Coastal Plant Species Response to Salinity Stress

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Dedication

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Chapter 1: Salt stress intensity and duration have independent and interactive effects on plant responses to salinity

Abstract.

Species-specific variation in response to stress is a key driver of patterns in community composition and ecosystem function. As climate change alters stress regimes, coastal plant communities must respond to elevated salinity due to the press stressor of sea level rise, in addition to the pulse stressor of more intense storms. Our study investigates the effects of presses and pulses of salinity stress on five glycophytic and five halophytic species to determine whether salinity intensity, duration, or their interaction best explain patterns in survival and performance. In a greenhouse salinity stress exposure experiment, salinity intensity best explained patterns of survival in glycophytic species, while the interaction between intensity and duration best explained survival in halophytic species. The interaction between intensity and duration also best explained biomass and chlorophyll production for glycophytic and halophytic species. There was interspecies variability in the magnitude of this interaction, with some species having a more pronounced interaction in biomass response (glycophytes *Persicaria maculosa*, *Sorghum bicolor* and *Glycine max*) and chlorophyll response (halophytes *Phragmites australis* and *Kosteletzkya virginica*). In leaf chlorophyll concentration, there were two distinct patterns in the interaction of salinity intensity and duration: for the majority of species, as duration increased, the effect of intensity on biomass became more negative, while for *P. australis* and *K. virginica*, the effect of intensity on chlorophyll became more positive with increasing duration, indicating a compensatory response. The

slope of the effect of salinity intensity on biomass at the highest duration (i.e. as a press stressor) was used to quantify and rank salinity tolerance. Using this indicator, species salinity tolerance levels matched those in the literature. In conclusion, by measuring species-specific response patterns to stress exposure, we were able to visualize the independent and interactive effects of salinity stress intensity and duration and found that these interactions varied by species type and in magnitude.

Introduction.

Community composition and ecosystem services are fundamentally underlain by species-specific variations in response to stress; quantifying this inter-species variation can assist with forecasting future distributions and predicting community dynamics. For example, inter-species variation in stress tolerance drives succession (Connell and Slatyer 1977, Griffiths and Orians 2004), spatial patterning such as zonation (Engels et al. 2011), ecological filters (Alexander et al. 2011), species interactions (Menge and Sutherland 1987, Bertness and Callaway 1994), and species distributions (Wethey 2002, Sommer et al. 2018). Stress events can be broadly categorized as press stressors (i.e. long-term changes in an environmental variable) and pulse stressors (i.e. shorter, punctuated events) (Li et al. 2022; Smith et al. 2009).

Salinity is a near universal stressor for terrestrial plants. Elevated salt concentrations induce osmotic stress, mimicking drought conditions and making it more difficult for plants to uptake the water necessary to perform vital functions, including photosynthesis and maintaining turgor pressure (Munns 2002). Additionally, salt can have toxic effects on plant tissues (Munns and Tester 2008); it can reduce germination,

nutrient uptake, seed production or crop yield, and growth, and ultimately cause senescence and plant death (Tanji and Kielen 2002, Bartels and Sunkar 2005, Munns and Tester 2008).

Salinity is a dynamic environmental variable that can occur as a press or pulse stressor (Jiang et al. 2012). Sea level rise (SLR) and saltwater intrusion (SWI; defined as the movement of saltwater into inland areas via groundwater) are press stressors that act over longer time scales, as salinity steadily rises when tidal waters and salt permeate the soil and porewater further inland during regular tidal events (Li et al. 2022). This press primarily impacts stress intensity, as salt concentrations gradually rise over decadal timescales. Storm events are a pulse stressor, with seawater overtopping soil during acute flooding events and punctuated rises in groundwater and porewater salinity at local and regional scales (Frazer et al. 2006, Nordio et al. 2022). The frequency and strength of storm events are both anticipated to increase due to elevated sea surface temperatures in tandem with gradually rising sea levels (Hanley et al. 2020). It is therefore imperative to study the interaction between salinity intensity and stress duration in order to anticipate its impact on coastal plant communities in the face of pulse and press stressors (Hanley et al. 2020; Harris et al. 2018).

