide the same habitat or ecosystem benefits (Vincent et al., 2013).

Megapools are also associated with an increase of marsh loss through gradual permanent conversion to open water (Himmelstein et al., 2021). Beyond gradual conversion, megapools also make marshes less resilient to storm events, which can lead to rapid acceleration of decline (Day et al., 2011). Marshes with megapools and ditches typically also have a lower lifespan and are less resilient to sea level rise (Ganju et al., 2020). This mix of both gradual and sudden events, or punctuated equilibrium, means there is no correlation between pond age and depth but instead with size, elevation decreases, and tidal connection width (Schepers et al., 2020). Open pools can also sometimes act as a habitat location for mosquito larvae which can present a public health risk (Wolfe, 1996).

When pool formation occurs, there appears to be a threshold or “tipping point” where the marsh can no longer recover because of several feedback loops, sometimes called “Runaway Marsh Fragmentation” (Vincent et al., 2021). Feedback loops can include increased fetch increasing wind driven erosion, sediment loss, subsidence, and biogeochemical changes

(Mariotti & Fagherazzi, 2013). Marshes with many smaller pools may be at the initial stages of pool formation before merging and approaching an end state of large pools (Himmelstein et al., 2021). Increased inundation can lead to vegetation dieback through changes of water and soil characteristics that decrease plant health. Rhizomal plants share nutrients and can oxygenate soil, and when dieoff occurs can also impact adjacent plants (Darby & Turner, 2008). In turn, vegetation dieback increases unconsolidated sediments as belowground biomass becomes oxidized and roots do not hold sediment down which can then be exported through erosion lowering elevation capital. As pond size increases, sediment export increases as wave action from tides and wind can increase erosion, which increases the size and depth of channels and ponds creating a cyclic interaction of evergrowing pools (Schepers et al., 2020). While in natural ponds elevation can remain more constant, ponds caused by increased inundation can continue to decline in elevation (Burdick et al., 2020).

Other potential causes of vegetation dieback include grazing, biogeochemical changes, submergence, or burning. Ditches in a marsh can alter hydrology, decreasing sediment accretion and creating uneven distribution (Day et al., 2011). This uneven distribution of sediment, in combination with spoil piles from construction of ditches, can create levees along edges of the ditches leading to submergence, accretion deficit, and biogeochemical changes (Wigand et al., 2017). This waterlogging stress in turn leads to vegetation dieback internally on the marsh, while exterior areas along edges of the ditches did not experience this (Schepers et al., 2020).

Common assessments of marsh degradation include assessing elevation, vegetation, hydrology, and soil through various metrics such as biomass, biogeochemistry, and soil strength. Marsh degradation has been found to be greater in areas with ponds, and lower in areas without ponds (Himmelstein et al., 2021). In comparison of two sites at varying degradation levels, one

study found that bulk density was higher in the 0-5cm range for the less degraded site than the more degraded site and had lower organic matter (Day et al., 2011). Similarly, Burdick et al., 2020 found that inundated sites had soils with decreased bulk density and carbon storage.

Additional measures can include those of productivity for belowground biomass, such as root cores (Blum, 1993). In *Spartina alterniflora* systems, a majority of root biomass is in the 0-10cm depth range with the biggest growth in July to September with less Nitrogen (N) found in belowground than above ground (Darby & Turner, 2008). Nitrogen and carbon are two important indicator elements for plant production in terms of nutrients as well as organic matter accumulation within a wetland. Because some research suggests that organic matter, bulk density, and carbon presence may be impacted by degraded marsh and vice versa, this has crucial implications for blue carbon calculations as well as understanding of ecosystem services (Day et al., 2011).

Hydrology characteristics are another important variable in understanding marsh degradation. The presence of additional ponding indicates changes in hydrology and can lead to stagnant tidally disconnected water that has different biogeochemistry. For instance, stagnant water may have lower dissolved oxygen, increased salinity, and increased sulfide concentration as compared to open water (Burdick et al., 2020; Himmelstein et al., 2021). Dissolved oxygen and sulfide concentrations can impact the distribution of vegetation, such as mangroves in the Bahamas (Nickerson & Thibodeau, 1985). Additionally, too regular inundation can prevent recolonization and accelerate decay of propagules of *Spartina alterniflora* as found in coastal Louisiana (Patterson et al., 1997).

Understanding these mechanisms are crucial to appropriately apply restoration solutions. For megapools that have lost extensive elevation, revegetation through plantings or time may be

impossible without hydrologic or restoration alterations (Schepers et al., 2020). Several “low tech restoration solutions” such as runnels or channel alterations may be appropriate for marshes that have lost moderate elevation capital as they work by increasing drainage and sediment flow (Campbell & Wang, 2019). Runnels can help to increase vegetation cover and therefore elevation, as well as lower inundation (Perry et al., 2021). More importantly, for microtidal marshes that rely on organic matter for elevation increases, they may facilitate revegetation where the tipping point has not surpassed (Vincent et al., 2013). For more degraded marshes, more extensive alteration to increase elevation capital through thin layer placement (also referred to as beneficial re-use of dredge material) may be necessary (Puchkoff & Lawrence, 2022).

The primary objective of this study was to better understand causes of megapool formation by examining different characteristics between varying levels of pool formation. To do this, characteristics of megapools were explored such as water, soil, vegetation, elevation, and more at three stages of formation: Fully Formed, Partially Formed, and Nonformed. The secondary objective of this study was to obtain baseline data regarding pool characteristics prior to the implementation of restoration solutions to allow for apt comparison before and after as compared to Nonformed. We hypothesized that Partially Formed megapools would be more similar to Fully Formed megapools than to Nonformed pools. We additionally hypothesized that vegetation presence, above ground biomass, elevation, and soil health will be greater in Fully Formed than Partially formed or Nonformed.

METHODS:

Study Site:

The study site for this project is Rum Pointe, a 40-acre privately owned property in Berlin, Maryland which is 24km south of Ocean City, MD (Figure 3-1). In this area, almost all

marshes were mosquito-ditched and are currently experiencing megapool formation (Figure 3-2). This site was selected due to its historical importance for nesting black rail, a species listed under the endangered species act, and for its potential to be restored. The marsh is mostly “low marsh” dominated by *Spartina alterniflora*. Areas of “high marsh” occupy a fraction of the site and are dominated by have *Spartina patens*, *Distichlis spicata*, *Iva frutescens* and *Phragmites australis*. Soil profiles were completed (see appendix 1 in Chapter 2 for more detail).

The marsh was separated into 62 marsh units which are areas of marsh separated by ditches, Sinepuxent bay, or high marsh. All marsh units were assigned a number (1-62), and any units with high marsh were excluded as well as units in the southern portion of the site (37-62) due to their challenging site access. All remaining units were field-categorized into three stages of formation, hereafter referred to as “megapool type”. 1) “Fully Formed” megapools were where a large center pool in a marsh unit was surrounded by vegetation to boundaries with less than 10% vegetation inside the open water area. 2) “Partially Formed” megapools were where several small to medium center pools were surrounded by vegetation with more than 25% open water and vegetation cover separating fragments. 3) “Nonformed” megapools were contiguous vegetated areas with less than 10% of open water within the marsh unit. Any units with intermediate categories were excluded. From this set of units, a random number generator was used to select 6 Fully Formed, 6 Partially Formed, and 3 Nonformed marsh units

Two transects and nine plots were established at each of the 15 study sites (Figure 3-3). For methodology on the 3 IRIS plots, see Chapter 2. A line intercept method was developed based on (Kercher et al., 2003; Shuman & Ambrose, 2003) where a baseline tape of 34m was laid out running east to west parallel with mosquito ditches on the southern portion of megapools. Using a random number generator, transect positions were determined where they

would be at least 5m from either side of the end and 5m from each other. Transects were then laid north to south at the random transect position with stakes placed at northern and southernmost point. To establish plots, elevations using an RTK Survey unit were taken across each transect and plot. Plots were established at High, Medium and Low elevations along each transect. High plots for all three formation stages were located at northernmost portion (34m) of the transect immediately adjacent to the stake and all Fully vegetated at high elevations. Medium plots were located at an intermediate elevation for all three formation stages and located right at the divide between vegetated and nonvegetated. Low plots were located at a low elevation for the Fully Formed and Partially Formed stages and located in open water inside the pool. Low plots for the Nonformed megapools were located at the same approximate location as the Fully Formed and Partially Formed low plots but were instead Fully vegetated.

Vegetation:

Starting at the southernmost portion (0m) of the transect, and walking northward (34m), presence or absence of each species in each 0.25-m long by 0.5m wide segment of the transect along 0.25-m of either side or any portion of the species was be noted using 1 (present) and 0 (absent) (Kercher et al., 2003; Shuman & Ambrose, 2003). Stakes were placed on the north and southern ends of these transects and latitude and longitude coordinates as well as elevation using the RTK survey unit were taken. Starting at the southernmost portion (0m) and walking northward (30-34m) presence or absence of each species or any portion of the species will be noted using 1 (present) and 0 (absent) in .25m increments. Bare Ground/Open water was recorded if it occupied greater than contiguous 15% coverage. Cover was determined at the 0m, 10m, 20m, and 30m marks using a 0.25m x 0.25m quadrat centered on the tape for percent cover using 5 categories: 0-10%, 10-25%, 25-75%, 75-95%, 95-100%.

Below Ground Biomass:

Root ingrowth cores were prepared by placing nursery grade peat saturated with water into mesh bags 5 cm x 30 cm packed with a disk attached to a dowel rod to remove water and stay rigid before being placed in a 60C oven for three days. Pilot holes were excavated in the marsh using a soil auger and then cores were placed into the ground using an aluminum 5 cm x 30 cm pipe for 1 year inserted in April 2022 and removed in March 2023. After cores were moved, they were refrigerated until processing. Processing included washing to remove sediment, and then separating roots by two sizes: fine and rhizomal, across three depths: 0-10, 10-20, 20-30.

Water Characteristics:

Using a YSI probe, salinity, temp, conductivity, %DO, DOmg/L, TDS, SPC, and pH were collected at each marsh unit, and in two ditches within the study area for comparison. This process was repeated along the 15 marsh units approximately 8 times a year when time and conditions allowed. This allowed for comparisons of ditches to study sites. It was important to collect these measurements during a variety of tidal times to get an accurate picture of impact or nonimpact of tidal fluctuations.

Soil Characteristics:

Soil samples were collected at the HML plots with a 3.8cm diameter aluminum corer at approximately 20 cm long. Samples were then dried to a constant mass 105C and sieved through a 2 mm sieve. A weighed subsample of each sieved soil was placed in crucible, which were ashed in the furnace at 550C for 4 hours, placed in a desiccator, and recorded to calculate for bulk density and organic matter (Heiri et al., 2001; Sutton-Grier et al., 2011). Samples were

analyzed for total carbon and nitrogen using a LECO C-N analyzer which measures combustion through an elemental analyzer.

Elevation:

Elevational data was collected across each transect up to the corresponding ditch using a survey grade Real Time Kinematics (RTK) positioning unit. Points were taken approximately every 2m or where a vegetational or other topographic change was noted across each 30m transect. This allowed for a cross sectional analysis of elevation to formulate a better understanding of within pool differences and between pool differences as well as making assumptions about the impact of lack of vegetation on elevation. It will also provide a baseline against which to measure elevation changes resulting from planting or runnel construction. Surveys were completed at low tide and care was taken in unvegetated areas to keep the unit on the surface of the soil.

Mosquitoes:

Mosquito data was collected using dippers, a tool used for easy collection of larvae and water, where a wooden handle is attached to a white plastic bowl measuring 5.08 cm by 11.43 cm (2" by 4.5") with a spout. A dip, refers to placing the dipper in the water on a 45 degree angle taking care to avoid creating big splashes or shadows which can cause disturbance to larvae and impact data. Along each transect where water was present, 2 dips were performed approximately 3 m apart for 8 samples total and assessed for larval presence 4 days after the lunar high tide during August and September. Maryland Department of Agriculture sent a mosquito biologist to perform identifications on site, and vials of ethanol alcohol labeled were prepared for collection of larvae for taking back to the lab.

Water Levels

Hobo water loggers were placed in each of the 15 locations in a well tip, as well as one air pressure logger, and one placed in open water in the closely located green point creek.

Loggers in the 15 locations collected water depth, duration of inundation, temperature, and tidal fluctuations with 15 minute intervals. The air logger helps calculate water depth by calculating air pressure to be used in the standardization equation. The open water in the creek showed normal tidal variation and allow for extrapolation of the difference of inundation in the locations as compared to the creek.

Data Analysis

Data were analyzed and plotted using R version "Spotted Wakerobin" using the R Studio 2022.07 interface.¹ Assumptions of normality and heterogeneity were assessed and met. A mixed-model analysis of variance (ANOVA) was used to test the effects on megapool type, date, and their interaction on species composition of vegetation using the lme4 package. For root cores, a three way ANOVA was used using the base R analysis testing the effects of elevation, megapool, and depth on the weight of root ingrowth cores nesting elevation within megapool. For YSI data, ranges, median, and mean were calculated. For water characteristic data, pressure was converted to water depth and standardized by sea level before being analyzed by megapool type. Due to the lack of mosquitoes found, no analysis was needed for mosquito data. For soil data, base R analysis and a regression using package ggpubr was used to compare relationships. For elevation data, a one way repeated measures anova using the lme4 package was used to test for elevation differences between megapool types. Post hoc means separation was performed using the Tukey test. Conversion of characteristic data for megapool (Nonformed, Partially Formed, Fully Formed) and elevation (High, Medium, Low) was converted to integer data by

classifying the least degraded marsh as 1 and the most degraded marsh as 3 with intermediate as 2. These values were then converted to establish an additional characteristic, degradation index, for the 9 crosses between each megapool and elevation type. This value was established by adding the value in each of the two variables and dividing by two. A correlation matrix was completed using the corrplot package inputting Rootcore, Plant Coverage, Bulk Density, Carbon Density, Organic Matter, Carbon, Nitrogen, Elevation, Degradation Index, Megapool, and Sulfide into one analysis allows for understanding of ecological interactions.

RESULTS:

Vegetation:

Megapool types were initially classified by vegetative cover and open water presence. Total plant cover varied between megapool types, confirming initial classifications ($p = 0.04$): Fully formed megapools (which included the surrounding vegetation in the marsh unit) had less than 40% plant cover, Partially Formed units had about 85%, and Nonformed units were almost 100% vegetated (Figure 3-4; Table 1). Megapool types differed in cover between 2021 and 2022 ($p = 0.003$), but the pattern of variation between megapool types differed between years, resulting in a significant interaction between megapool type and date (Table 1); Partially Formed megapools increased in total cover between 2021 and 2022 ($p = 0.0014$), while neither Nonformed nor Fully formed megapools increased in total cover between 2021 and 2022 ($p = 0.9992$, and $p = 0.7080$). Fully Formed and Partially Formed megapools as well as Fully Formed and Nonformed megapools were significantly different from one another in total cover in both 2021 and between 2021 to 2022 ($p < 0.001$ for all years and combinations of megapools) (Table 4). Nonformed and Partially Formed were not different from one another in total cover in 2021 ($p = 0.1932$) or 2022 ($p = 0.5557$) or across years ($p = 0.6099$, $p = 0.1645$).

Unlike in total plant cover, open water did not vary between megapool type ($p=0.263759$) nor did megapool and date interaction ($p=0.2642777$) (Table 1). Open water did still vary by date ($p=0.00060$). That being said, within the megapool stage types there were still relationships. Fully formed and Partially Formed megapools increased in open water between 2021 and 2022 ($p=0.7080$ Fully, $p=0.0014$ Partially). However, though the box plot shows a large change in range for Nonformed between 2021 and 2022, no difference was found ($p = 0.9992$). Fully Formed and Partially Formed as well as Fully formed and Nonformed were significantly different from one another in open water in both 2021 and 2022 as well as across years, again confirming the initial classifications ($p<0.001$ for all years and combinations) (Table 2). Nonformed and Partially Formed were not different from one another in open water in 2021, ($p=0.1932$) or 2022 ($p=0.5557$) or across years ($p=0.6099$, $p=0.1645$).

Elevation:

The range of variation in elevation for Fully Formed megapools from (0.29, -0.03) is much higher than Partially Formed (0.25, 0.02) or Nonformed (0.22, 0.08) (Figure 3-5). Additionally, Fully Formed has consistent and overlapping elevations between the two transects in each megapool, whereas the elevation profiles in Partially Formed and Nonformed differ considerably between transects. There was considerable variability between individual megapools. Mean elevation differed between megapool types ($M<0.0001$. Table X), and was lower in the Fully formed megapools than in the Partially and Nonformed pools ($p=<0.0001$) (Table 6), which did not differ ($p = 0.41$). Mean elevation differed by megapool formation stage ($p=2.2e-16$) on elevation (Table 5). Elevation was lower in the Fully Formed than in the Nonformed or Partially Formed ($p=<0.0001$, $p=<0.0001$) (Table 6). Nonformed and Partially Formed are not different from each other ($p=0.4141$) which can be seen visually on the averaged

graph of the three types (Figure 3-6). These Figures all showcase adjusted average profiles to account for variation in transect length by standardizing the shorter transects to the standard long width.

Root Cores:

Root biomass decreased with decreasing elevation ($P < 0.0001$), but differences between megapool formation stages were minor with root biomass decreasing in Fully formed megapools ($P = 0.0882$) (Figure 3-7, Table 7). There was no effect of the interaction between megapool:elevation on total weight of roots ($p=0.7100$) (Table 7). Investigating these relationships more with a Tukey's test, it was found that low elevations had lower root biomass than medium and high elevations, which were similar ($p=0.9292$) and were different from low elevations where low elevations had almost no biomass ($p<0.0001$). Within the low elevation group, though Partially low appears to have more total weight of roots it was not different from Fully or Nonformed low (Table 8). Most roots occurred at 0-20 cm depth; nly 2 out of 90 sample contained roots at 20-30 cm.

Soil Characteristics:

Organic Matter was impacted by megapool ($P<0.001$) , and the interaction of megapool and elevation ($P=0.014$) however it was not impacted by elevation ($P=0.35$) (Table 9). Similarly, bulk density was impacted by megapool ($P<0.001$) and Elevation ($P<0.001$), however not the interaction between the two (Table 10). For both Organic Matter and bulk density, lower elevations had lower levels, and Fully formed megapools were lower than Nonformed megapools, though Partially formed levels were higher. Organic Matter vs Bulk Density regression was modeled by both elevation and megapool at all three levels high, medium, low and by formation stages Nonformed, Fully, and Partially (Figure 3-8) to account for the impact of different variables as found in the ANOVA (Table 9, 10). Soil organic matter content by

Organic Matter was inversely related to bulk density across all elevation and megapool stages ($R^2 \sim 0.4$), but the strength of the relationship was greater at high elevations ($R^2 = 0.66-0.97$) than at medium and low elevations ($R^2 = 0.15-0.59$). Also, organic matter content was higher in some of the Control megapools (greater than about 50%) than in Partially and Fully formed megapools (generally $<50\%$) (Figure 3-8).

Organic Matter had a linear relationship with carbon ($R^2=0.38$), however when high values of Organic Matter above 55 and above 25 for carbon were excluded, the relationship between Organic Matter and carbon was even stronger ($R^2=0.74$) (Figure 3-9). Similarly for nitrogen, Organic Matter against nitrogen in a regression was $R^2=0.3$ however when high values of Organic Matter above 55 and Nitrogen above 1.2 were excluded this improves to $R^2=0.48$ (Figure 3-10). Separating out Organic Matter vs elevation and megapool, Nonformed, Fully, and Partially are all different from one another while elevation appears more similar (Figure 3-11, Table 9). Similarly, looking at bulk density modeled by all 9 elevation and megapool factors, megapool and elevation both impacted bulk density ($P<0.001$, $P<0.001$) (Table 10). Here, high elevations had greater bulk density than medium or low and Partially was greater than Nonformed and Fully (Figure 3-12).