Controlled greenhouse experiments, coupled with field observations, have demonstrated that plant ecophysiology responses to increased salinity are species-specific, with marked differences in how species can tolerate and recover from seawater inundation events (Li et al. 2022; Parida and Das 2005; Spalding and Hester 2007; Howard and Mendelssohn 1999). Different plant species may also have different thresholds of tolerance based on the duration of salinity exposure (Li et al. 2022). Some

non-halophytic plant species can recover after short-term exposure to elevated salinity levels as long as post-pulse conditions allow for a recovery period, i.e. adequate soil conditions with lowered interstitial salinities (Flynn et al. 1995; Goodman et al. 2010). This is a source of resilience for coastal plant communities experiencing pulses of salinity. Yet, if elevated salinity persists over time and becomes a press stressor, or the post-pulse environment is not suitable for plant recovery, less salt-tolerant species are filtered out by elevated salinity stress (Li et al. 2022; Flynn et al. 1995). Interspecific variation in tolerance and recovery patterns between species can result in a shift in community composition, as salinity filters the plant community assemblage. This, in turn, can alter ecosystem function (Gedan and Fernández-Pascual 2019; Howard and Mendelssohn 1999; Anderson et al. 2022). Understanding which plant species are tolerant to different combinations of salinity intensity and duration can offer insight into how communities are likely to shift in composition, and therefore function, over time (Howard and Mendelssohn 1999).

To investigate how pulses and presses of salinity stress affect the survival and fitness of coastal plant species, I manipulated two variables of salinity exposure that represent the pulse and press stressors occurring in coastal ecosystems: 1) *intensity*, or the concentration of salt in water, measured in parts per thousand (PPT), and 2) *duration*, or the length of time that plants were exposed to salt water, measured in days per month (DPM) (Harris et al. 2018). We also calculate a novel stress index that integrates salinity stress intensity and duration, which we call the salt stress exposure (SSE) index.

The SSE index was inspired by the growing degree days (GDD) index, which has intuitive units and has been extremely useful in relating temperature and organismal

physiology. Growing degree days is a way to understand and quantify the number of days where growth is able to occur by summing the number of degrees over a baseline temperature that accumulate each day over a growing season, the summation of which describes the total growing time that falls within benign operating conditions (Gilmore and Rogers 1958). Every degree over the threshold temperature contributes to the growth of the plant, and GDD is highly correlated with plant production and yield. Building on the additive framework of GDD, we estimate SSE by multiplying PPT of the intensity treatment by DPM of the duration treatment to accumulate salinity exposure stress-time. SSE therefore combines intensity and duration into a single variable, as a simple representation of the interaction between intensity and duration.

As species vary greatly in their ability to tolerate salinity, this study aimed to test salinity tolerance in a group of coastal and upland species that exhibit a diverse array of adaptations to salinity (Zhao et al. 2020). Species fall into two main groups defining their salt tolerance: halophytes and glycophytes. Halophytic plants have evolved specialized adaptations to cope with saline conditions, such as synthesizing organic solutes and accumulating stress metabolites such as proline (as well as ions such as calcium and potassium) to maintain osmotic balance between the internal and external environments (Munns and Tester 2008; Flowers and Colmer 2008; Zhao et al. 2020). Additional adaptations for salt tolerance include low rates of sodium and chlorine ion transport to the leaves and compartmentalizing these ions to prevent toxic buildup in the cytoplasm or cell walls (Munns 2002). A select number of halophytes are also able to exude excess salt from salt glands in their leaves to prevent toxicity (Bradley and Morris 1991; Li et al. 2018). Having one or more of these adaptations allows halophytic species to avoid

salinity toxicity from an excessive buildup of salt ions. Glycophytes lack these adaptations and are therefore more susceptible to salinity stress.

Coastal and tidal wetland species are halophytic and adapted to repeated inundation events and elevated levels of salinity. Coastal and wetland species tested within this experiment and expected to exhibit higher salt stress tolerance include *Kosteletzkya virginica, Panicum amarum, Paspalum floridanum, Phragmites australis,* and, the moderately salt tolerant *Panicum virgatum*. We expected these species to maintain biomass and chlorophyll production at elevated levels of salinity (both intensity and duration). The upland plant species tested within this study – *Brassica napus, Glycine max* (a genetically-modified, chloride-excluding variety by Syngenta), *Persicaria punctata, Persicaria maculosa,* and *Sorghum bicolor* – are glycophytes, originating from areas of little to no salinity. In coastal regions these species are increasingly experiencing salinity levels and inundation events to which they are not well-adapted. We therefore expected to see less salinity tolerance in these upland species, specifically lower biomass and lower chlorophyll levels in response to increasing salinity intensity and duration.

By developing physiological response curves upon exposure to various levels of salinity intensity and duration, this study investigated the following research questions:

1) Which metric(s) of salinity exposure best explains variation in survival and plant ecophysiology (biomass and chlorophyll production) of halophytic and glycophytic species?

2) Can species tolerance to salinity stress be sufficiently explained by a simplified variable that combines intensity and duration into a single salinity stress metric, the SSE index?

Methods.

Experimental design

Two salt stress exposure experiments were conducted in the Harlan Greenhouse in George Washington University's Science and Engineering Hall. The first experiment ran from March 15 through May 9, 2021 and tested five glycophytes (along with the moderately salt-tolerant *Panicum virgatum*): *Brassica napus*, *Glycine max*, *Persicaria maculosa*, *Persicaria punctata*, *Sorghum bicolor*. *P. maculosa* is an introduced species in the United States, and the soybean *G. max*, *B. napus*, and *S. bicolor* are crops, which were included as species of interest due to the growing threat of rising salinity levels on coastal agriculture. The second greenhouse experiment ran from May 15 through July 16, 2021 and tested five halophytes: *Kosteletzkya virginica*, *Panicum amarum*, *Panicum virgatum*, *Paspalum floridanum*, and *Phragmites australis*. The species *P. virgatum* was included in both the glycophyte and the halophyte experiments to bridge the results and compare across experiments. *P. australis* is a pervasive invasive species that is dominant in many coastal wetland systems along the eastern United States; all other halophytes were native species in the Mid-Atlantic region. The selected set of study species encompasses a variety of ecological niches and habitat preferences, and several of them have known halophytic adaptations (Table 1). Therefore, we expected to see different

responses to salinity stress between species, as well as between the glycophytic and halophytic species groups.

All plants used in this study were germinated from seed in George Washington University's Harlan Greenhouse. Seeds for the glycophyte experiment were sourced from the same sources as in de la Reguera (2020), with the exception of *P. maculosa*, which was collected from an urban, roadside population in northwest D.C., and *P. punctata*, which was collected from the understory of a maritime forest in Brownsville Preserve, Nassawaddox, Virginia. Seeds for the halophyte experiment were provided by the conservation agronomist and manager of Cape May Plant Materials Center with the USDA-NRCS, Chris Miller.

After noticing fungal growth on seeds in the germination chamber, seeds for the halophyte experiment were treated with fungicide prior to germination. To stimulate germination, seeds for both experiments were spread on top of a wet potting mix (2:1 of potting mix:sand) and placed into a warm, humid germination chamber. When the majority of seeds began to sprout, they were removed from the germination chamber and trays were placed on heated mats until seedlings developed true leaves, at which point, each plant was individually repotted and tagged with a unique identifier to facilitate tracking throughout the experiment. The glycophytes were repotted into 10 x 10 x 10 cm cubic pots, and placed into flat trays. Three individuals of each of the six glycophyte species were placed into each tray. In the halophyte experiment, seedlings were repotted into conical pots, and placed in a plant stand set atop six cylindrical containers. Each cylindrical container held five cones, one individual of each of the five halophytic

species. All plants within a tray or container received the same water, and therefore the same stress treatments.