Carbon levels varied by megapool ($P<0.001$) where Partially formed had less Carbon than Fully formed Megapools and Nonformed had more than either. Carbon levels did not vary between high, medium, or low elevations or the interaction of elevation and megapool ($P=0.42$, $P=0.097$) (Table 11, Figure 3-13). Similarly, Nitrogen Varied by megapool ($P<0.001$) where Partially formed had less Carbon than Fully formed Megapools and Nonformed had more than either. Like Carbon, Nitrogen levels did not vary between high, medium, or low elevations ($P=0.52$), however was significant ($P=0.022$) (Table 12, Figure 3-14). Looking at Carbon

Density (CD), a combination of Carbon and Bulk Density, both elevation and megapool were significant ($P=0.012$, $P=0.026$) however not their interaction ($P=0.30$) (Table 13). The trend shown visually depicts Nonformed highest in CD, Partially slightly lower, and Fully formed with even less CD for megapool. In terms of elevation, high elevations have the most CD, followed by medium elevation, and then low elevation (Figure 3-15).

Mosquitoes:

No mosquito larvae were observed throughout all sampling days, marsh units, transects, and dips (Table 14). Two sampling efforts occurred on August 17th and September 14th, 2022, 4-5 days after the lunar high tide. On each sampling date, 2 transects were completed throughout all 15 marsh units, with 8 dips for larvae at each transect were performed by Maryland Department of Agriculture Mosquito Control Division. This demonstrates that mosquito larvae were essentially absent from the marsh during the sampling time.

Correlation Matrix:

This heat map correlation matrix analysis details several relationships of interest and the corresponding table details (Figure 3-16, Table 15). BD was negatively correlated with Organic Matter ($R^2=0.62$, $P<0.001$) and positively correlates with CD ($R^2=0.51$, $P<0.001$). Organic Matter corresponded with nitrogen ($R^2=0.55$, $P<0.001$) and carbon ($R^2=0.62$, $P=0.348$) though the confidence is less certain with a higher p value. Megapool corresponded with degradation (0.68, $p=1$) and Elevation corresponds with degradation 0.73, $p=1$. Degradation negatively correlated with plant coverage ($R^2=0.81$, $P=0.052$) and root cores ($R^2=0.56$, $P<0.001$) as well as positively with sulfides ($R^2=0.59$, $P=0.029$). Plant coverage and root cores correlated positively with one another ($R^2=0.611$, $P<0.001$) and also negatively with elevation ($R^2=0.80$, $P<0.001$; $R^2=-0.64$, $P<0.001$). Some relationships such as megapool corresponding with sulfide ($R^2=0.66$,

P=0.94) and Carbon with Nitrogen ($R^2=0.93$, $P=0.63$) had strong correlations but may not be realized due to their low confidence (Figure 1-16, Table 15).

DISCUSSION:

Vegetation:

The original classifications of Megapool stage were based on the amount of vegetation and open water present, so it is not surprising that the results of vegetation analysis showed that Megapool stage type had an impact on coverage for both total cover and open water presence. This validates the initial classifications. However, notably in assessing within megapool stage types, Nonformed and Partially formed were not different from one another in total cover or open water, while Fully formed and Partially formed as well as Fully formed and Nonformed were. The results suggest that Nonformed areas are more similar to Partially formed and vice versa than either are to Fully formed. It is also surprising to note that there were changes from the year 2021 to 2022 as shown by the impact of date, such as strong relationship increases in open water in Fully and Partially formed pools while less strongly correlated increases in Nonformed. Further exploration should occur in future years to assess whether this trend of increasing open water will continue potentially leading to the conversion of Nonformed to Partially formed, and Partially to Fully, or whether this phenomenon is only temporary or from sampling bias (Vincent et al., 2021). Beyond permanent conversion, there are other potential causes of temporary dieback such as changes in microbial communities which can alter vegetation and soil geochemistry through reduction and methane production (Kim et al., 2023). Additionally, areas where dieback has occurred previously for various reasons are more likely to experience dieback in future years. This dieback can have recovery however the extent is limited

and once a tipping point is surpassed it can lead to permanent conversion (Crawford & Stone, 2014).

Notably while Partially formed areas had an increase in total cover, they also had an increase in open water. The sampling methodology of a linear point intercept method allows for increases in open water and total cover as areas where more than 50% cover of open water and more than 5% coverage of plants are still noted as having both vegetation and open water. This effect is one drawback of the linear point intercept method (Canfield, 1941). Anecdotal notes from the field showed presence of small spotty dieback areas of above ground biomass that had converted to open water adjacent to alive biomass, though again the permanence of this conversion is unknown. Under this theory, it could help explain why the increase of total cover and open water occurred in Partially formed areas.

Initially, exploration occurred of individual species composition, however because of the dominance of *Spartina alterniflora* and relatively few instances of other species, focus was switched to assessing total cover. The few instances of *Spartina patens* were immediately adjacent to ditches where elevation was slightly higher than the surrounding transect. In the past, *Salicornia virginica* dieback has been used as an indicator of heavy metal such as cadmium presence and is high salinities and inundation (Rosso et al., 2005; Seliskar, 1983). Research has found that *Salicornia virginica* thrives in soils with high salt concentrations (Bogeborg et al., 2011). However, there appeared to be no pattern of presence of *Salicornia virginica*. Post restoration, species composition should be explored more.

Elevation:

The range of variation was greater for Fully formed pools with more low spots present and at lower elevations than Partially or Nonformed. This could have to do with both the age and

also lack of vegetation present. Vegetation is known to assist in soil organic matter accumulation (Delaune & Pezeshki, 2003). Pond age has been correlated with elevational decreases as unconsolidated sediments in areas without vegetation get eroded, decreasing elevation capital (Schepers et al., 2020). Similarly, more saturated areas are also associated with declines in elevation even when vegetation is present (Burdick et al., 2020). These saturated area declines could help explain why there were still some low points present in partial and Nonformed, though the low areas were more variable in these two formation stages than Fully formed.

Using the runaway marsh fragmentation theory posited in Vincent et al., 2021 where small pools connect gradually until reaching an end stable state, it tracks that the Fully formed pools are so different from the partial areas where small pools are connected and explains low elevation areas in dieback areas within smaller ones. Concerningly though this does point to the potential that even Nonformed areas may have the initial signs of pool fragmentation and could convert into pools shortly, especially as there was no difference found between Partially and Nonformed pools. Additionally, as posited by Coverdale et al., 2013 vulnerable marsh can be triggered more easily and rapidly by disturbance into degradation. These differences in elevation also point to the transitory nature of megapools, as compared to natural pools which still have higher elevation than anthropogenic pools and maintain a more constant elevation (Vincent et al., 2013). In Louisiana, where canals dominate the landscape similar to ditches, a similar subsidence pattern was noted as to Maryland with ditches, where anthropogenic canals widened naturally, caused subsidence and vegetation dieback, leading to eventual conversion of open water (Turner & Mo, 2020).

Root Cores:

When assessing the differences by megapool and elevation on root cores, it is interesting that megapool type does not have a big effect on belowground biomass. Instead, elevation appears to be the biggest factor which shows up again on the correlation matrix. Interestingly, there is not a difference in biomass between medium and high elevations, however low elevations which has close to zero biomass were different from high and medium. However, within the three megapool types there was no difference between the lower elevations. This pairing of medium and high differs from soil characteristics where medium and low elevations pair more closely together. This is most likely related to the plant coverage present in medium elevations, with medium plots having between 40-60% vegetative cover. Plant and root coverage also correlated with the degradation index. Unsurprisingly, below ground biomass and above ground biomass also correlate. Belowground biomass production is crucial for accumulation of organic matter in a marsh which can increase elevation. However, the amount of accumulation can differ by ecosystem and tidal range, such as in southern marshes which have more sediment accretion from deposition in the water column than from organic matter. When vegetation is lost, this can lead to sediment loss through decreased sheer strength and structure causing additional conversion to open water (DeLaune & Pezeshki, 1994).

Within the 90 samples, only 2 had roots in the 20-30 area with the rest concentrated in the upper 20cm. Gallagher, 1975 found that in *Spartina alterniflora*, 69% of biomass below ground is located in upper 15 cm of 35cm cores while Seliskar, 1983 found 65% in the upper 10 cm can be an indicator of saturated conditions. The soil moisture and aeration impact biomass (Seliskar, 1983). However, *Spartina alterniflora* may allocate fewer resources to belowground biomass when under stress, which can also lead to quicker decomposition after dieback (Crosby et al., 2017). While high levels of salinity can lead to lower amounts of above and below ground

biomass production, *Spartina alterniflora* can tolerate high levels of salinity (Hester et al., 2001). Crawford & Stone, 2014 found no impact on dieback of ph/salinity, pointing to a different major contributor to dieback. One potential suggestion is sulfide, which correlates with megapool type.

Soil Characteristics:

In general, plant coverage and root cores correlated with elevation but not megapool whereas soil characteristics correlated more with megapool than elevation. The relationship between Bulk Density, Organic Matter, Carbon, and Nitrogen were apparent with similar relationships to each type of megapool and elevation as well as with one another. Organic Matter positively correlated with nitrogen and carbon and inversely correlated with bulk density. In the literature, bulk density is inversely correlated with organic matter, carbon, and Organic Matter (a measure of organic matter), and organic matter, carbon, and Organic Matter are positively correlated with one another, however by how much varies with vegetation, nutrients, and hydrology (Puppim et al., 2022).

While the presence of nutrients like nitrogen in soil can lead to increases in plant growth, elevation capital has a larger effect on growth than presence of nutrients (Wilsey et al 1992). Soil Organic Carbon is typically higher in higher vegetated areas than lower non vegetated areas as the presence of *Spartina alterniflora* can help stabilize and increase carbon, on the other than bulk density is lower in vegetated soils than non-vegetated areas (J. E. Liu et al., 2007). The relationship for Carbon, Nitrogen, and ORGANIC MATTER where Nonformed was equal or greater than Fully formed which were greater than partial, and bulk density was the inverse with partial greater than Nonformed or Fully and high elevations greater than medium or low was initially perplexing. However, by exploring the Carbon Density (CD), this mechanism can be explained. Saturation and vegetative cover and species impact soil bulk density and soil organic

matter (Wang et al., 2016). It is suggested that the effect of pH and salinity have relatively low impact on dieback, however dieback from previous years can leave a legacy effect where decreased bulk density and lower carbon can make areas more vulnerable to dieback (Crawford & Stone, 2014).

In looking at Carbon Density, the nonformed megapools and high elevations have the highest Carbon Density, they also have the strongest relationship between Organic Matter and BD. However, in more degraded areas, such as Fully formed and low elevations, there is lower Carbon Density and less strong Relationship between Organic Matter and BD. This points to more degraded marsh having potentially experienced greater destabilization of the marsh, loss of sediment and mineral layers, and dilution and decomposition of organic matter. Simultaneously, areas with higher levels of mineral sediment may accrete faster and stay vegetated decreasing destabilization (Schepers et al., 2020). This interconnected relationship helps to explore the potential decline in carbon concentration in more degraded areas, which is concerning as carbon storage is a key ecosystem service of the marsh. While these initial results point to decreased carbon concentration, it is important to note that typical carbon storage protocols require depths of at least 1m and that the relatively shallow upper 30cm taken here may not be deep enough to draw robust conclusions about carbon storage.

Mosquitoes:

No mosquito larvae were found across all sampling efforts, and the literature as well as discussions with local mosquito experts revealed that this is not abnormal. The protocol used of two sampling dates 4-5 days after the lunar high tide with 30 transects (2 at each of the 15 marsh units) and 8 larvae dips along each performed by expert mosquito inspectors with the Maryland Department of Agriculture Mosquito Control Division was extensive enough to have unearthed larvae if they were there. The 4-5 day wait is due to the lifecycle of mosquitoes and that after

this many days larval hatching is expected making ease of sampling, dippers are a common tool used for assessment of this life stage (James-Pirri et al., 2009). Mosquito larvae are typically found in high marsh habitat and wet spots in upland areas and more infrequently in low marsh areas (Rochlin et al., 2012). In a study of 19 locations in a ditched marsh, only 3 locations had mosquito larvae all adjacent to high marsh (Rochlin et al., 2012). The conversion of high marsh into low marsh in this habitat from marsh migration due to sea level rise has left much of this habitat unviable for mosquitoes (Coverdale et al., 2013). Additionally, marsh management practices that favor increased flooding such as runnels or open water marsh management decrease mosquito populations rather than those that prevent flooding like ditches (Ruber & Gilbert, 1994). While these pools and ditches have decreased flooding, they are still tidally inundated and the presence of fish and birds within these pools would help to eat larvae and prevent presence should the occasional larvae appear (Talbot et al., 1986).

CONCLUSION:

The recent increase in megapool formation, pools in close proximity to mosquito ditches, has led to concern due to conversion to open water and vegetation dieback which can alter ecosystem services of a marsh. Here we sought to explore characteristics of megapools in three stages: Fully formed, Partially formed, and Nonformed and at three elevational profiles high (Fully vegetated), medium (Partially vegetated), and low (not vegetated) in Partially and Fully formed megapools. Fully formed and Partially formed megapools increased in open water between 2021 and 2022. Interestingly, Nonformed and Partially formed were not different from one another in open water. Fully formed megapools had a larger range of elevation than Partially or Nonformed and had a lower mean elevation. Partially and Nonformed pools did not differ from one another in elevation. Elevation impacted the total weight of root core below ground

biomass, with high and medium elevations not different from one another while low elevations were with much lower below ground biomass. In the correlation matrix, plant coverage and root cores were correlated with one another and with elevation, but not megapool. Sulfide and degradation index also correlated with plant coverage, root core, and megapools.

Soil characteristics of Organic Matter, Bulk Density, Carbon, Nitrogen, and Carbon Density were all more impacted by megapool type than elevation. Nonformed megapools and high elevations had less variation in the relationship between Organic Matter and bulk density. Interestingly, for Carbon and Nitrogen, Nonformed and Fully formed were more similar than Partially formed which was less, and positively correlated with Organic Matter which had more distinction between Nonformed and Fully with Nonformed higher than Fully and higher than partial. Bulk density had an inverse relationship with Organic Matter where Partial was greater than Nonformed which is greater than full. The reason for this assortment in bulk density, Organic Matter, carbon and nitrogen likely has to do with the pattern displayed in carbon density, where more degraded areas of marsh of medium and low elevations then Partially and Fully formed megapools, have lower carbon density where degradation removes materials lowering their bulk density and therefore making concentrations alone unreliable.

Mosquitoes were not found in any megapool or elevation levels. In terms of plant coverage, open water presence, root cores, and elevation, Nonformed and Partially formed megapools at high and medium elevations are more similar than Fully formed or low elevations. These complex interactions between megapool and elevation point to how vegetation dieback and marsh degradation manifests differently through different variables and characteristics.

FIGURES:



Figure 3-1. This map of the field site shows the location of the study site and marsh unit numbers. The color of the marsh unit numbers corresponds to the stages of pool formation for the 15 study locations.



Figure 3-2.

A photo of a megapool adjacent to a ditch at Rum Point along with *Spartina alterniflora* marsh grasses

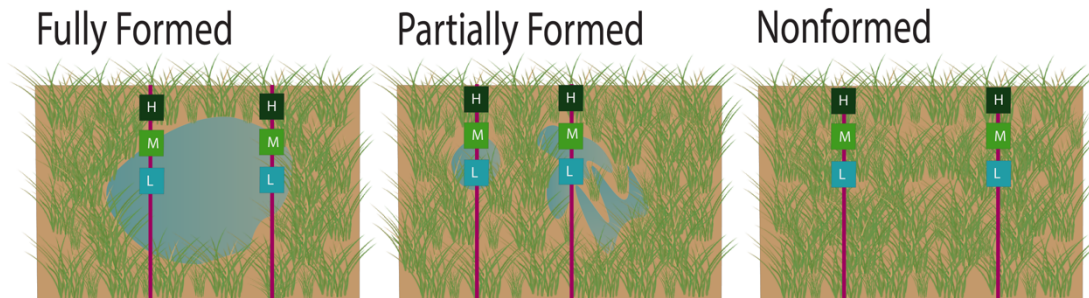


Figure 3-3.

This figure shows the Experimental design. Megapools were assigned one of three categories (Fully, Partially, or Nonformed). Within each marsh unit, 2 transects were established and 3 plots were established along each. High signifies Fully vegetated and highest elevation. Medium elevations for Partially and Fully Formed megapools signifies area between vegetated and non vegetated divide, and low signifies open water or bare ground with no vegetation at lowest elevation. For Nonformed, low and medium elevations were lower than the high areas but not different from each other in elevation and were still Fully vegetated.

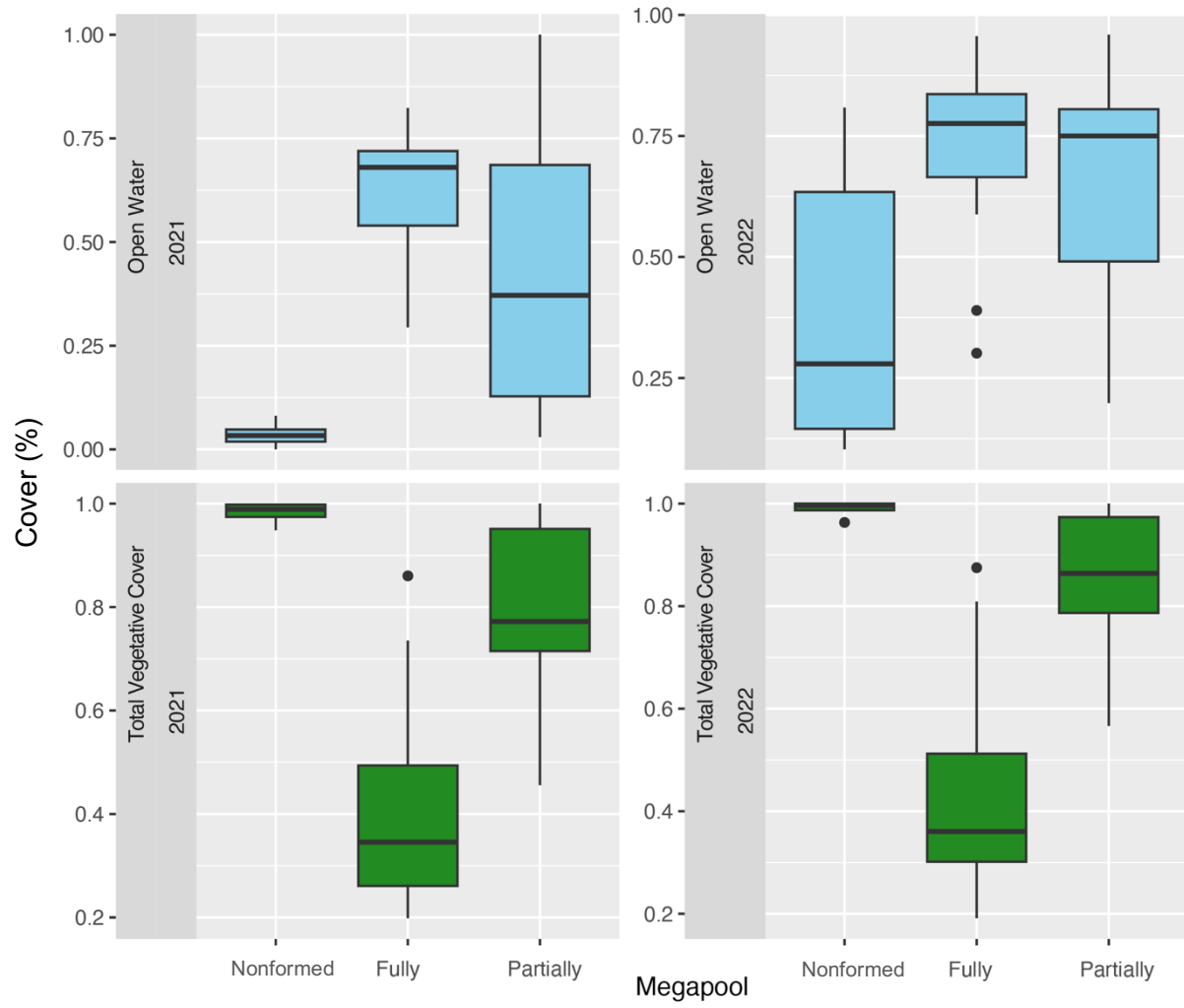


Figure 3-4.