The tray or container served as an experimental replicate and was randomly assigned to a combination of salt intensity and duration (Figure 1). Salinity duration was controlled by the amount of time plants were subjected to salt water versus fresh water. Salinity intensity was controlled by the salt concentration of the salt water in parts per thousand (PPT). We exposed species to a range of salinity intensities that was appropriate for their expected salt stress tolerance (0-6 PPT for the glycophyte experiment; 0-24 PPT for the halophyte experiment). In the glycophyte experiment, intensity treatments were 0 (freshwater control), 1, 2, 4, and 6 PPT, and duration treatments were 0, 5, 10, 20, or 30 days of salinity exposure per month (DPM). Intensity treatments for the halophyte experiment were 0 (freshwater control), 6, 12, or 24 PPT, and duration treatments were 0, 5, 15, or 30 DPM. The glycophyte experiment included 25 combinations of salinity intensity and duration, while the halophyte experiment included 16 combinations. Saltwater was made by mixing Instant Ocean crystals with DI water in large containers to reach the target concentration, and plants were bottom watered. Water was changed every 3 days to prevent salinity buildup in soils due to evaporation. During the water change for the halophyte experiment only, soil was gently flushed with freshwater for 15 seconds to prevent the buildup of salinity in the soil, which proved to be a challenge during the glycophyte experiment (Supplemental Figures S1 and S2). In both experiments, trays were rotated among greenhouse benches at each watering to prevent greenhouse position effects.

A handheld salinity meter (Pocket Pro+ Multi 1) was used to check the source water salinity of each treatment. The salinity of each tray or container was also measured before each water change. A full experimental rotation was 30 days; at the end of each 30-day period, all treatments were set to their original PPT. Therefore, the 5 DPM treatment received 5 days of its appropriate salt treatment, then 25 days of fresh DI water, etc. (Fig. 1). The freshwater control received constant freshwater and the 30 DPM treatment received constant saltwater of the prescribed intensity. The glycophyte experiment ran for 60 days, or two 30-day cycles, and the halophyte experiment ran for 90 days, or three 30-day cycles.

Performance measurements: survival, biomass, and chlorophyll

We screened for salt tolerance by measuring survival, biomass, and chlorophyll response to increasing salinity. We monitored survival by checking each plant on every watering. Plants with no green aboveground tissue were scored as dead and removed from the experiment. At the end of the experiment, all plants were removed from their pots, and gently agitated in DI water to remove soil from roots. Biomass was separated into aboveground live, aboveground dead, and belowground components during harvest. All biomass was dried at 60 degrees Celsius for at least 72 hours, and then weighed. Chlorophyll measurements were made in the halophyte experiment only. In the week prior to harvesting for biomass, we made three measurements of chlorophyll concentration in each plant's third, or second if only two leaves, oldest leaf using a chlorophyll meter (model CCM-200 plus, Opti-Sciences). The three readings were then averaged to estimate plant chlorophyll concentration.

Data analysis

Outliers were removed from the dataset via visual analysis prior to data transformations and model analysis. Additionally, any plants that died due to physical damage from handling (i.e. broken stems or torn leaves) were removed from the experiment and excluded from analysis. Plant mortality was coded in a binary variable with 1 indicating dead and 0 indicating alive; therefore a positive coefficient value indicated an increase in plant mortality probability.

Total biomass and aboveground biomass were transformed to a standard normal distribution (mean of 0, standard deviation of 1) using the following equation:

$$B_y = x - \text{mean}(x) / \text{sd}(x)$$

where x is biomass of species y. This procedure allowed comparison of biomass effects between species that vary in size and growth rate.

In order to answer our first research question and determine the metric(s) of salinity that best explained variation in survival and ecophysiology, we used a model selection framework. We performed model selection for each experiment separately, as we expected the glycophyte and halophyte groups to exhibit different responses to salinity stress. For each experiment, we ran a series of models that included intensity (PPT), duration (DPM), intensity and duration (as independent or interactive variables), or only the salt stress exposure index (SSE) to identify the metric that best explained

patterns of plant fitness and survival in response to experimental salinity stress in greenhouse manipulations. SSE was calculated by multiplying the PPT treatment by the DPM treatment to generate a single linear scale of salinity stress. General linear mixed-models were built using the glm() function in R. In addition to intensity and duration predictor variables, all models included species as a fixed factor to allow patterns to vary between species. Tray was incorporated into all models as a random factor with a zero intercept. Including tray as a random slope allowed for differences in biomass response to salinity between trays. Models that included both DPM and PPT as fixed predictor variables allowed both to individually vary by tray. Standardized total biomass and standardized above ground biomass met both normality and homogeneity of variance assumptions, and chlorophyll data was log-transformed to meet these assumptions of normality. The model with the lowest AIC value was designated as the model of best fit for each experiment. This model selection framework also served to answer our second research question; should SSE, which combined intensity and duration, produce the model of best fit, this simplified variable can sufficiently explain species tolerance to salinity stress, rather than incorporating both intensity and duration.

We ran the model of best fit that corresponded with each metric of plant ecophysiology for each of the species to test for significant predictor coefficients to identify where salinity explained patterns in survival and ecophysiology. Standardized total biomass and standardized aboveground biomass of each species were checked for normality and homogeneity of variance assumptions prior to running these species-specific models and log- or square-root transformed when required.

Lastly, simple linear regression models were used to predict total biomass as a function of PPT, as a measure of each species' sensitivity to salinity, with a more negative slope indicating greater salinity sensitivity. For these regression models, the data from the highest duration treatment, 30 DPM, and lowest duration treatment, 5 DPM, were run separately, and the results were compared against known information about the species' salt tolerances from published literature.

Results.

Effect of salinity on survival and fitness

Survival

The glycophyte and halophyte experiments had different models of best fit for explaining patterns in survival. In the glycophyte experiment, the model that best explained variation in survival between species and across our experimental salinity treatments was the model with intensity alone as a fixed predictor (Table 2). Patterns in survival for our glycophytic species were therefore best explained by salinity intensity, rather than duration or their interaction. In the halophyte experiment, the model of best fit for survival was the model with intensity and duration and their interaction as fixed predictors (Table 2); intensity and duration therefore have both independent and interactive effects on whether halophytes will survive a salinity regime.

When the model of best fit was run for each species, intensity failed to significantly explain patterns in any of our glycophytes despite it being the model of best fit. However, intensity did significantly explain a decline in survival for one of our native

halophyte species, *K. virginica* (p = 0.00314) within the full model of intensity, duration, and their interaction (Table 3). The majority of the remaining species (glycophytes: *P. punctata*, *P. maculosa*, *B. napus*, *S. bicolor*, *G. max*; halophytes *P. amarum* and *P. australis*), maintained high levels of survival across all treatments of intensity and duration.

Biomass

Unlike survival, the same model of best fit best explained patterns in plant ecophysiology between both the glycophyte and halophyte experiments. The model that included salinity intensity, duration, and their interaction best explained variation in standardized total biomass, standardized aboveground biomass, and chlorophyll (Tables 3, 4, and 5).

When the model of best fit was run individually for each species to test for significant coefficients, intensity and duration had a significant interactive effect on total biomass in 7 of 10 species (Table 3): glycophytes *P. maculosa*, *G. max*, and *S. bicolor*, and halophytes *P. amarum*, *P. virgatum*, *P. floridanum*, and *K. virginica*. For all glycophytes and halophytes except *K. virginica*, the effect of PPT on biomass became more negative with increasing duration. In other words, prolonged salinity duration magnified the negative effect of salinity intensity on biomass. This effect was most pronounced in the glycophytic species *P. maculosa*, *S. bicolor*, and *G. max*.

Total biomass of native wetland plant *K. virginica* was negatively affected by increasing salinity intensity to a similar magnitude across all levels of duration, but produced greater biomass at low intensity salinity (6 PPT) than in the freshwater control, indicating a nonlinear effect of salinity on total plant production. ANOVA analysis found

significant differences in average biomass of *K. virginica* by intensity ($F_{3, 91} = 12.95$, $p < 0.001$). The 6 PPT treatment had a significantly higher biomass than the control, 12 PPT, and 24 PPT treatments, and the control and 12 PPT treatments had significantly higher biomass than the 24 PPT treatment (Figure 4).

Halophyte *P. australis* and glycophytes *B. napus* and *P. punctata* produced the same biomass in all salinity stress treatments, regardless of intensity or duration. This indicated that the selected levels of stress were not of high enough magnitude to significantly affect biomass production for these species, at least on the short timescale of these experiments (90 days for *P. australis*, 60 days for *P. punctata* and *B. napus*).