Open water (blue) and Total cover of plant vegetation (all species combined, green) shown across the three stages of megapool formation and averaged across each of the 15 sites (left). Raw data shown in the scatter plot (right)

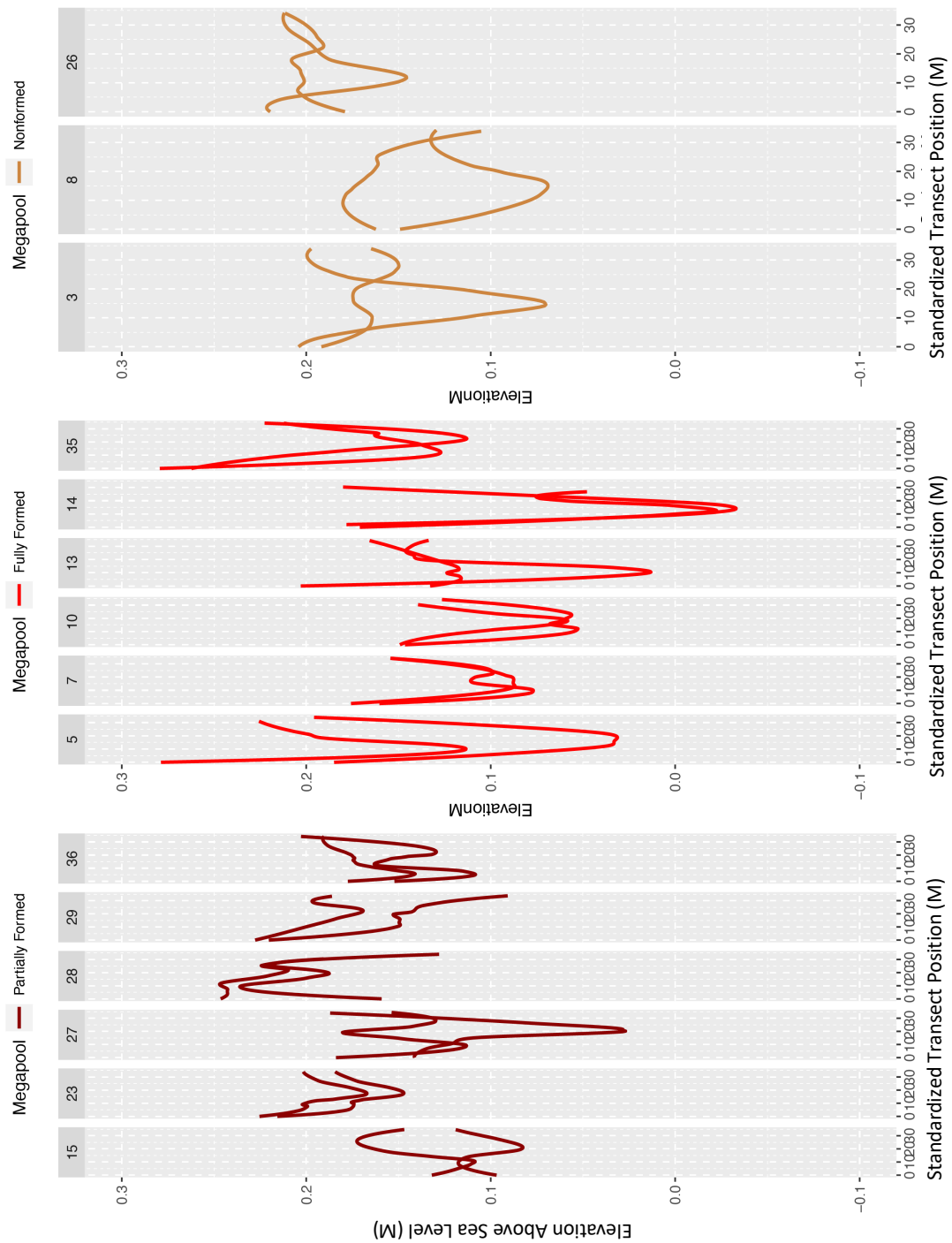


Figure 3-5.
The raw elevation for all 30 transects across each of the 15 marsh units separated megapool.

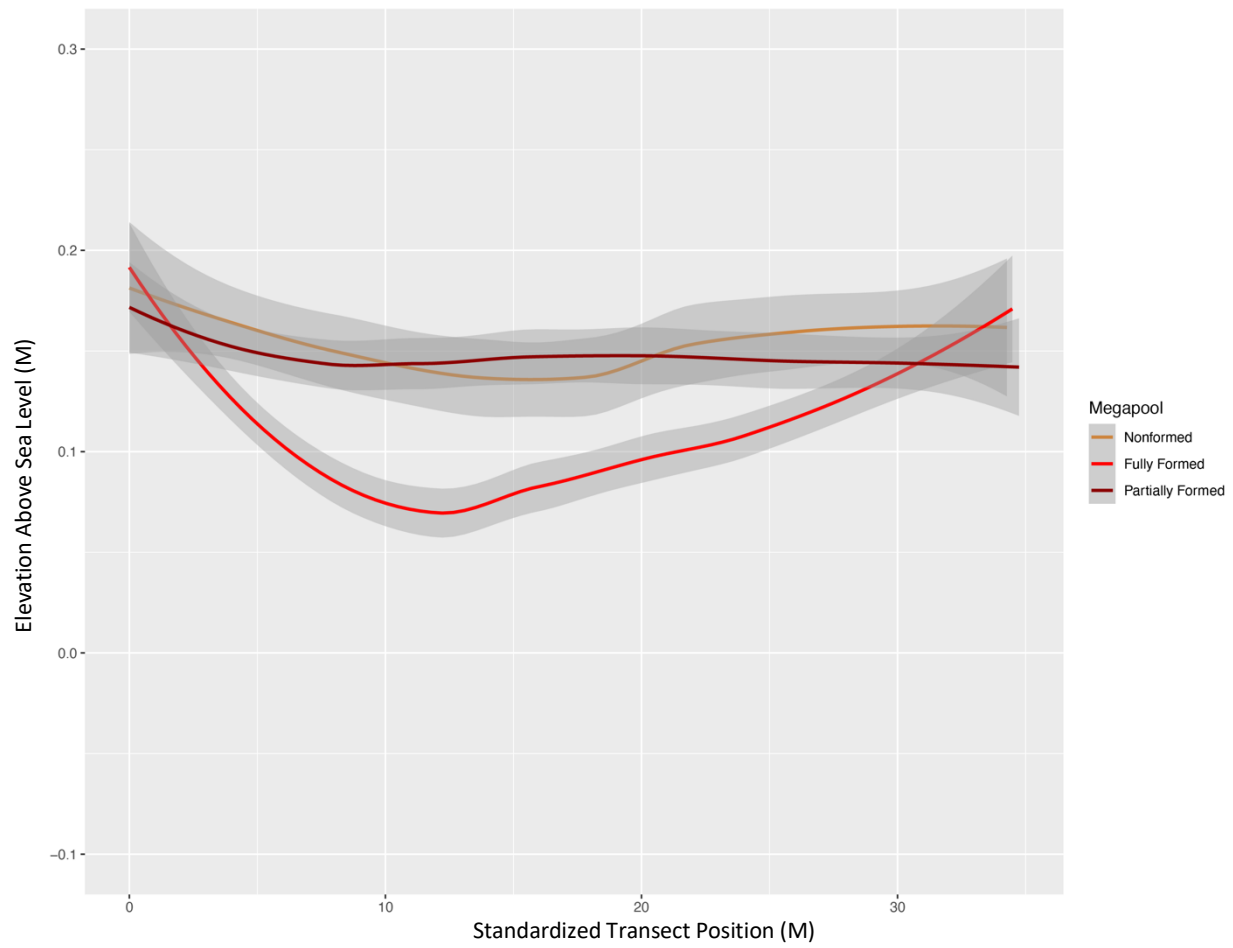


Figure 3-6.

Elevation averaged across the 30 transects by the three megapool formation levels. Variation on Partially and Nonformed is not significant but Fully formed is.

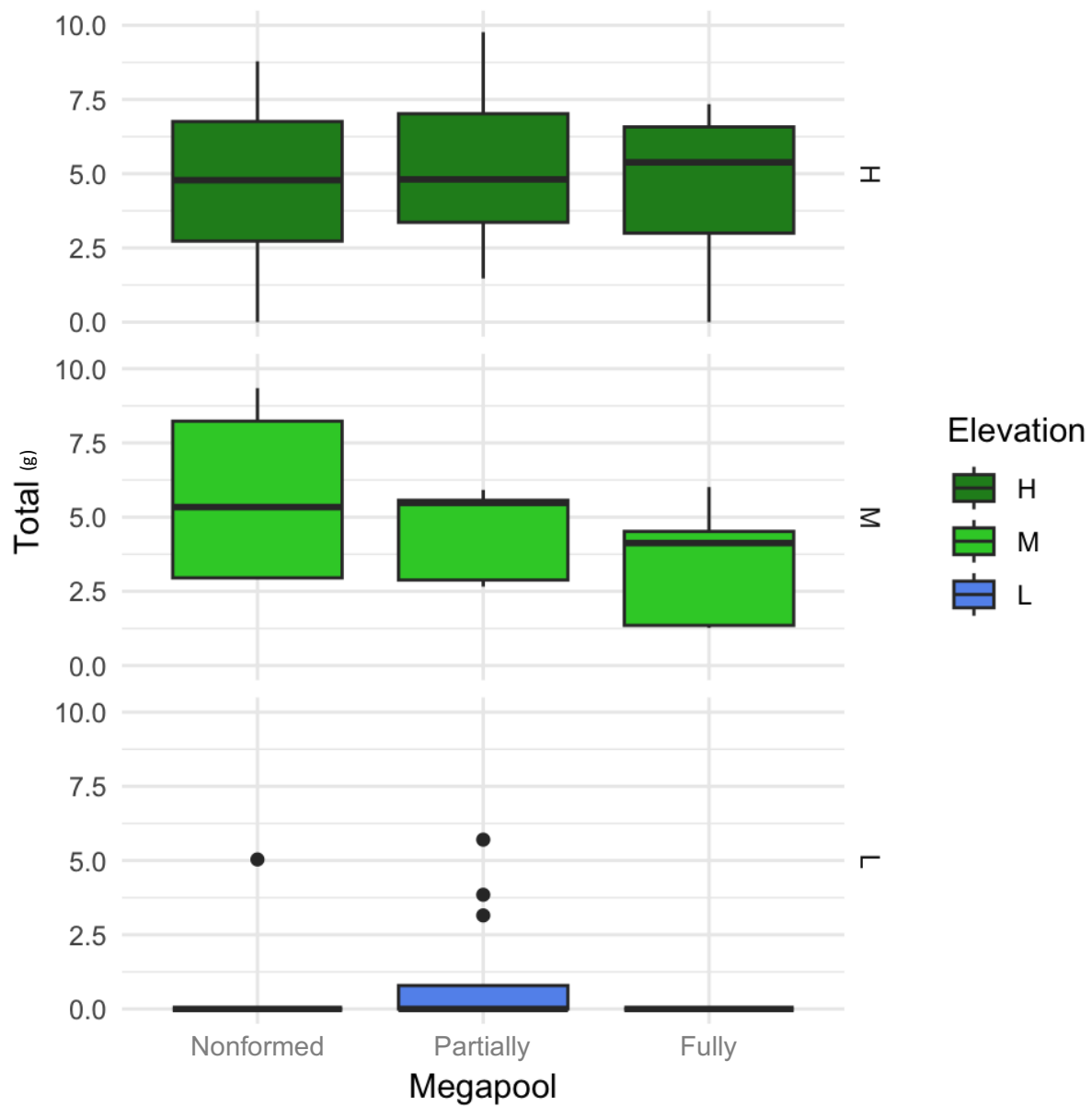


Figure 3-7

Total Belowground biomass (g) from root cores separated by three elevations (high, medium, low) and three megapool formation levels from 0-30cm depths.

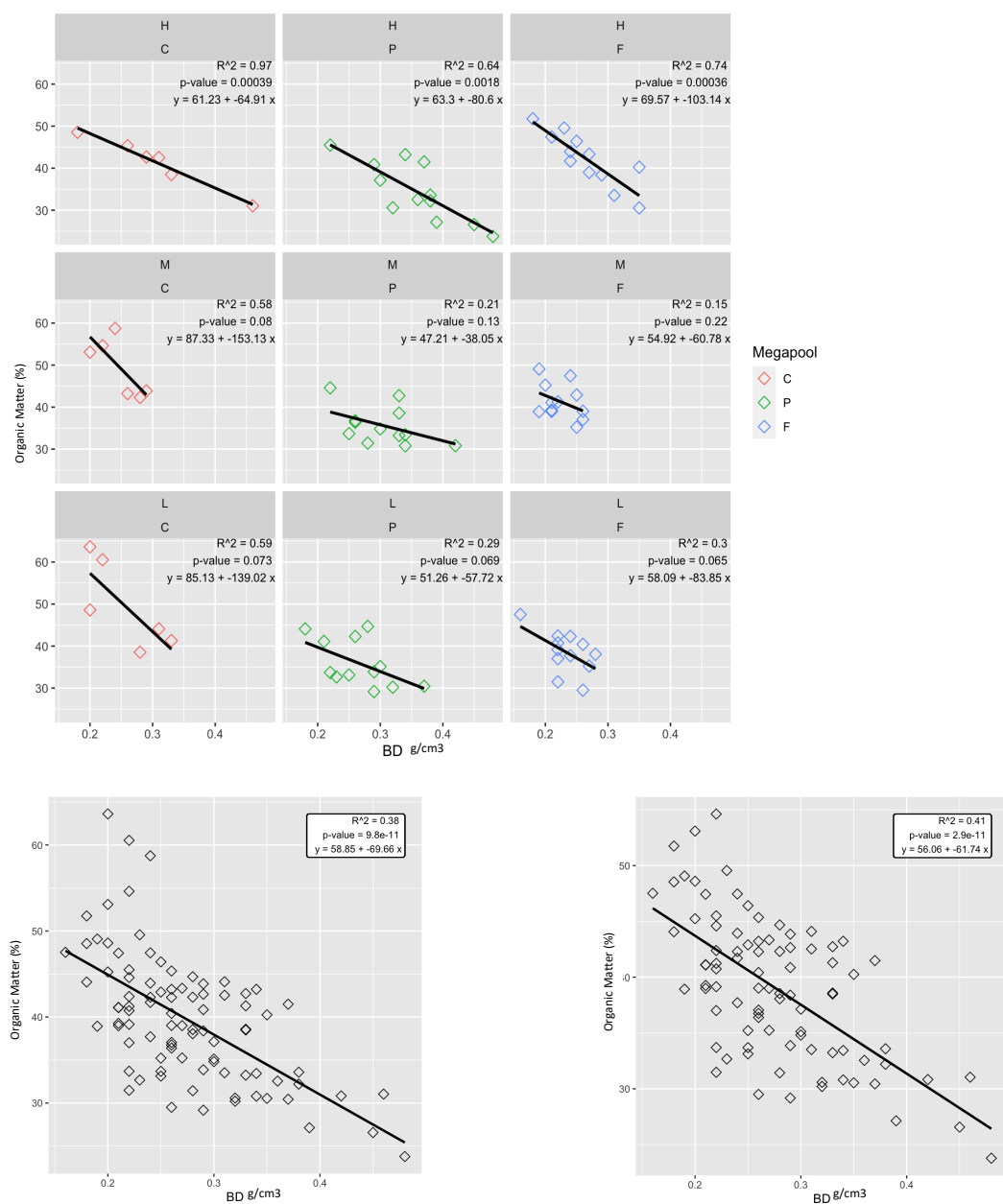


Figure 3-8

Organic Matter vs Bulk density split by elevation and megapool type as well as overall

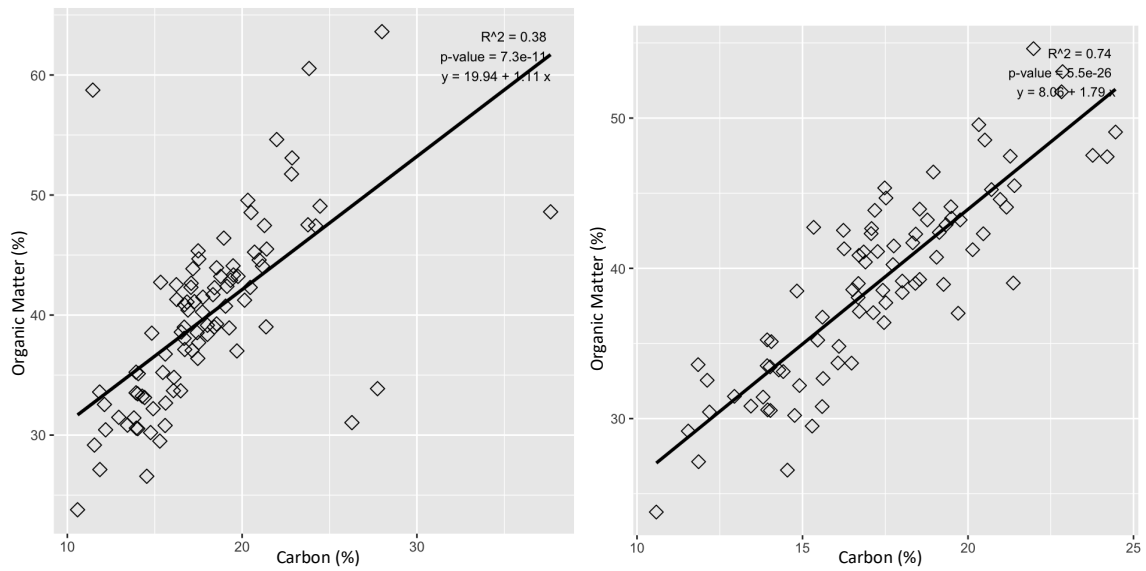


Figure 3-9

Figure on the left showcases all data, figure on the right removes high outliers (organic matter greater than 60 and C greater than 25 which artificially skewed data)

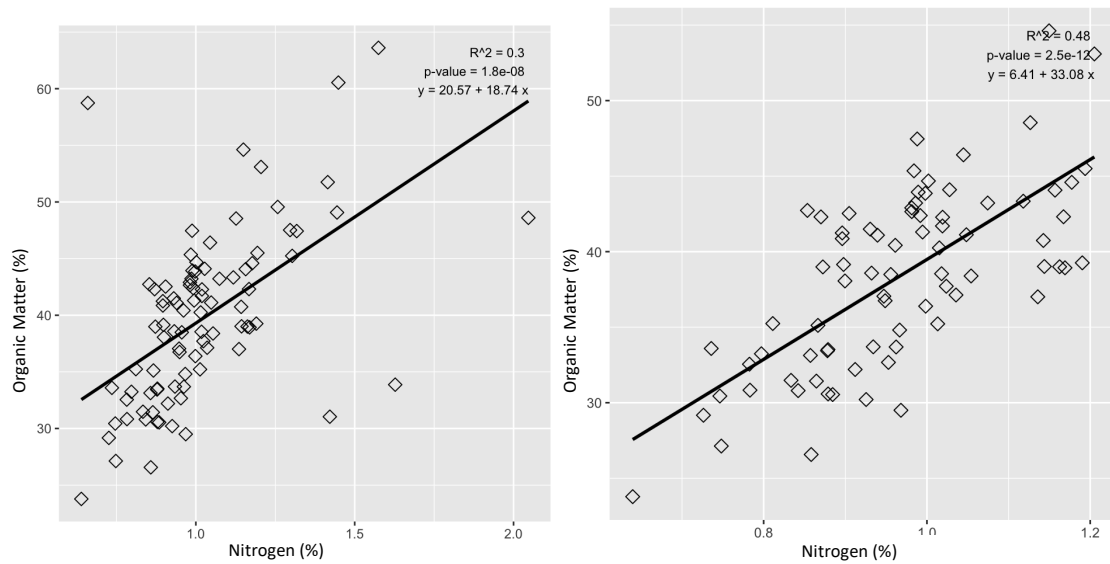


Figure 3-10

Figure on the left showcases all data, figure on the right removes high outliers (organic matter greater than 60 and N greater than 1.2 which artificially skewed data)

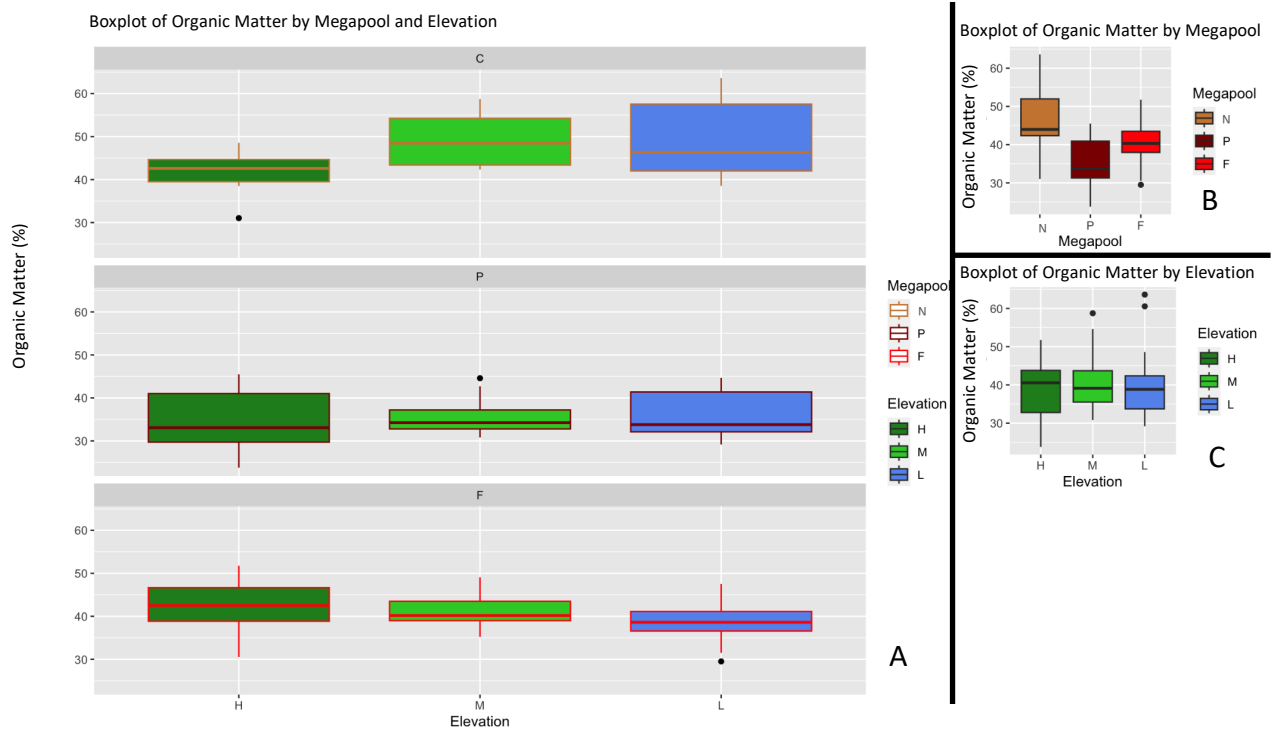


Figure 3-11

Figure on panel a showcases all 9 variations of 3 elevation types (High, Medium, and Low) x 3 megapool types (Nonformed, Partially Formed, Fully Formed) by Organic Matter, figure on panel B showcases only the 3 megapool levels and figure bottom right showcases only at the 3 elevation levels.

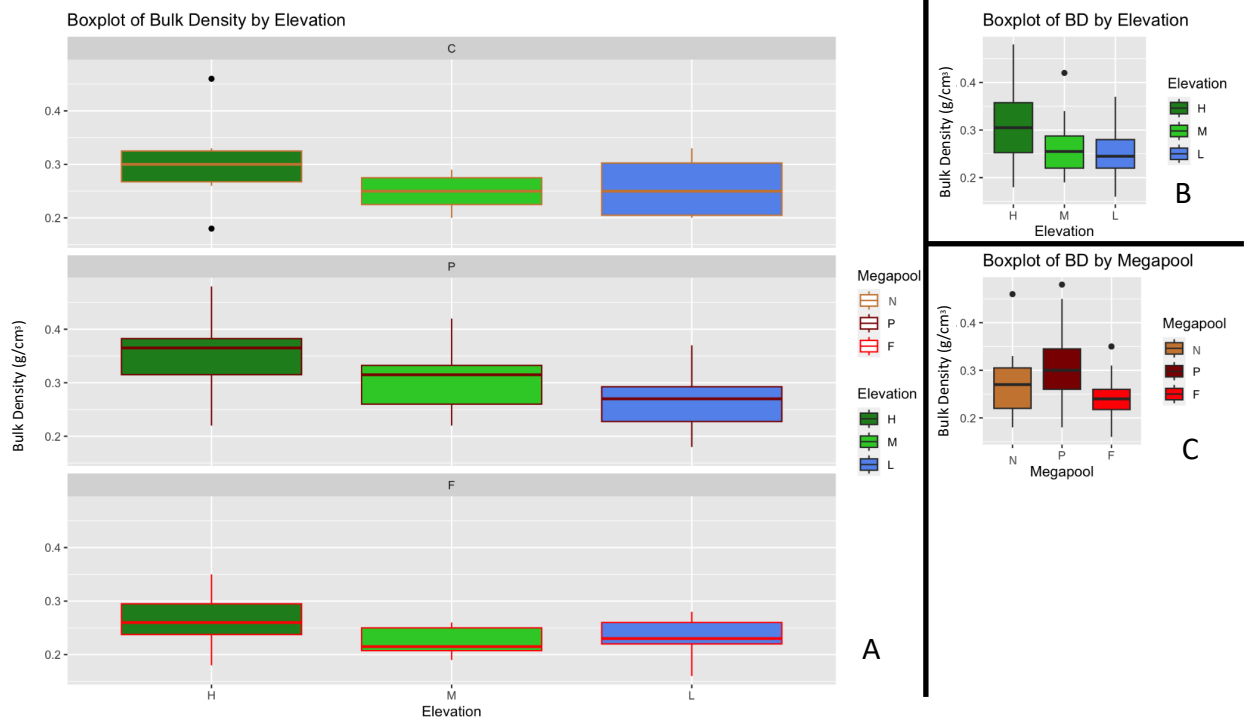


Figure 3-12

Figure on panel a showcases all 9 variations of 3 elevation types (High, Medium, and Low) x 3 megapool types (Nonformed, Partially Formed, Fully Formed) by Bulk Density, figure on panel B showcases only the 3 megapool levels and figure bottom right showcases only at the 3 elevation levels.

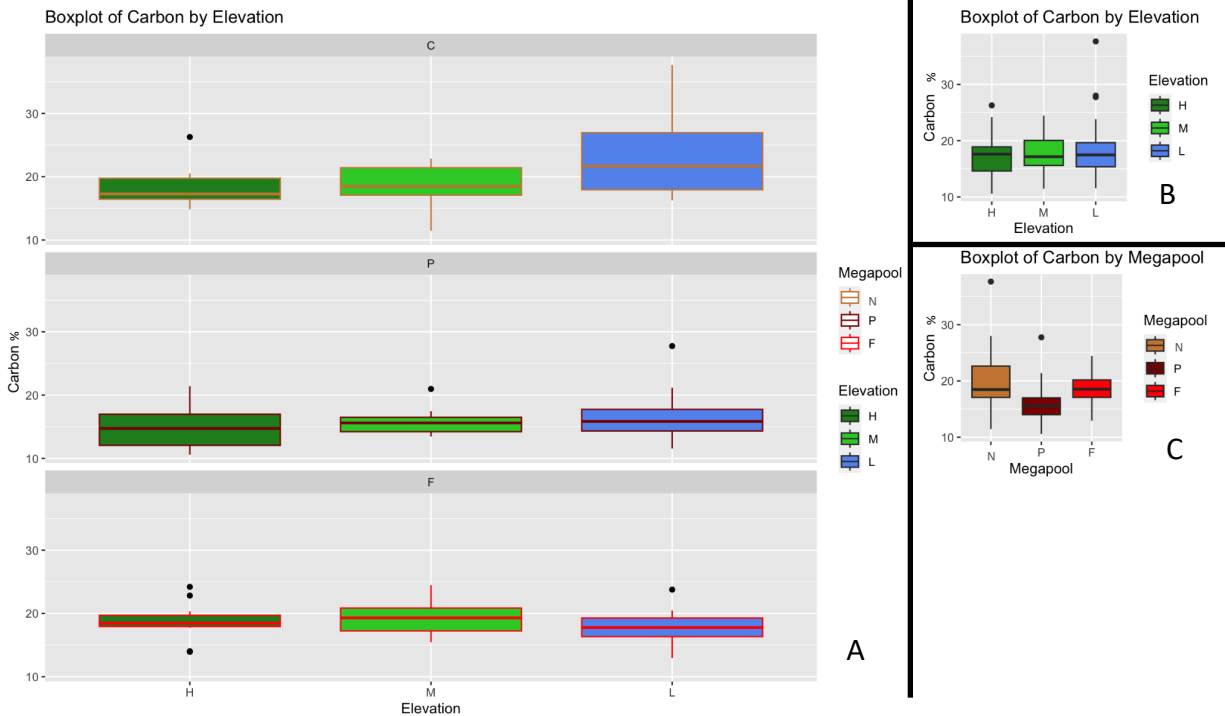


Figure 3-13.

Figure on panel a showcases all 9 variations of 3 elevation types (High, Medium, and Low) x 3 megapool types (Nonformed, Partially Formed, Fully Formed) by Carbon, figure on panel B showcases only the 3 megapool levels and figure bottom right showcases only at the 3 elevation levels.

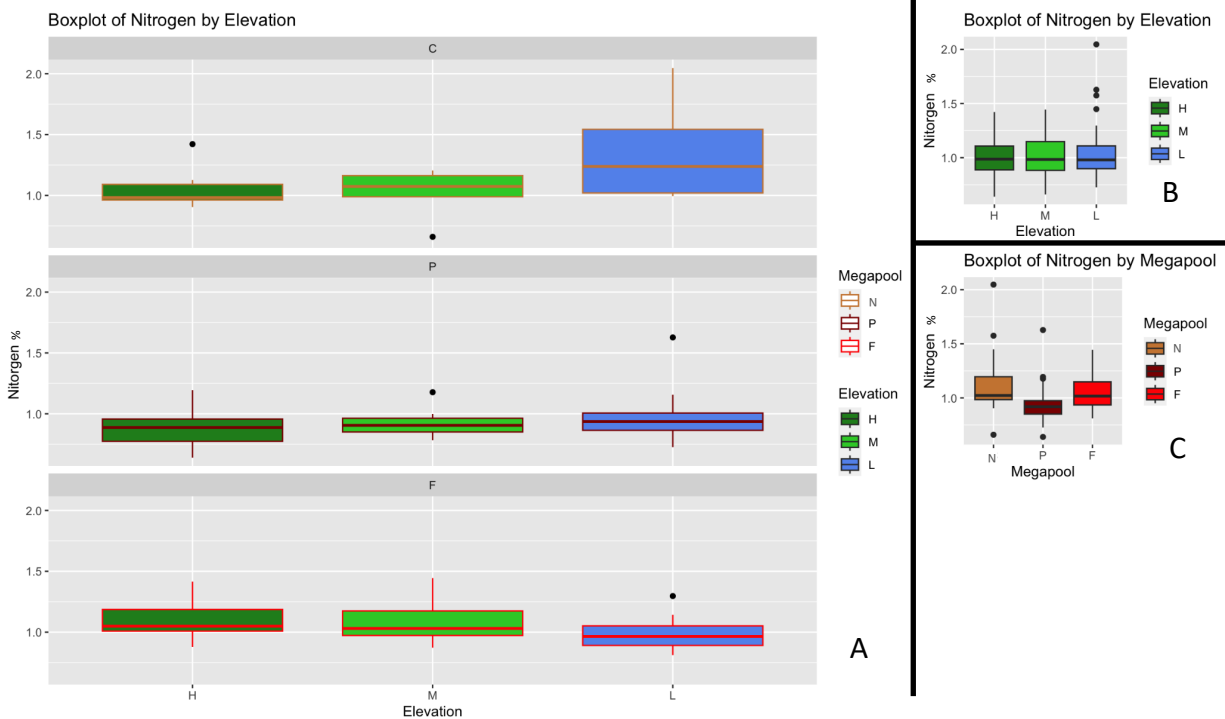


Figure 3-14.

Figure on panel a showcases all 9 variations of 3 elevation types (High, Medium, and Low) x 3 megapool types (Nonformed, Partially Formed, Fully Formed) by Nitrogen, figure on panel B showcases only the 3 megapool levels and figure bottom right showcases only at the 3 elevation levels.

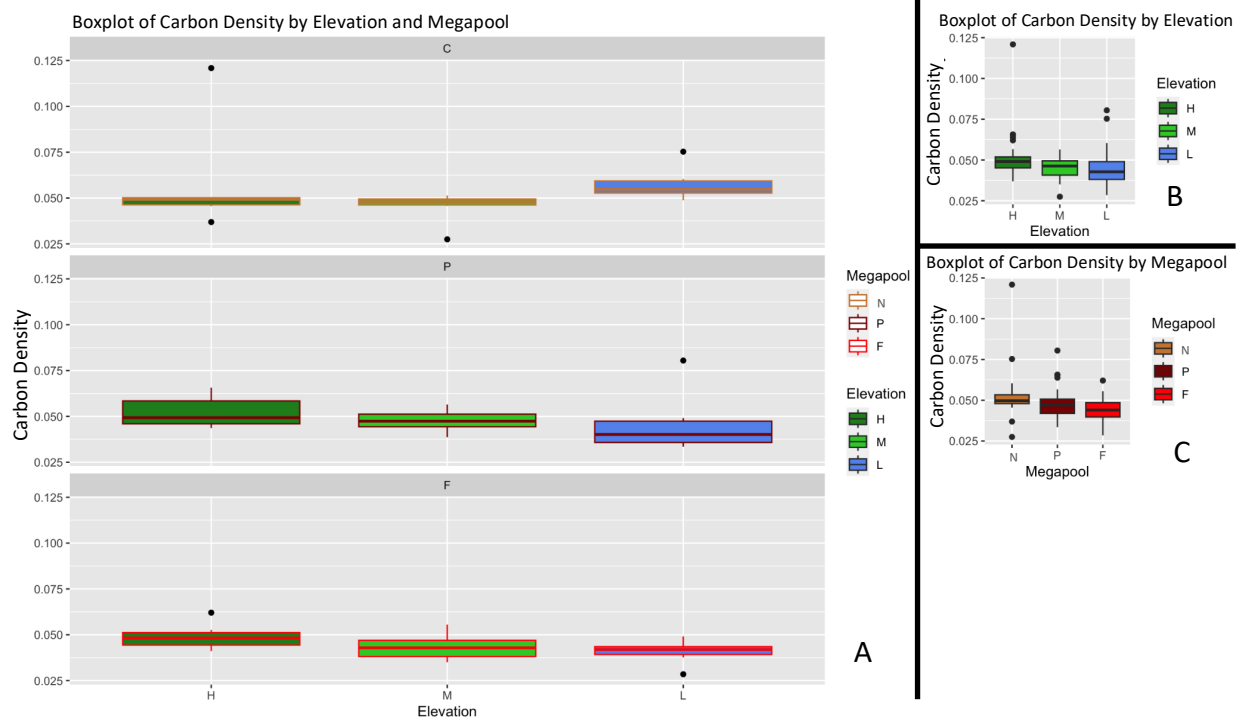


Figure 3-15.

Figure on panel a showcases all 9 variations of 3 elevation types (High, Medium, and Low) x 3 megapool types (Nonformed, Partially Formed, Fully Formed) by Carbon Density, figure on panel B showcases only the 3 megapool levels and figure bottom right showcases only at the 3 elevation levels.

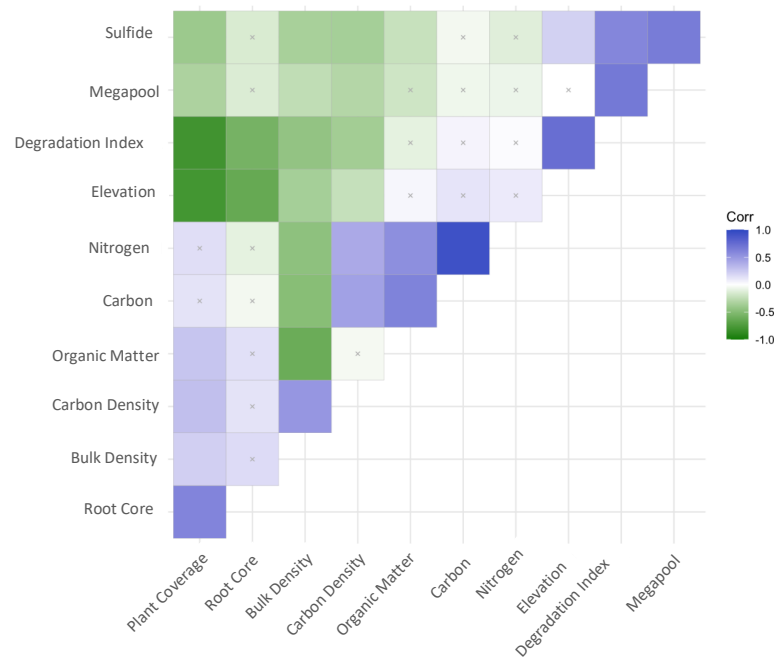


Figure 3-16.

Correlation heatmap of Sulfide, Megapool, Degradation Index, Elevation, Nitrogen, Carbon, Organic Matter, Carbon Density, Bulk Density, Root Core (Belowground Biomass), and Plant Coverage. This correlation matrix is hierarchically clustered, and correlations with $p > 0.05$ are indicated with an “x”.

TABLES:

Table 3-1. Results of the Open Water Analysis of Variance Table assessing influence of megapool, date, and their interaction.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Megapool	0.0096454	0.0048227	2	27.009	3.5949	0.041280
Date	0.0138918	0.0138918	1	27.013	10.3553	0.003344
Megapool:Date	0.0096665	0.0048333	2	27.009	3.6028	0.041025

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(> t)
(Intercept)	-1.39E+00	1 4.275e+	7.04E+03	-0.325	0.748
MegapoolF	-2.90E+00	1 5.235e+	7.02E+03	-0.554	0.5844
MegapoolP	-1.22E+00	2 5.235e+	7.02E+03	-2.325	0.0279
Date	7.35E+00	3 2.115e-	7.04E+03	0.348	0.7307
MegapoolF:Date	1.41E+00	2 2.590e-	7.02E+03	0.543	0.5917
MegapoolP:Date	6.01E+00	2 2.590e-	7.02E+03	2.321	0.0281

Table 3-2. Results of the Open Water Tukey's Test assessing influence of each type of megapool, date, and their interaction.

Contrast		Estimate	SE	df	t.ratio	p.value
C Date2021 - F	Date2021	0.57179	0.0844	28.3	6.771	<.0001
C Date2021 - P	Date2021	0.20194	0.0844	28.3	2.391	0.1932
C Date2021 - C	Date2022	-0.00735	0.0211	27	-0.348	0.9992
C Date2021 - F	Date2022	0.55038	0.0844	28.3	6.518	<.0001
C Date2021 - P	Date2022	0.13447	0.0844	28.3	1.592	0.6099
F Date2021 - P	Date2021	-0.36984	0.0689	28.3	-5.364	0.0001
F Date2021 - C	Date2022	-0.57914	0.0844	28.3	-6.858	<.0001
F Date2021 - F	Date2022	-0.02141	0.015	27	-1.432	0.708
F Date2021 - P	Date2022	-0.43731	0.0689	28.3	-6.343	<.0001
P Date2021 - C	Date2022	-0.2093	0.0844	28.3	-2.479	0.1645
P Date2021 - F	Date2022	0.34843	0.0689	28.3	5.054	0.0003
P Date2021 - P	Date2022	-0.06747	0.015	27	-4.512	0.0014
C Date2022 - F	Date2022	0.55773	0.0844	28.3	6.605	<.0001
C Date2022 - P	Date2022	0.14183	0.0844	28.3	1.68	0.5557
F Date2022 - P	Date2022	-0.4159	0.0689	28.3	-6.032	<.0001

Table 3-3. Results of the Total Cover Analysis of Variance Table assessing influence of megapool, date, and their interaction.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Megapool	0.14018	0.07009	2	26.822	1.4012	0.2637593
Date	0.75506	0.75506	1	26.92	15.095	0.0006015
Megapool:Date	0.13996	0.06998	2	26.822	1.399	0.2642777
Fixed effects:						
	Estimate	Std. Error	df	t value	Pr(> t)	
(Intercept)	-710.7819	261.0274	26.6414	-2.723	0.0113	
MegapoolF	502.3064	319.692	26.6974	1.571	0.1279	
MegapoolP	197.2335	319.692	26.7167	0.617	0.5425	
Date	0.3517	0.1291	26.6414	2.724	0.0112	
MegapoolF:Date	-0.2483	0.1582	26.6974	-1.57	0.1282	
MegapoolP:Date	-0.0974	0.1582	26.7167	-0.616	0.5432	

Table 3-4. Results of the Total Cover Tukey's Test assessing influence of each type of megapool, date, and their interaction.

contrast	Date	estimate	SE	df	t.ratio	p.value
C Date2021 - F	Date2021	0.57179	0.0844	28.3	6.771	<.0001
C Date2021 - P	Date2021	0.20194	0.0844	28.3	2.391	0.1932
C Date2021 - C	Date2022	-0.00735	0.0211	27	-0.348	0.9992
C Date2021 - F	Date2022	0.55038	0.0844	28.3	6.518	<.0001
C Date2021 - P	Date2022	0.13447	0.0844	28.3	1.592	0.6099
F Date2021 - P	Date2021	-0.36984	0.0689	28.3	-5.364	0.0001
F Date2021 - C	Date2022	-0.57914	0.0844	28.3	-6.858	<.0001
F Date2021 - F	Date2022	-0.02141	0.015	27	-1.432	0.708
F Date2021 - P	Date2022	-0.43731	0.0689	28.3	-6.343	<.0001
P Date2021 - C	Date2022	-0.2093	0.0844	28.3	-2.479	0.1645
P Date2021 - F	Date2022	0.34843	0.0689	28.3	5.054	0.0003
P Date2021 - P	Date2022	-0.06747	0.015	27	-4.512	0.0014
C Date2022 - F	Date2022	0.55773	0.0844	28.3	6.605	<.0001
C Date2022 - P	Date2022	0.14183	0.0844	28.3	1.68	0.5557
F Date2022 - P	Date2022	-0.4159	0.0689	28.3	-6.032	<.0001

Table 3-5. Results of the Elevation Analysis of Variance Table assessing influence of Megapool.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Megapool	2.221	1.1105	2	539.25	56.355	< 2.2e-16 ***

Table 3-6. Results of the Elevation Tukey's Test assessing influence of each type of Megapool: Nonformed (N), Fully (F), and Partially (P)

contrast	estimate	SE	df	t.ratio	p.value
N - F	0.1632	0.0191	482	8.561	<.0001
N - P	0.0272	0.0215	551	1.268	0.4141
F - P	-0.136	0.0163	549	-8.316	<.0001

Table 3-7. Results of the Root Core Biomass Analysis of Variance Table assessing influence of megapool, elevation, and their interaction.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	2	25.9	12.948	2.5063	0.0882 .
Elevation	2	360.21	180.104	34.862	1.658e-11
Megapool:Elevation	4	11.07	2.767	0.5355	0.71
Residuals	77	397.8	5.166		

Table 3-8. Results of the Root Core Biomass Tukey's Test assessing influence of each type of megapool, elevation, and their interaction.

Megapool	diff	lwr	upr	p adj
P-C	0.4870487	-1.086179	2.06027607	0.7406165
F-C	-0.7486377	-2.296543	0.79926709	0.4830721
F-P	-1.2356864	-2.564263	0.09289014	0.0736811
Elevation	diff	lwr	upr	p adj
M-H	-0.2279941	-1.72054	1.264551	0.9292426
L-H	-4.3130254	-5.722622	-2.903428	0
L-M	-4.0850313	-5.50946	-2.660603	0
Megapool:Elevation	diff	lwr	upr	p adj
P:H-C:H	0.65947	-3.086743	4.40568348	0.999731
F:H-C:H	-0.08862727	-3.770431	3.59317659	1
C:M-C:H	1.12326	-3.269565	5.51608468	0.9960545
P:M-C:H	0.73986	-3.006353	4.48607348	0.9993712
F:M-C:H	-1.24142727	-4.923231	2.44037659	0.9759815
C:L-C:H	-4.006075	-7.923959	-0.08819117	0.0411401
P:L-C:H	-3.57710833	-7.204364	0.05014727	0.056227
F:L-C:H	-4.6356	-8.21605	-1.05514966	0.0027858
F:H-P:H	-0.74809727	-3.917821	2.42162682	0.9977402
C:M-P:H	0.46379	-3.509669	4.43724943	0.9999883
P:M-P:H	0.08039	-3.163926	3.32470604	1
F:M-P:H	-1.90089727	-5.070621	1.26882682	0.606335
C:L-P:H	-4.665545	-8.106662	-1.22442819	0.0014169
P:L-P:H	-4.23657833	-7.342774	-1.13038221	0.0012935
F:L-P:H	-5.29507	-8.346479	-2.24366118	0.0000141
C:M-F:H	1.21188727	-2.700905	5.12467964	0.9858927
P:M-F:H	0.82848727	-2.341237	3.99821136	0.9954146
F:M-F:H	-1.1528	-4.246134	1.94053397	0.9564169
C:L-F:H	-3.91744773	-7.28833	-0.54656518	0.0110995
P:L-F:H	-3.48848106	-6.516685	-0.4602772	0.0122742
F:L-F:H	-4.54697273	-7.518952	-1.57499358	0.0001847
P:M-C:M	-0.3834	-4.356859	3.59005943	0.9999973
F:M-C:M	-2.36468727	-6.27748	1.54810509	0.5963265
C:L-C:M	-5.129335	-9.265043	-0.9936273	0.0049876
P:L-C:M	-4.70036833	-8.561877	-0.83885987	0.0063423
F:L-C:M	-5.75886	-9.576436	-1.94128366	0.0002392
F:M-P:M	-1.98128727	-5.151011	1.18843682	0.5515239
C:L-P:M	-4.745935	-8.187052	-1.30481819	0.0010879
P:L-P:M	-4.31696833	-7.423164	-1.21077221	0.000964
F:L-P:M	-5.37546	-8.426869	-2.32405118	0.00001
C:L-F:M	-2.76464773	-6.13553	0.60623482	0.1965097
P:L-F:M	-2.33568106	-5.363885	0.6925228	0.267001
F:L-F:M	-3.39417273	-6.366152	-0.42219358	0.0135348
P:L-C:L	0.42896667	-2.88225	3.74018286	0.9999737
F:L-C:L	-0.629525	-3.889401	2.6303514	0.9994665
F:L-P:L	-1.05849167	-3.96262	1.84563705	0.9616989

Table 3-9. Results of the Organic Matter Analysis of Variance Table assessing influence of megapool, elevation, bulk density, and their interaction.

Response: Organic Matter					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	2	1604.5	802.25	41.0158	1.288e-12
Elevation	2	41.58	20.79	1.063	0.35078
BD	1	1429.46	1429.46	73.0824	1.448e-12
Megapool:Elevation	4	263.69	65.92	3.3704	0.01384
Megapool:BD	2	49.12	24.56	1.2557	0.29103
Elevation:BD	2	15.18	7.59	0.388	0.67985
Megapool:Elevation:BD	4	125.21	31.3	1.6004	0.18353
Residuals	72	1408.29	19.56		

Table 3-10. Results of the Bulk Density (BD) Analysis of Variance Table assessing influence of megapool, elevation, and their interaction.

Response: BD					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	2	0.083791	0.041895	14.6604	3.681e-06
Elevation	2	0.058647	0.029323	10.2611	0.0001066
Megapool:Elevation	4	0.012898	0.003224	1.1283	0.3490338
Residuals	81	0.231475	0.002858		

Table 3-11. Results of the Carbon Analysis of Variance Table assessing influence of megapool, elevation, and their interaction.

Response: C					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	2	270.39	135.193	9.6669	0.0001718
Elevation	2	24.84	12.422	0.8882	0.4153535
Megapool:Elevation	4	113.85	28.461	2.0351	0.0971791
Residuals	81	1132.79	13.985		

Table 3-12. Results of the Nitrogen Analysis of Variance Table assessing influence of megapool, elevation, and their interaction.

Response: N					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	2	0.63322	0.31661	8.1963	0.0005732
Elevation	2	0.05027	0.02514	0.6507	0.5243861
Megapool:Elevation	4	0.46671	0.11668	3.0205	0.0224344
Residuals	81	3.12887	0.03863		

Table 3-13. Results of the Carbon Density (CD) Analysis of Variance Table assessing influence of megapool, elevation, and their interaction.

Response: CD					
	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	2	0.0011068	0.00055341	4.6572	0.01217
Elevation	2	0.0009057	0.00045287	3.8111	0.02619
Megapool:Elevation	4	0.0005854	0.00014635	1.2316	0.30403
Residuals	81	0.0096251	0.00011883		

Table 3-14. Mosquito Survey Results (Sum of Larvae) for each marsh unit, megapool type (Nonformed, Partially, Fully), and transect across two different sampling dates and multiple dips.

Marsh Unit	Megapool	Transect	August 17 th 2021	September 14 th 2021
3	N	1	0	0
3	N	2	0	0
5	F	1	0	0
5	F	2	0	0
7	F	1	0	0
7	F	2	0	0
8	N	1	0	0
8	N	2	0	0
10	F	1	0	0
10	F	2	0	0
13	F	1	0	0
13	F	2	0	0
14	F	1	0	0
14	F	2	0	0
15	P	1	0	0
15	P	2	0	0
23	P	1	0	0
23	P	2	0	0
26	N	1	0	0
26	N	2	0	0
27	P	1	0	0
27	P	2	0	0
28	P	1	0	0
28	P	2	0	0
29	P	1	0	0
29	P	2	0	0
35	F	1	0	0
35	F	2	0	0
36	P	1	0	0
36	P	2	0	0

Table 3-15. Correlation Matrix R Values assess the relationship between 10 variables

	var1	var2	r value	p value
1	BD	OM	-0.61658372	0.000 ***
2	BD	Megapool	-0.25094332	0.000 ***
3	BD	Elevation	-0.36325665	0.017 *
4	BD	DegredationIndex	-0.43734971	0.000 ***
5	BD	C	-0.49415797	0.000 ***
6	BD	N	-0.46435688	0.000 ***
7	BD	CD	0.51007653	0.000 ***
8	BD	S	-0.3496451	0.000 ***
9	BD	Plant	0.22126366	0.001 ***
10	BD	Rootcore	0.15754106	0.036 *
11	OM	Megapool	-0.18751648	0.000 ***
12	OM	Elevation	0.03603036	0.077
13	OM	DegredationIndex	-0.10013619	0.736
14	OM	C	0.61979566	0.348
15	OM	N	0.55159597	0.000 ***
16	OM	CD	-0.03691062	0.000 ***
17	OM	S	-0.21630429	0.73
18	OM	Plant	0.27436428	0.041 *
19	OM	Rootcore	0.1393372	0.009 **
20	Megapool	Elevation	0	0.494
21	Megapool	DegredationIndex	0.67566392	1
22	Megapool	C	-0.06036687	0.644
23	Megapool	N	-0.07356163	0.000 ***
24	Megapool	CD	-0.29701775	0.000 ***
25	Megapool	S	0.66119931	0.94
26	Megapool	Plant	-0.3304093	0.585
27	Megapool	Rootcore	-0.13961974	0.09
28	Elevation	DegredationIndex	0.73720978	1
29	Elevation	C	0.1249975	0.000 ***
30	Elevation	N	0.09233779	0.572
31	Elevation	CD	-0.23094844	0.491
32	Elevation	S	0.2051172	0.004 **
33	Elevation	Plant	-0.79599747	0.000 ***
34	Elevation	Rootcore	-0.64284202	0.001 **
35	DegredationIndex	C	0.05136166	0.000 ***
36	DegredationIndex	N	0.01836938	0.24
37	DegredationIndex	CD	-0.37094163	0.387
38	DegredationIndex	S	0.59796293	0.029 *
39	DegredationIndex	Plant	-0.81006277	0.052
40	DegredationIndex	Rootcore	-0.56824544	0.000 ***
41	C	N	0.93472409	0.631
42	C	CD	0.45205035	0.864
43	C	S	-0.04883336	0.000 ***
44	C	Plant	0.12554723	0.000 ***
45	C	Rootcore	-0.04676261	0.000 ***
46	N	CD	0.41126288	0.000 ***
47	N	S	-0.11702548	0.000 ***
48	N	Plant	0.14558186	0.648
49	N	Rootcore	-0.10107013	0.238
50	CD	S	-0.36259494	0.000 ***
51	CD	Plant	0.29814283	0.272
52	CD	Rootcore	0.12536484	0.171
53	S	Plant	-0.39968023	0.000 ***
54	S	Rootcore	-0.14745991	0.004 **
55	Plant	Rootcore	0.61152156	0.000 ***

CHAPTER FOUR: SURVIVAL AND BIOMASS OF SPARTINA ALTERNIFLORA PLANTINGS IN RELATION TO ELEVATION, POOL FORMATION STAGE, AND PLANT SPACING

INTRODUCTION:

Vegetation cover is a common metric of salt marsh restoration success and can be accomplished through seeding, planting, transplanting, or natural recolonization. Sites that are planted may have greater vegetation coverage for the first seven years after restoration compared to unplanted sites, and after three years are typically comparable in biomass to reference marshes (Bergen et al., 2000). Traditionally, restoration plantings are spaced by a standard distance per species to increase survival and prevent competition, rooted in agricultural and forest ecosystem research (Gedan & Silliman, 2009). For *Spartina alterniflora*, standard spacings vary from 30-40cm (Bergen et al., 2000). However recent research has shown that in some ecosystems for some plants, planting seedlings in a clumped patch rather than spaced may yield higher survival and biomass due to the difficult environments of salt marshes (Silliman et al., 2015).

Salt marsh ecosystems create challenging environments for plants due to regular inundation and high salinities. Zonation patterns are impacted by these environmental conditions which create stress, as well as by competition with other plants (Pennings & Callaway, 1992). *Spartina alterniflora*, smooth cordgrass, is a common salt marsh species along with *Spartina patens*. In low marsh areas with more inundation, *Spartina alterniflora* typically outcompetes *Spartina patens*, however in high marsh settings the opposite is true (Bertness, 1991).

Spartina alterniflora is able to propagate both sexually through seed and asexually via rhizomes. Seeds allow for spreading to new areas through seed dispersal, most commonly via water, whereas rhizomes allow for propagation and expansion in existing patches. While seeds are immediately viable, they grow better after a period of cold stratification and with periods of

aeration (Xiao et al., 2009). *Spartina alterniflora* is able to survive in a variety of salinity and inundation levels as it can oxygenate the roots and rhizomes as well as exclude salt, however in more challenging environments like anoxic high salinity salt marshes, above ground biomass is lower compared to in other conditions (Bertness, 1991).

Plants that are stressed produce less above ground biomass, which is important as greater biomass typically correlates with a greater proportion of flowering shoots, making above ground biomass an important variable (W. Liu & Pennings, 2019). Allometric regression equations allow for above ground biomass calculations based on height, width, or diameter of a plant, such as *Spartina alterniflora*. The equation used here will be established by collecting plants at a site and measuring the height of all leaves on a plant specimen, and then drying the specimen at between 50-65 C before weighing the sample and running a regression analysis between weight and total sum of all leaves (Hill et al., 2018). Sum of leaf lengths, also called sum height, will be referred to as total leaf height here and is sufficient alone without width or diameter to establish a relationship (González Trilla & Morandeira, 2013). The benefits of this method are that it shortens processing time and expenses, and that other than initial development of the equation, it is nondestructive sampling. The challenges with this method can be establishing an initial relationship accurately, allometric equation relationships can differ by site and time of year, and in early spring may present as polynomial rather than linear models (Morris & Haskin, 1990).

Belowground biomass of *Spartina alterniflora* is typically 3:1 to above ground, with positive correlation to nutrients; however, this relationship is negatively correlated with high salinity, and may be closer to 2.5:1 in highly saline environments. A value of 2.64:1 was found for a site located in Virginia with similar salinity and tidal range, and will be used for this project (Gross et al., 1991; Hill & Roberts, 2017). In one location in Louisiana, with a higher tidal range

than our field site, the root:shoot ratio was found as 2.6:1, and noted to be slightly lower than northeastern marshes (Darby & Turner, 2008). When possible, equations should be established individually for each site as microvariations in salinity, tidal regime and more can drive variation in the relationship of root:shoot, though notably there was consistency across seasons for belowground unlike above ground (Hill & Roberts, 2017). Belowground biomass like rhizomes vary due to environmental characteristics, such as reducing conditions which can lead to high decomposition rates of roots in salt marshes, or by impacting energy allocations of the plant or from site conditions (Benner et al., 1991).

Along with colonization, rhizomes of *Spartina alterniflora* are mutualistic and facilitative, sharing nutrients and signals between plants and aiding adjacent plants through ecosystem impacts (Bruno & Kennedy, 2000). Silliman et al., 2015 had a paper assessing impact spacing on *Spartina alterniflora* and posited that clumped spacing improves survivorship, biomass, stem density, and expansion at low elevations while it may have no impact or detrimental effects at higher elevations. Roots oxygenate soil surrounding them, benefiting adjacent roots and plants, as well as preventing erosion stress and decreasing wave energy (Silliman et al., 2015). Clumped plants may also more effectively trap sediment and mitigate impacts of heavy wind on root dislodging. Additionally, belowground biomass production varies by age as initial implementation focuses on stress avoidance, later transitioning to colonization efforts (van de Ven et al., 2023). However, the benefits of clumped plantings have not always been replicable in other ecosystems or with different species such as a *Frankenia salina* or *Jaumea carnosa* (Tanner et al., 2023).

Project Rationale & Objectives

Marsh restoration techniques such as channel alteration, beneficial reuse, and living shorelines are costly and sometimes difficult to construct due to permitting and site factors like depth of channels and nearby infrastructure (Raposa et al., 2022). In recent years, a focus on low technology restoration techniques has increased with techniques like targeted plantings, ditch filling, and runnels. Additionally, there has been pushback from community members and regulators about the needs for such extensive marsh restoration in vegetation dieback areas, and whether this is merely temporary dieback or if plantings would be sufficient (Besterman et al., 2022). Likely, these larger interventions are necessary because sites require sufficient elevation capital to be sufficient to support plant growth (Cahoon et al., 2019).

The objective of this study was twofold 1) to assess whether in some situations, specifically at high elevations and Partially formed megapool stages as compared to low elevations and Fully formed megapool stages, whether plantings would be sufficient to revegetate areas 2) to investigate the impact of different elevations (high and low) and spacings (clumped and dispersed) on plant survival and growth under the understanding that facilitation of clumped plantings may improve survival. To explore this relationship, four pools, two Fully formed megapools and two Partially formed megapools, were selected from the broader 15 in the above two chapters for inclusion into the planting study, and forty plots established across all. We hypothesized that clumped plants will have higher survival than dispersed, Partially formed megapools greater than Fully formed megapools, and higher elevations greater than lower elevations. We also hypothesized that plants will not be sufficient to revegetate entire pools as elevation capital is too low even at higher elevations and that additional more extensive restoration will be required to revegetate pools.

METHODS:

Study Site:

This project occurred at the 40-acre site known as Rum Pointe, approximately 24 km south of Ocean City, MD, and is a part of a larger research project assessing megapools, elevation, and restoration solutions. For more on the experimental design and location, see Chapter 2 and 3. For this experiment, four pools, two Fully formed megapools and two Partially formed megapools, were selected from the broader 15 in the above two chapters for inclusion into the planting study. Nonformed megapools were excluded from this experiment as they already had vegetation present.

Experimental Design:

RTK (Real-time kinematic) satellite positioning equipment was used to measure elevations, and then locations were selected to establish plots along elevational gradients. Each elevation plot, high or low, contained two sub-plots, clumped or dispersed, creating a block of four subplots (Figure 4-1). Fully formed megapools received two blocks, creating eight subplots each. Partially formed megapools received three blocks creating twelve subplots each. This difference in blocks was due to plant availability and the expectation that Partially formed megapools would have more variation. In total, across the two Fully formed megapools and two Partially formed megapools there were forty subplots, creating a nested factorial design (Figure 4-1).

Plantings:

All plants were nursery grown *Spartina alterniflora* grown from a local native seed source. Seedlings were planted by making a pilot hole using a trowel and then putting the seedling into the hole, and placing sediment just over the immediate top of the plant. Four

hundred plants were purchased with three hundred and sixty plants needed for across the forty subplots. Blocks were established as 3m by 1m for each elevation with four stakes placed at each corner. Within each block were two 1m by 1m subplots, with a 1m by 1m buffer zone in between. Within each dispersed spacing subplot, plants were planted in a 3x3 grid, spaced 33cm between each plant. Additionally, plants had a 33cm buffer zone to the edge of the subplots, ensuring a minimum distance of 33cm from any existing vegetation. The spacing of 33cm was used as it is approximately equivalent to 13 inches where standard planting spacing in the literature and used by practitioners is 12-16 inches (Bergen et al., 2000). Within the clumped plot, seedlings were planted touching one another where all 9 plants were placed into one hole again in a 3 x 3 row formation but with no spacing (Figure 4-1). This design was modified from (Silliman et al., 2015).

To keep track of plants, facing north plants were labeled 1-9, with row one: 123, row two: 456, row three: 789. Within the first three days after planting, plots were monitored for any plants that went missing from high tide events. Five seedlings of the three hundred and sixty received a replacement plant, plots were again checked two weeks after planting and no missing plants were noted. All plants were planted at low tide April 12th, 2022. Due to time of year restrictions from nesting shorebirds, the site was inaccessible during May 1st- July 15th so additional data was not collected. Plants were measured and the subsample for drying collected during the next available time frame, on August 11th, 2022.

Height, Allometric Above Ground Biomass Data Collection, and Survival:

Leaf heights were measured from the ground to the top of the leaf. Before plantings, all leaves on 10 plants from each tray (100 plants per tray) were measured and were homogenous, so initial height of eaves of each plant in each subplot were not measured. In mid-August,

approximately three and a half months after planting, all leaves were measured per plant, and plant survival outcomes were noted. One plant of each of the 9 plants was harvested at the base, measured again, and weighed before and after drying at 70 C for 7 days. excluded from the study were 8 paired subplots due to their low survival rates (less than 25%) and 4 paired subplots were excluded from the study due to safety concerns with accessing the plots as the pool was experiencing collapse leading to 12 subplots of 40 total excluded for n=28. Total leaf height was calculated per plant where all leaves were added together by plant to obtain one value (Figure 4-1). This methodology was modified from (Hill et al., 2018).

Belowground Biomass Root Cores:

An aluminum hollow pipe measuring in diameter 9.525 by 30.48 cm (3.75 inches by 12 inches) was used to collect belowground biomass. Using a mallet with a board placed across the top, the pipe was gently tapped into the ground, and then twirled four times and rocked back and forth. The root core was then gently placed into a ziploc bag. On the dispersed plantings, the pipe was placed around 1 singular plant, and with plants spaced this captured 1 plant's worth of below ground biomass. For the clumped plantings, the plant on the corner labeled 1 or 7 depending on the survival had belowground biomass collected. Roots were then sorted by live and dead, washed, dried at 70C, and then weighed.

Due to an oversight, while both belowground biomass and aboveground biomass were collected from the same plants, we were not able to pair specimens on an individual plant basis, however we were able to pair them via plot type. Additionally, due to challenges with the root core methodology results (See Discussion), we instead opted to pair the field ratio of 2.64:1 from (Gross et al., 1991) for the root:shoot ratio.

Data Analysis:

Data were analyzed and plotted using R version "Spotted Wakerobin" using the R Studio 2022.07 interface.¹ An analysis of variance (ANOVA) was used to test the effects of classifications spacings, elevation, and megapool on below ground biomass, above ground biomass, and survival using the base R package, aov. For the allometric equation and calculation of weight, base R analysis and a regression using package ggpubr was used to establish relationships and calculate values. Conversion of characteristic data for megapool (Nonformed, Partially Formed, Fully Formed) and elevation (High, Medium, Low) was converted to integer data by classifying the least degraded marsh as 1 and the most degraded marsh as 3 with intermediate as 2, and for the spacings Clumped as 1 and Dispersed as 2. Assumptions of normality and variance heterogeneity were met.

RESULTS:

Allometric Above Ground Biomass:

A regression between total leaf height and weight for field collected values found a strong positive relationship ($r^2=0.88$, $p<0.001$), with an equation of $y=-0.04 + 0.01 \cdot x$ (Figure 4-3). They were visually clustered closely together with little variation, and had a best fit of linear. The sum of length measurements sampled in the field without weight were then plugged into this equation to establish calculated values for weight biomass (Figure 4-4), establishing predicted values.

Survival:

Survival of planted plants varied between megapool types with higher survival rates in Partially formed megapool than Fully formed megapool (Figure 4-5). The dispersed spacings had similar levels by elevation ($p= 0.549$) and spacing ($p=0.34714$), with only megapool type having

influence ($p=0.01161$) (Table 4-1). The clumped plantings had more variation but similar survival in the low and high elevations.

Predicted Aboveground Biomass:

These trends continued for the most part in the predicted biomass, with Partially formed megapools having greater biomass than Fully formed megapools in the dispersed plantings at both high and low elevations and low clumped plantings, but not high clumped plantings (Figure 4-6). Megapool again had the greatest impact on biomass ($p=0.04127$) and elevation and spacing appearing to have no influence ($p=0.5669$, $p=0.44228$) (Table 4-2). These same trends were also present in the total leaf height per plot, the raw data used to predict the calculated weight (Table 4-3, Figure 4-7).

Measured & Predicted Belowground Biomass:

Measured Belowground biomass was impacted only by elevation, where low was lower than high ($p=0.022$) (Table 4-4). Partially formed megapools had slightly greater below ground biomass, this trend was not significant. Instead, elevation with high plots having more biomass than low plots was present (Figure 4-8). Predicted below ground biomass was higher in Partially Formed than Fully Formed Megapools ($p=0.041$), but elevation and spacing had no effect ($p=0.657$, $p=0.442$) (Table 4-5, Figure 4-9).

DISCUSSION:

Seedlings planted in Partially formed megapools had greater survival than Fully formed megapools, but elevation and spacing had no effect on survival. Not only was survival lower in Fully Formed Megapools, but they also had lower predicted aboveground Biomass, predicted belowground Biomass, and measured Total Leaf Height. Surprisingly, planting elevation and planting spacing did not affect survival, predicted biomass, predicted belowground biomass, or total leaf height. Silliman et al., 2015 found that clumped plants did better at high elevations, and

that low elevations are too challenging for impacts, could be similar here. Data from other papers has historically found that higher elevations have higher survival and biomass than lower elevations (Bertness & Ewanchuk, 2002). We had therefore hypothesized that clumped plantings at high elevations would have higher survival and growth than low dispersed plantings. High Fully Formed Clumped plots did do slightly better than High Partially Clumped plots or Low Clumped and Dispersed plots at both Fully and Partially Formed megapools. This stronger impact of megapools is likely due to the differences in environmental characteristics like bulk density and sulfide levels (See Chapter 3) at these different megapool formation stages and same elevation classification.

The calculated values using the sampled belowground biomass as compared to above ground biomass for root:shoot ratio varied from 0:1 to 66.41:1, with an average of 7.55:1, much higher than any other value found in the literature (raw data). The highest root:shoot ratio for short form *Spartina alterniflora* was 3.71:1 which is still much lower than the above ratios (Gross et al., 1991). There were already concerns that the sampling protocol and lab processing would overestimate the roots per plant, and as these values were so high it was important to explore this issue. The root core methodology used for this project pulled a standardized circumference core to a standard depth around an experimental plant. However, particularly for clumped plants but also dispersed plants, this could pull up living roots from adjacent plants. Additionally, the soil is high in organic matter and has many dead roots present in it. Because there were several weeks between sampling and lab processing, it was challenging to assess which roots were dead and which were alive.

Though in the first year energy allocation of plants may differ focusing on below ground biomass production, the ratio still should not be that high (W. Liu & Pennings, 2019; van de Ven

et al., 2023). Rather we instead opted to use the above ground biomass data to extrapolate below ground biomass which we are more confident in. However, this does make the large assumption that the relationship of root:shoot would be the same in this environment, and there can be variation based on nutrient availability and salinity (Hill & Roberts, 2017). One other potential problem with this approach is that in our field measurements of root cores in Chapter Three, almost all low elevation cores across all three megapool types had zero or very low belowground biomass. Because our predicted below ground biomass calculations were based off of above ground biomass total leaf height and predicted total biomass using leaf height, below ground biomass for low elevation cores may be overestimated here. This could be due to decomposition of roots in these low elevation systems, as in highly reducing conditions with high sulfides and low dissolved oxygen organic matter decomposition can occur. It could be due to differences in energy allocation towards above ground biomass (van de Ven et al., 2023). Additionally, though the trend of megapool type impacting the data is still prominent here, it is because the original data set also had this trend.

Although the measured total leaf height against sample biomass were linearly related, about one third of the predicted data set exceeded the initial range of the measured biomass model. The assumption, then, is that the relationship would stay the same linear equation at higher weights and total leaf heights. Some studies have supported that at lower biomass levels, the allometric relationship is linear however at higher weight and total leaf height levels or at different times of year like early spring it can become a polynomial relationship (Morris & Haskin, 1990). This could be a potential flaw or area for room of improvement, it would be good to collect data at larger values to extend the model and assess the continuity of the relationship. While measuring the total leaf height was time and labor intensive, it was an effective way to

assess belowground biomass. One challenge that arose in the field was separating which leaves came from which plant on the clumped plots, however because the total leaf height was later converted to per plot rather than per plant, any misattribution to another plant would no longer be a relevant issue. The total leaf height and biomass data for the plants used to establish the allometric model were excluded from analysis by megapool, elevation, and spacing because there were so few replicates, and the sampling methodology was designed for equation establishment rather than assessing impacts.

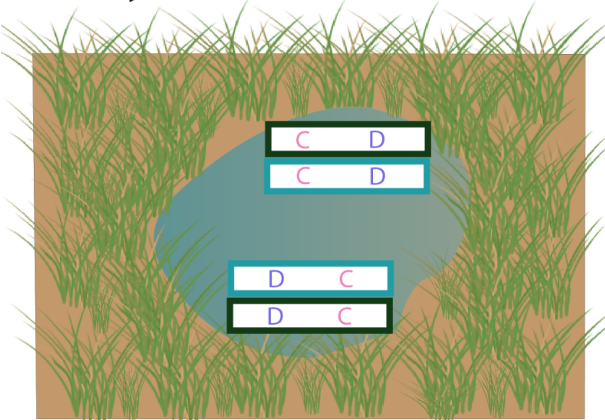
The initial goal of the planting was twofold, the first being to explore the impact of different elevations and spacings on survival and growth. The secondary goal was to explore if in some situations plantings may be sufficient to recolonize an area without another intervention. While planting survival was fairly high in Partially formed pools, on a spatial level, the plants took up relatively little surface area and did not seem to spread much. Further assessment in additional years should be drawn to make further conclusions on whether pools that receive even small amounts of plantings recolonize faster.

CONCLUSION:

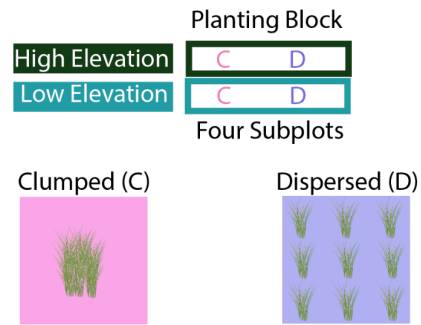
Vegetative cover is an important metric in marsh health and restoration success. Concerns about the need for more intensive restoration led to curiosity about whether plantings might be sufficient in high elevations and in Partially formed megapools. Additionally, there was interest in assessing survival and biomass of *Spartina alterniflora* plantings at different spacings to test if facilitation was more important than competition here. This led to the design of an experiment assessing plantings in relation to elevation, pool formation stage, and spacings. Establishing an allometric linear regression model between Measured Total Leaf Height and Measured Biomass Weight had a strong linear correlation, allowing us to Predict Biomass Weight of other Measured Total Leaf Height Data. We found that Predicted Biomass Weight and its input Measured Total Leaf Height Data were impacted only by megapool type with Partially formed megapools having higher biomass and leaf height by plot. Similarly, Calculated Belowground Biomass using the Predicted Biomass Weight a root:shoot ratio of 2.64:1 was also only impacted by megapool type. While Field Collected Belowground Biomass was impacted only by elevation, concerns about biases in the data set led to use of the Calculated Belowground Biomass data set instead. As Predicted Biomass Weight and Calculated Belowground Biomass were all based off of Measured Total Leaf Height Data, it makes sense that they would have similar effects from variables. However Survival, a metric collected separately, was also impacted only by megapool type, confirming the impact of this effect. Across all analyses, spacing had no effect on survival, aboveground biomass, or belowground biomass. While plantings had reasonable survivals, we noted that the slow pace of recolonization and dieback present still may lead to the need for more aggressive interventions, such as runnels.

FIGURES:

Fully Formed

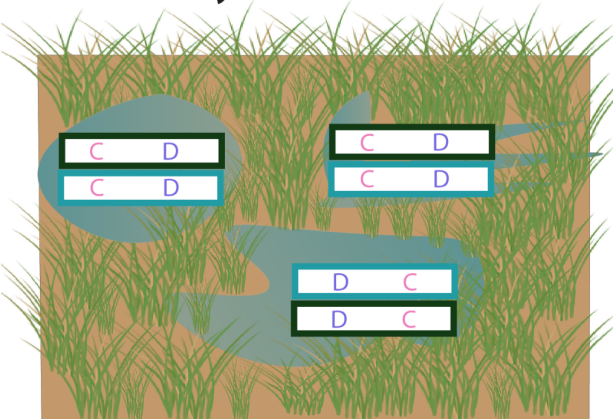


Legend



Subfigure B

Partially Formed



Total Leaf Height

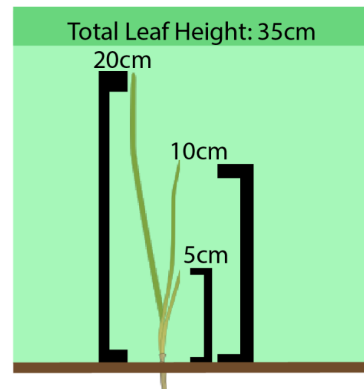


Figure 4-1.

There were two Partially formed megapools, which received 3 blocks, and two Fully formed megapools, which received 2 blocks. Each block contained a high elevation and low elevation plot, which in turn each contained a clumped and dispersed subplot. Total Leaf Height, subfigure B, was calculated by summing each individual leaf height per plant.



Figure 4-2.

April 12th, 2022. This photo shows a high elevation block at the top, along with two subplots, dispersed towards the left and clumped towards the right. At the bottom, is a low elevation block, along with two subplots, dispersed towards the left and clumped towards the right. Wooden stakes and flags denote the edge of the plots.

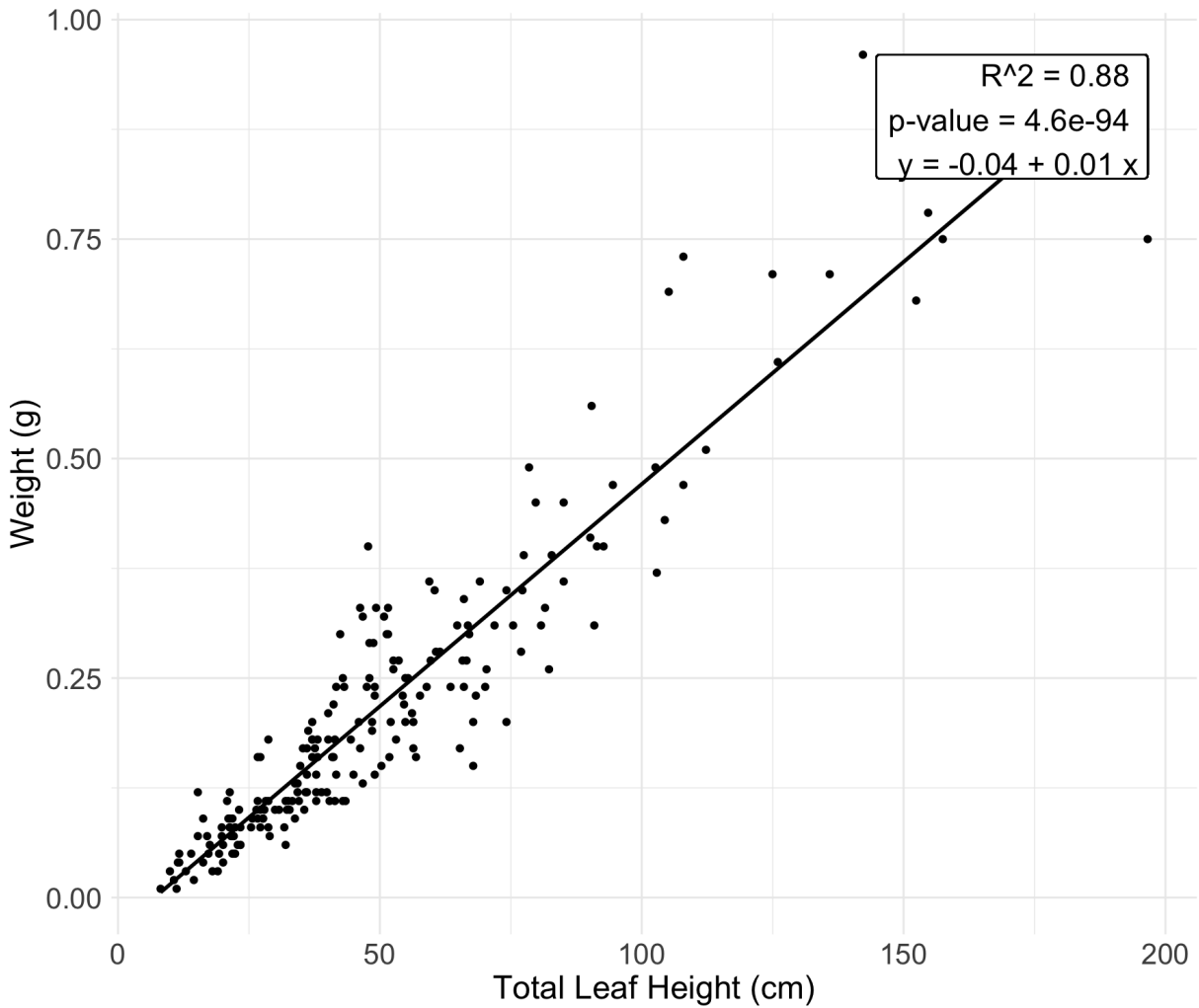


Figure 4-3.

Weight (g) and Total Leaf Height (cm) for leaves of *Spartina alterniflora* were collected in field and then dried and weighed. This graph depicts a regression modeled as linear (best fit) of Weight (g) vs. Sum of Length (cm).

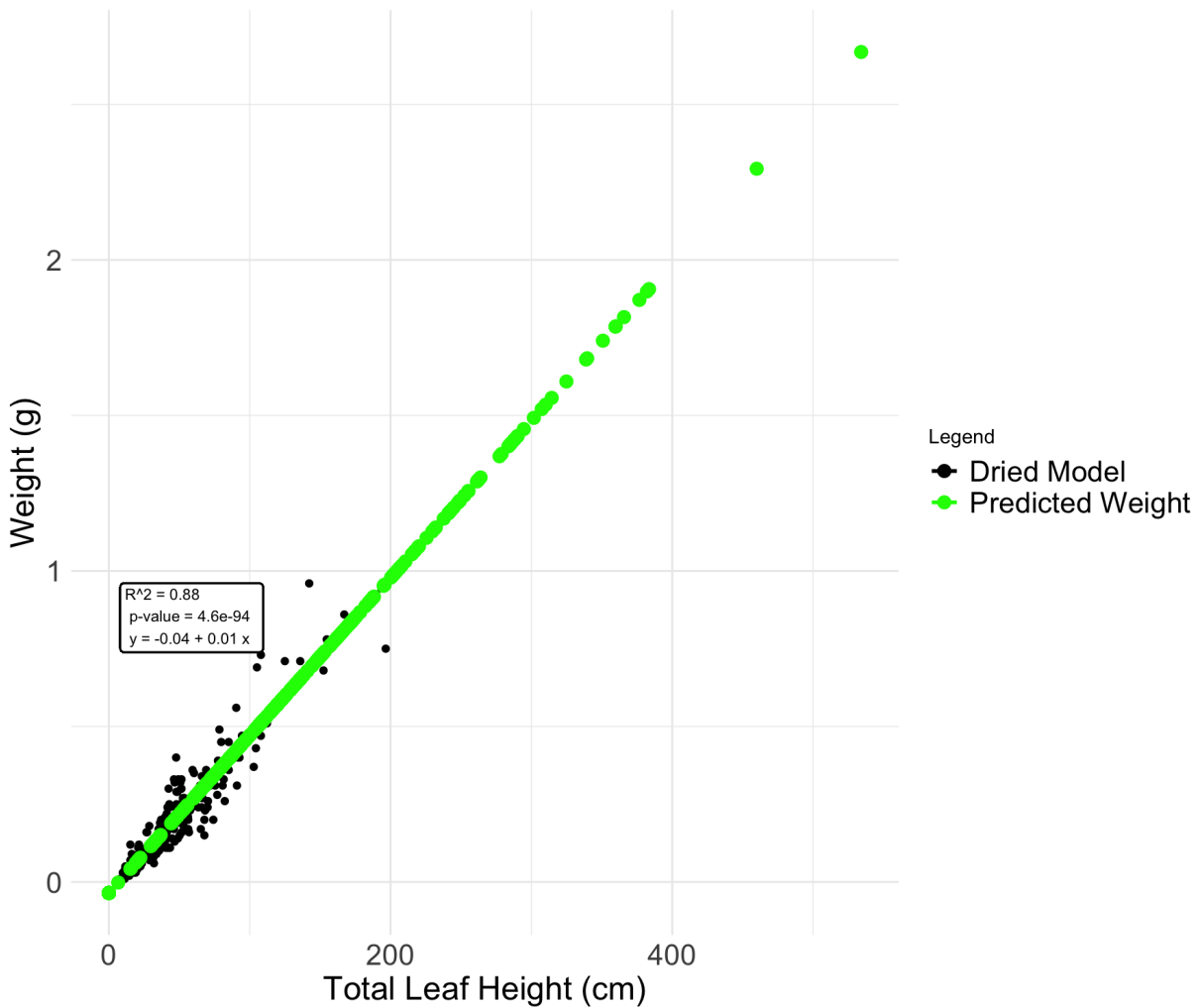


Figure 4-4

Weight (g) and Total Leaf Height (cm) for leaves of *Spartina alterniflora* were collected in the field and then dried and weighed, shown in black; along with predicted Weight (g) for leaves of *Spartina alterniflora* measured in the field to obtain Total Leaf Height, shown in green. This graph depicts a regression modeled as linear (best fit) of Weight (g) vs. Total Leaf Height (cm) with both the dried and calculated predicted values.

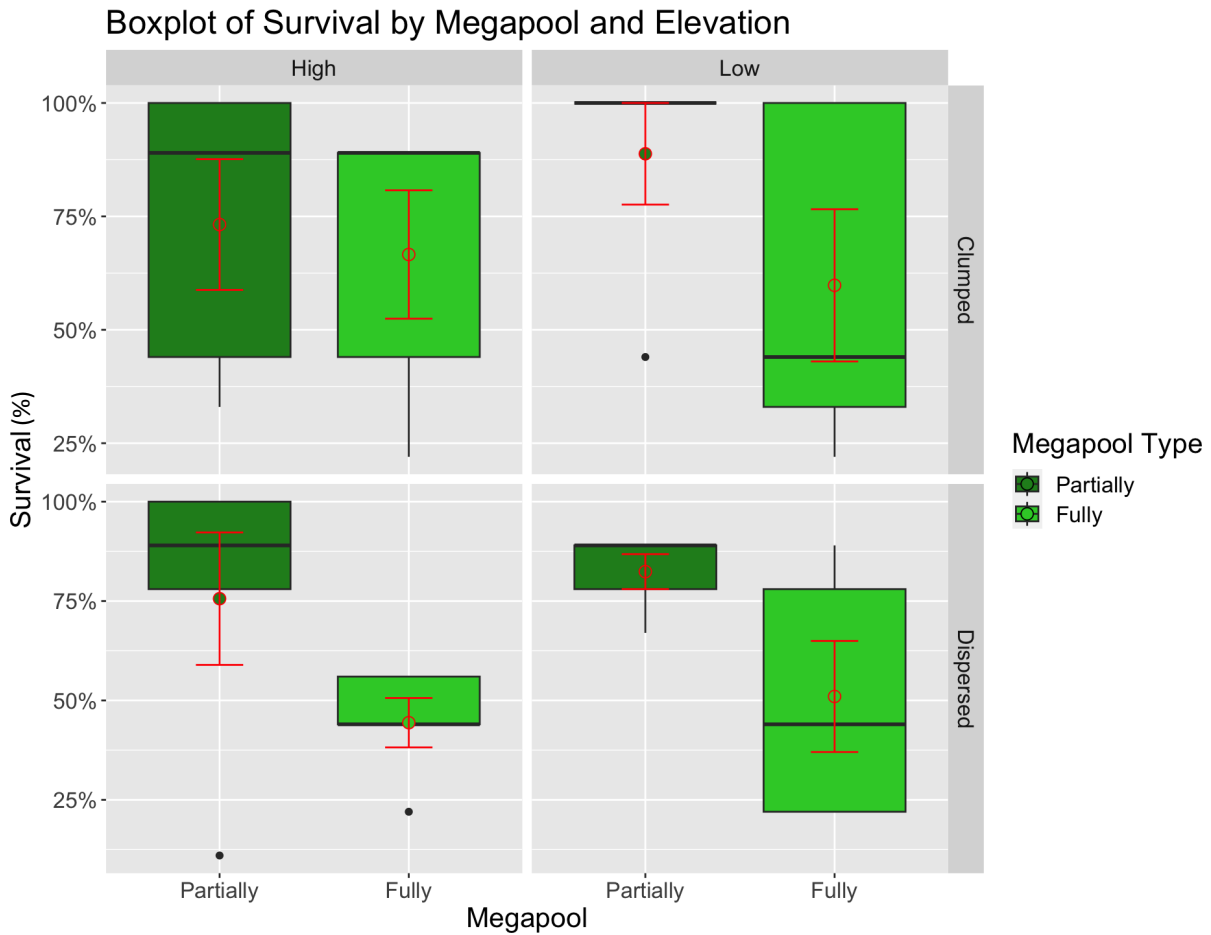


Figure 4-5.

Box plot of percent survival of plants within each plot as stratified by megapool formation level (Partially or Fully), spacing (clumped or dispersed), and elevation (high or low). Here the boxplot shown in black shows the Minimum, Lower Quartile 1, Medium, Upper Quartile 1, and Maximum, and the red shows the standard error and mean.

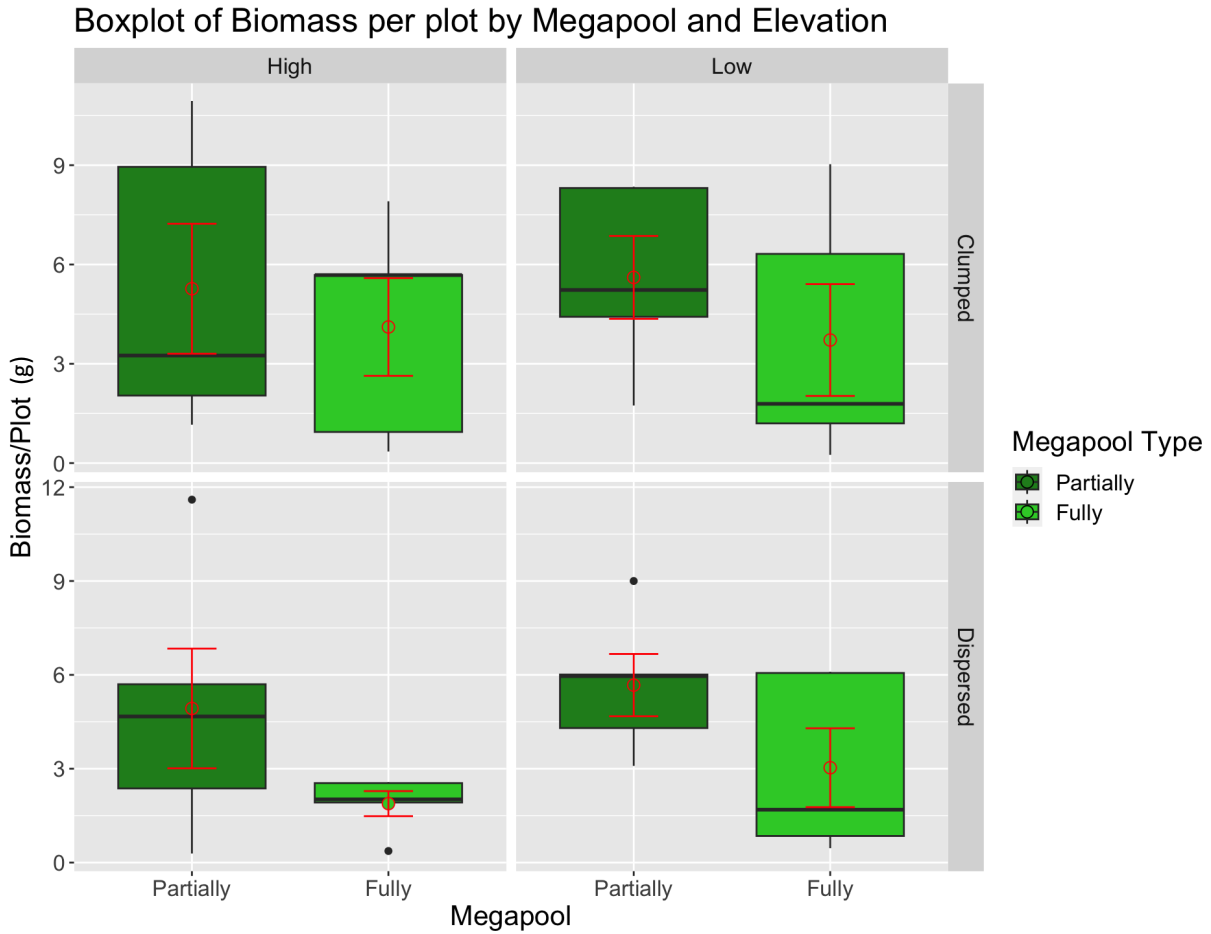


Figure 4-6.

Box plot of predicted aboveground biomass of each plot, using the field sampled Total Leaf Height and calculated model regression shown in Figure 4, as stratified by megapool formation level (Partially or Fully), spacing (clumped or dispersed), and elevation (high or low). Here the boxplot shown in black shows the Minimum, Lower Quartile 1, Medium, Upper Quartile 1, and Maximum, and the red shows the standard error and mean.

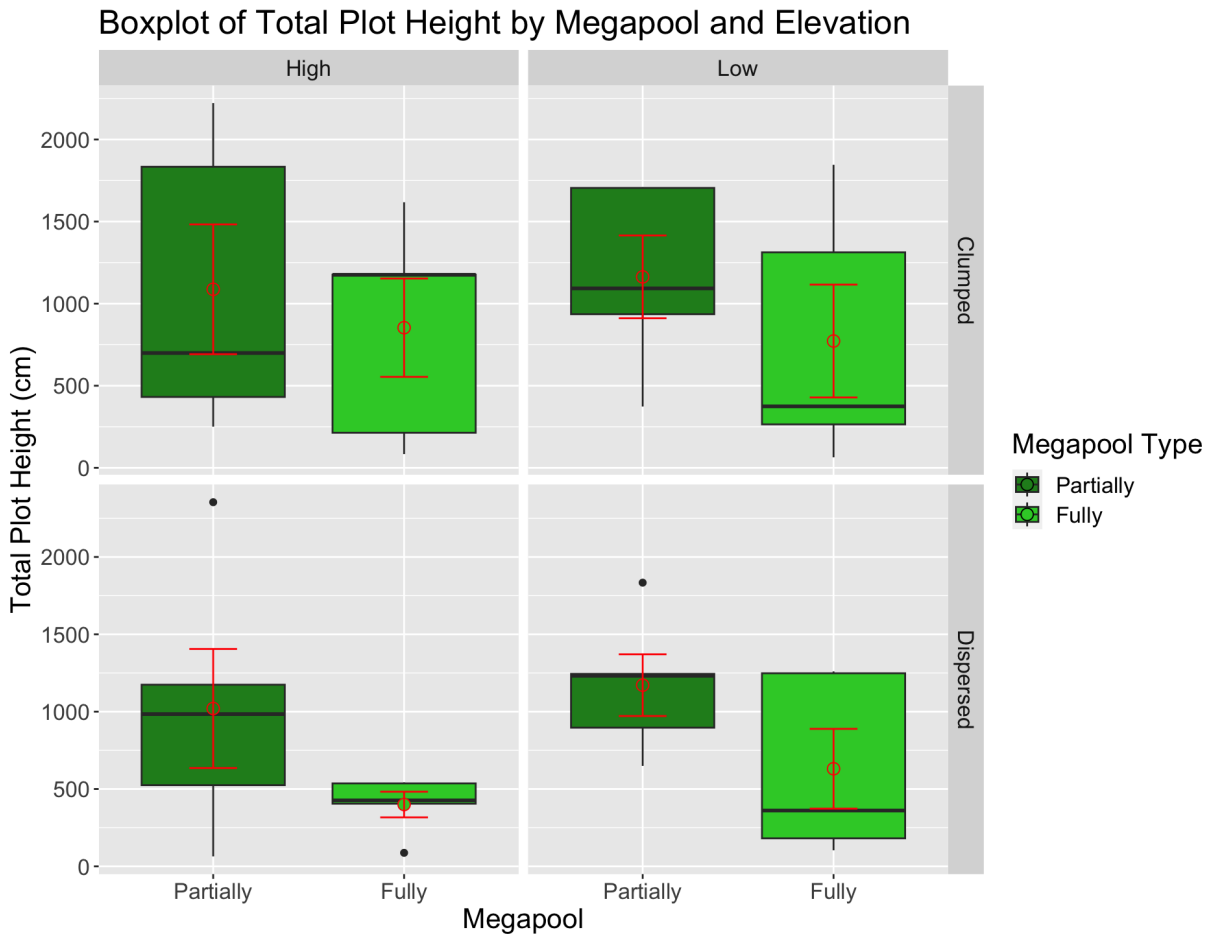


Figure 4-7.

Box plot of measured Total Leaf Height of each plot, using the field sampled Total Leaf Height which was used to calculate Figure 3, as stratified by megapool formation level (Partially or Fully), spacing (clumped or dispersed), and elevation (high or low). Here the boxplot shown in black shows the Minimum, Lower Quartile 1, Medium, Upper Quartile 1, and Maximum, and the red shows the standard error and mean.

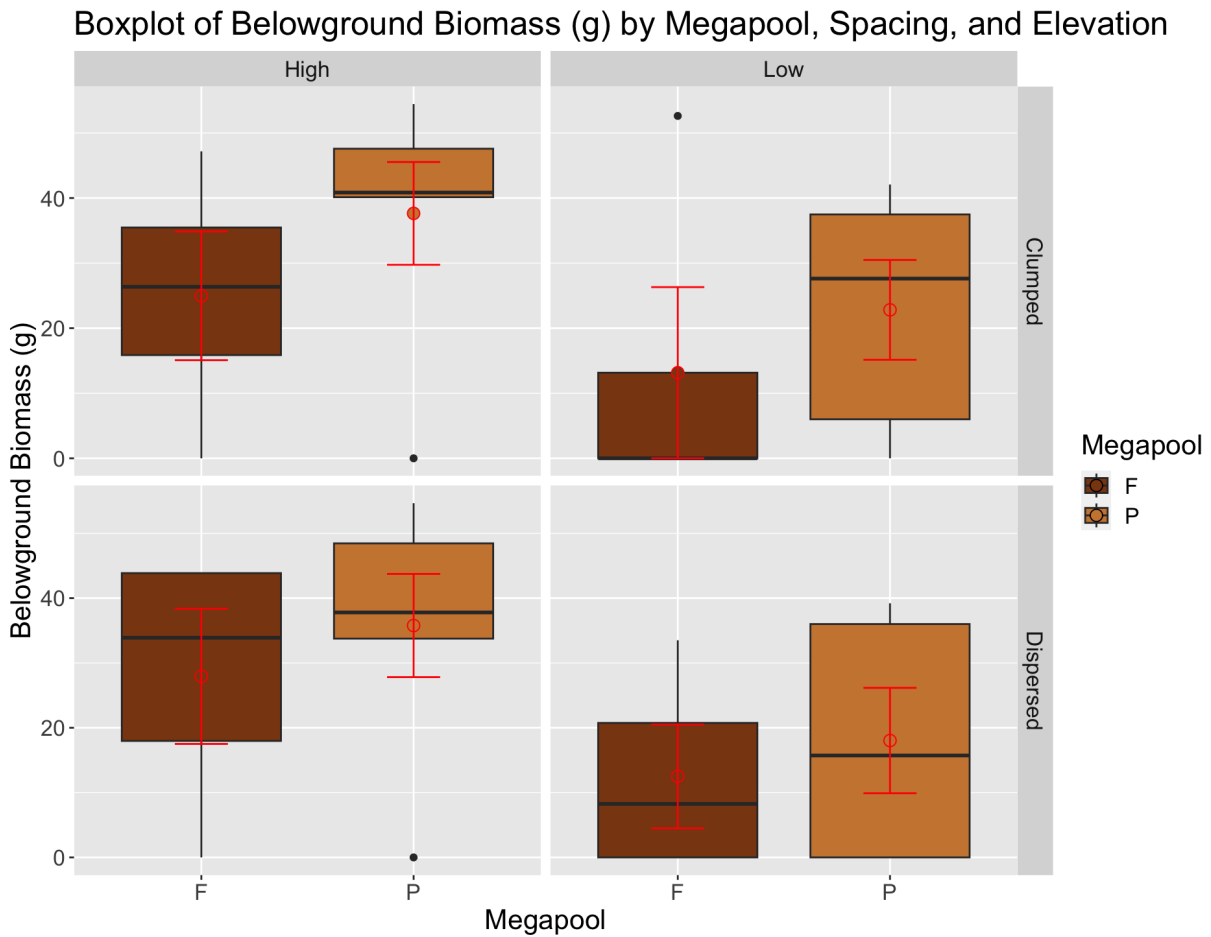


Figure 4-8.

Box plot of field sampled belowground biomass, using the data collected from root cores, as stratified by megapool formation level (Partially or Fully), spacing (clumped or dispersed), and elevation (high or low). Here the boxplot shown in black shows the Minimum, Lower Quartile 1, Medium, Upper Quartile 1, and Maximum, and the red shows the standard error and mean.

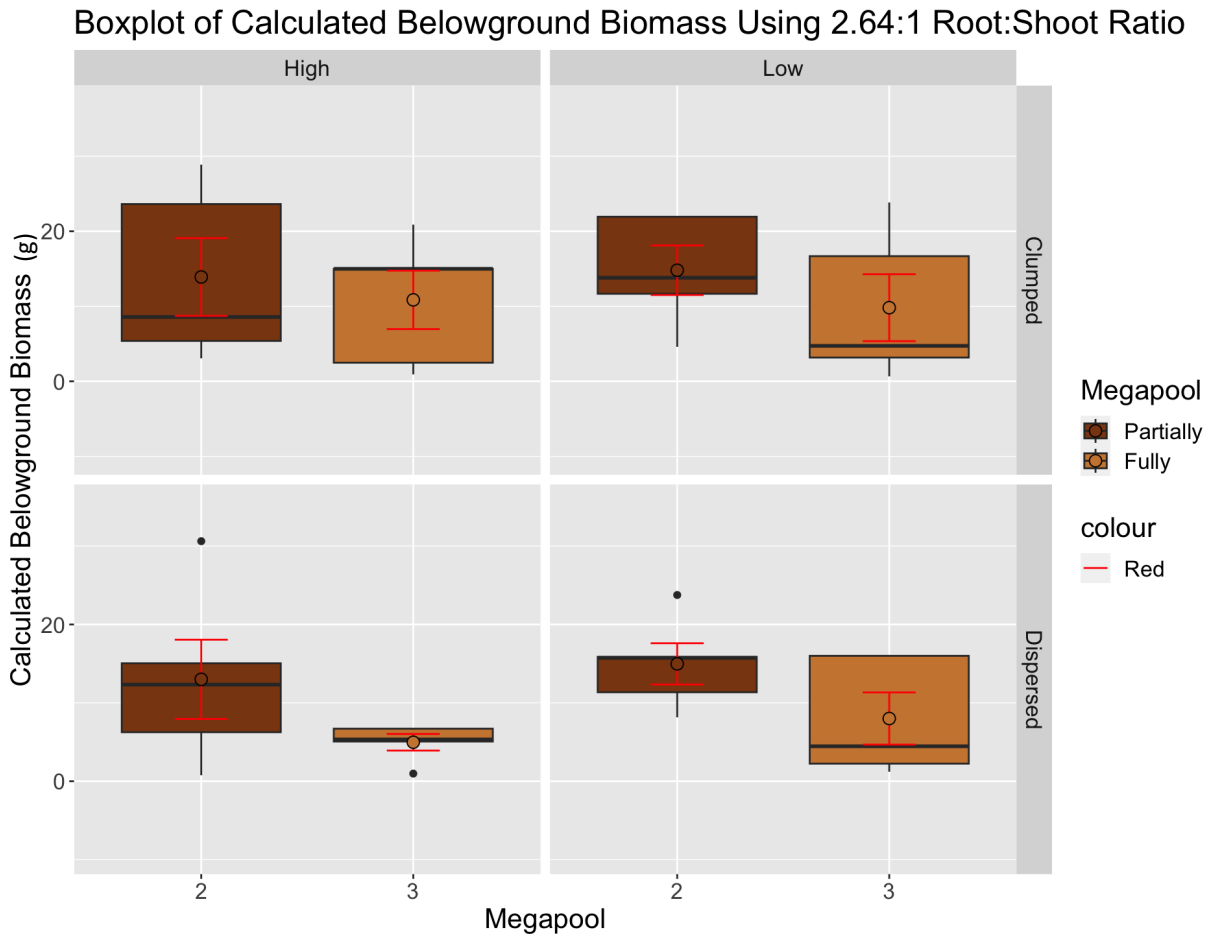


Figure 4-9.

Box plot of Calculated belowground biomass, using the calculated above ground biomass using the measured total leaf height data, as stratified by megapool formation level (Partially or Fully), spacing (clumped or dispersed), and elevation (high or low). Here a ratio of 2.64:1 for root:shoot was used. Here the boxplot shown in black shows the Minimum, Lower Quartile 1, Medium, Upper Quartile 1, and Maximum, and the red shows the standard error and mean.

TABLES:**Table 4-1.**

Percent Survival Analysis of Variance Table of each Plot against megapool, elevation, and spacing and their interactions.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	1	0.6027	0.6027	7.1672	0.01161
Elevation	1	0.0308	0.0308	0.3663	0.5493
Spacing	1	0.07656	0.07656	0.9105	0.34714
Megapool:Elevation	1	0.03192	0.03192	0.3796	0.54217
Megapool:Spacing	1	0.04556	0.04556	0.5418	0.46704
Elevation:Spacing	1	0.00132	0.00132	0.0157	0.90099
Megapool:Elevation:Spacing	1	0.0308	0.0308	0.3663	0.5493
Residuals	32	2.69092	0.08409		

Table 4-2.

Estimated Calculated Predicted Biomass of each Plot against megapool, elevation, and spacing analysis of variance table.

	Df	Sum Sq	Mean Sq	F value
Megapool	1	47.63	47.633	4.5219
Elevation	1	2.12	2.121	0.2013
Spacing	1	6.38	6.376	0.6053
Megapool:Elevation	1	0.07	0.07	0.0066
Megapool:Spacing	1	4.34	4.336	0.4116
Elevation:Spacing	1	2.37	2.367	0.2247
Megapool:Elevation:Spacing	1	0.81	0.809	0.0768
Residuals	32	337.09	10.534	

Table 4-3.

Field Collected Total Leaf Height of each Plot against megapool, elevation, and spacing analysis of variance table.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	1	308967	308967	4.6287	0.03909 *
Elevation	1	13753	13753	0.206	0.65296
Spacing	1	41492	41492	0.6216	0.43625
Megapool:Elevation	1	569	569	0.0085	0.927
Megapool:Spacing	1	27863	27863	0.4174	0.52283
Elevation:Spacing	1	14558	14558	0.2181	0.64365
Megapool:Elevation:Spacing	1	5459	5459	0.0818	0.77674
Residuals	32	2135988	66750		

Table 4-4.

Biomass of belowground root cores (g) against megapool, elevation, and spacing and their interactions analysis of variance table.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	1	764.3	764.27	1.9034	0.17726
Elevation	1	2317.7	2317.7	5.7724	0.02225
Spacing	1	23.5	23.47	0.0585	0.8105
Megapool:Elevation	1	16.9	16.94	0.0422	0.83856
Megapool:Spacing	1	48.1	48.13	0.1199	0.73143
Elevation:Spacing	1	25.6	25.6	0.0638	0.80227
Megapool:Elevation:Spacing	1	0.3	0.29	0.0007	0.97858
Residuals	32	12848.5	401.52		

Table 4-5.

Predicted Belowground Biomass Anova of Calculated Belowground biomass using root:shoot ratio of 2:64:1 of each Plot against megapool, elevation, and spacing as well as their interactions.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Megapool	1	331.98	331.98	4.5219	0.04127
Elevation	1	14.78	14.78	0.2013	0.65669
Spacing	1	44.44	44.44	0.6053	0.44228
Megapool:Elevation	1	0.49	0.49	0.0066	0.93567
Megapool:Spacing	1	30.22	30.22	0.4116	0.52571
Elevation:Spacing	1	16.5	16.5	0.2247	0.63871
Megapool:Elevation:Spacing	1	5.64	5.64	0.0768	0.78341
Residuals	32	2349.36	73.42		

CHAPTER FIVE: CONCLUSIONS, IMPLICATIONS, AND NEXT STEPS FOR MEGAPOOLS AND RESTORATION POTENTIAL

SUMMARY:

Historic marsh management strategies, such as mosquito ditching, have left marshes more vulnerable to the already damaging impacts of sea level rise. In many ditched coastal salt marshes, large megapools, or ponded areas of vegetation dieback, have formed. Concerns arise that this interior marsh loss through megapools may not provide the same ecosystem services as vegetated areas of marsh, are more vulnerable to storms, and should be of concern in restoration ecology. Here, we sought to explore differences in environmental characteristics in varying pool formation stages (Full Formed, Partially Formed and Nonformed/Control) and Elevations (High-Fully Vegetated, Medium-Partially Vegetated, Low-Unvegetated) as well as potential restoration solutions.

In Chapter 2, using IRIS films, we found that sulfide concentrations increase with depth in all megapool and elevation types, with Nonformed areas having similar concentrations across all three elevation stages. We found that Fully and Partially Formed megapools had higher sulfide levels in Low and Medium than High Elevations, and that Fully Formed megapools had more sulfides than Partially formed megapools. Sulfide concentration correlated with plant coverage, root cores, and megapool type.

In Chapter 3, we explored vegetation transects more closely, and found an increase in open water in Fully formed and Partially formed megapools between 2021 and 2022, and Nonformed and Partially formed megapools were not different from one another in open water coverage in 2022. Fully formed megapools had lower elevations and more range of elevations than Partially or Nonformed, which did not differ from one another in elevation. For root cores,

high elevations had more than medium which had more than low, and correlated with plant coverage as well as with elevation, but not with megapool. As no mosquitoes were found, they were excluded from analysis. Organic Matter, Bulk Density, Carbon, Nitrogen, and Carbon Density were all more impacted by megapool type than elevation where more degraded areas of marsh of medium and low elevations then Partially and Fully formed megapools, have less carbon density where degradation removes materials lowering their bulk density. In terms of plant coverage, open water presence, root cores, and elevation, Nonformed and Partially formed megapools at high and medium elevations are more similar than Fully formed or low elevations. These results support a close relationship between pool stages of formation, elevation, vegetation health, and sulfide levels.

In Chapter Four, we explored the impact of different spacings (clumped, dispersed), megapool types (Fully, Partially), and elevations (high, low) on survival and growth of *Spartina alterniflora* seedlings to assess if plantings may be a viable restoration strategy. We found a strong allometric linear relationship between Measured Total Leaf Height and Measured Biomass Weight, allowing us to Predict Biomass Weight of other Measured Total Leaf Height Data. While we field collected Belowground Biomass and an elevation effect was found, concerns about data biases led to usage of a 2.64:1 root:shoot ratio to Calculate Below Ground Biomass. For Predicted Biomass Weight and Calculated Belowground Biomass, based off of Measured Total Leaf Height Data, all three were impacted solely by megapool type. Similarly, plant survival was also only impacted by megapool type. For all of these metrics, Partially formed megapools had a higher biomass than Fully formed pools, and no impact of spacing type or elevation level were found. While plantings had reasonable survivals we noted that the slow

pace of recolonization, dieback present, and high cost still may lead to the need for more aggressive interventions, such as runnels.

IMPLICATIONS:

Salt marshes are experiencing rapid change and there are a number of ongoing research and restoration projects underway. For instance, in Maryland, there are at least 3 projects that will be constructed in the next year implementing runnels and other low tech restoration, and 4 more to be hopefully constructed in the following 2 years. In Delaware and New Jersey, there are 15 projects slated for construction in the next two years. There are a number of ongoing partnerships to enhance salt marsh restoration and monitoring. The research obtained here is crucial to add to the growing body of knowledge for megapools and environmental characteristics, as well as best practices for protocols on a local level. Focused monitoring and sharing knowledge is crucial for restoration projects to understand lessons learned and demonstrate success.

Lessons Learned and Future Research Needs for Salt Marsh Restoration:

While there are many existing protocols and methodologies and monitoring for salt marshes, here were the variables and methodologies that we found most useful as well as lessons learned. Specifically, this may be useful for looking at before and after effects of restoration.

- Marsh Units: Dividing the marsh into “marsh units”, so called numbered areas divided by environmental features like ditches, open water, or high marsh and upland, allowed for easy reference to specific areas, labeling, and prevented miscommunications.
- Regular Landowner and Stakeholder Communications: A monthly meeting was held with updates for all stakeholders as part of an existing meeting and this was a useful way to provide updates, share resources, and get feedback from everyone involved. Additionally,

landowners received email updates quarterly, and were given at least 14 days' notice before fieldwork happened. This frequent communication strengthened the project.

- Paired Elevational and Vegetation Transects: For this project, we established 30 transects with 2 in each marsh unit. In the future, for restoration purposes it may be less time and labor intensive to establish fewer longer transects that cross over multiple marsh units. We found the linear point slope method to be a useful and quick way to assess vegetational changes. In the future, it may be good to note specific numeric changes of large swaths of vegetation to dieback areas rather than solely the general numeric category they fall under (i.e. vegetation dieback starts at 1.4m rather than between 1.25m-1.5). Transects may not be the best or only method for assessing restoration success, though similar sampling should be kept before and after restoration for accuracy.
- Water/Water Characteristics: Implementing hobo water loggers and taking regular salinity, DO, and temperature measurements in megapools and adjacent ditches was useful to explore inundation and water characteristics. One challenge we encountered was the harsh salt marsh conditions led to frequent lapses in data from malfunctioning loggers. Rather than wait 6 months to collect data (when loggers would be full), it would be better to capture data every 3 months so less data was lost in the event of an issue. Additionally, more frequent scheduled collection of water characteristics may lead to better analysis and more firm conclusions than our ad hoc method.
- Soil Characteristics/IRIS: Bulk density and organic matter were useful assessments of marsh health as was sulfide levels. Calculating sulfide levels was effective and easy with IRIS strips, and the data supports sulfide playing a key role in vegetation dieback characteristics. Including this in future studies is useful.

- Deeper depths for Carbon Stock: Initial assessments of Carbon density at 30cm showed variation between Megapool and elevation. Going to deeper depths to assess Carbon stocks could be an important next step.
- Add shear strength assessment: Shear Strength was not directly tested, including an assessment of shear strength would be useful and should be included in future projects.
- Add Nekton: Nekton surveying at Rum Pointe was completed in 2023 as part of the post monitoring study, however was not initially included in the 2021 and 2022 baseline data collection. In future projects, it would be useful to include this metric to understand if megapools are providing habitat for nekton, and how that usage may change over time with restoration.
- Equations: Root core data, Carbon and Nitrogen calculations, and establishing an allometric equation were very labor and time intensive. For future projects with a focus specifically on restoration outcomes in similar environments, equations calculated here or found in the literature may be sufficient. Additionally, metrics of vegetation presence absence may be sufficient for some research studies rather than above and belowground biomass.
- Plantings: Seedlings were time intensive and only took up a very small amount of area of the megapool. Planting seeds or waiting for natural recolonization may be more cost effective. That being said, for areas in public view such as a marsh unit next the landowners house plantings may be effective for outreach purposes.
- Runnel Shapes: Preliminary results from studies in New England as well as discussions with hydrologic experts have led to implementation of sinuous “S-Shaped” runnels rather than linear ones. It could be useful to assess different types of runnels shapes, widths,

depths and results, specifically how runnels may impact tidal flood and ebb cycles along with sediment import and exportation.

NEXT STEPS:

In January of 2023, 35 runnels were placed at the field site Rum Pointe, with 8 of the 15 study marsh units receiving runnels (Figure 5-1). The 3 Nonformed units did not receive runnels, while 4 of the 6 Partially and Fully formed units did receive runnels. The 2 Partially and 2 Fully formed that did not receive runnels were intentionally excluded from the study as part of the before-after-control-impact design, where the ones that did not receive runnels can be compared to those that did. A portion of the data collected in Chapters 2, 3, and 4 will be collected again after runnels have been implemented though on a smaller scale to assess changes from runnels. Soil characteristics and belowground biomass will likely not be repeated.

Summer 2023:

- Chapter 3: Vegetation, Elevation, Mosquitoes, and Water characteristics were repeated
- Chapter 4: The planting experiment was also replicated

Summer 2024:

- Chapter 3: Vegetation, Elevation, Mosquitoes, and Water characteristics will be repeated assuming funding and capacity.
- Chapter 4: The planting experiment will be monitored, no additional plantings will occur

Summer 2025:

- Chapter 2: If funding allows, repeat subset of IRIS Sulfide data
- Chapter 3: Vegetation, Elevation, Mosquitoes, Water, and Water characteristics will be repeated assuming funding and capacity.
- Chapter 4: The planting experiment will be monitored, no additional plantings will occur

FIGURES:



Figure 5-1

This image shows constructions of a runnel on the lower left, where plant and root matter is being manually shoveled out, placed in five gallon buckets for transport, and used to build hummocks inside a megapool.

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