Ranking species by salt sensitivity scores

Overall, the glycophyte species, with the exception of *P. punctata*, lost more biomass per unit increase in PPT than the halophytes (Table 6). From least to most salt-sensitive, the species ranked as follows (h: halophyte, g: glycophyte, c: crop): *P. punctata* (g), *P. virgatum* (h), *P. australis* (h), *P. amarum* (h), *K. virginica* (h), *P. floridanum* (g), *B. napus* (g, c), *S. bicolor* (g, c), *P. maculosa* (g), and *G. max* (g, c) (Table 6).

Crop species *B. napus* and *S. bicolor* had similar magnitudes of biomass change per unit PPT, approximately -0.1 g/PPT (Figure 2). Introduced glycophyte *P. maculosa* and crop species *G. max* were the least tolerant, with slopes of > -0.5 g/PPT. Glycophyte *P. punctata* was unique in that it had a non-negative slope between total biomass and intensity at 30 DPM.

P. virgatum experienced a stronger negative effect of PPT on biomass during the halophyte experiment (-0.030 g/PPT) than the glycophyte experiment (0.004 g/PPT). This was likely a function of the low maximum salinity intensity (6 PPT) in the first

experiment that did not have as strong an effect on *P. virgatum* (Table 1). Overall, we conclude that the effect of salinity intensity on biomass production in *P. virgatum* and other halophytes is likely non-linear.

Chlorophyll

Chlorophyll measurements were only collected in the halophyte experiment. Similar to biomass, the response of chlorophyll to the individual and interactive effects of salinity intensity and duration was species-specific, but three of five species tested (natives *K. virginica* and *P. floridanum*, and invasive *P. australis*, Table 5) exhibited a significant interaction between PPT and DPM. For both *K. virginica* and *P. australis*, the slope between PPT and chlorophyll became more positive with increasing DPM. In other words, as duration increased, the effect of intensity changed from negative at 5 DPM (more intense stress reduced chlorophyll concentration) to positive at 30 DPM (more intense stress increased chlorophyll concentration). For *K. virginica* and *P. australis*, the highest concentrations of chlorophyll occurred in individuals grown in the most stressful combination of intensity and duration. The native wetland species *P. floridanum* exhibited a different type of interactive effect. For *P. floridanum*, the effect of intensity on chlorophyll became less positive with increasing duration (Figure 3). Additionally, and unlike *K. virginica* and *P. australis*, in *P. floridanum*, chlorophyll concentrations were always lower at higher salinity intensity, across all levels of duration.

In *P. amarum*, chlorophyll concentration was significantly explained by DPM alone ($p = 0.0133$), with shorter duration salinity exhibiting lower levels of chlorophyll across all levels of intensity. However, *P. virgatum* chlorophyll concentration was not significantly explained by intensity, duration, or their interaction.

Discussion.

SSE index performance

In both the glycophyte and halophyte experiments, the SSE index failed to outperform other models of salinity stress in explaining effects on fitness and survival. Unfortunately, SSE was too simplistic of a metric to adequately describe salinity stress effects for either group of species. Rather, the independent and interactive effects of salinity intensity and duration are important in explaining patterns in plant response to stress, and these effects were species-specific across both halophytes and glycophytes.

Salinity stress effects varied based on performance metric and tolerance class

The model of best fit for explaining patterns in survival varied between our two salinity tolerance classes. The intensity model (PPT) was the best fit to explain patterns in survival among glycophytes; we therefore determined intensity to be the primary metric of salinity that drives lower tolerance class plants' ability to survive salt stress. In other words, patterns in survival for species that are more susceptible to salinity (i.e. do not have known salt tolerance adaptations) are best explained by how much salt plants are exposed to, rather than how long they are exposed. Our glycophyte experiment concluded after 60 days, meaning we cannot draw conclusions on which predictors best explain effects of salinity on long term survival beyond our study period; for example, the reductions in biomass and chlorophyll production that we observed in response to salinity exposure may result in mortality over longer time periods.

While intensity alone best explained patterns of survival for glycophytes, it was the independent and interactive effects between intensity and duration that best explained survival in our higher tolerant halophyte species. The independent and interactive effects between intensity and duration are therefore both important in determining the likelihood of survival for species with known salinity tolerance mechanisms.

Though the model of best fit for survival varied by tolerance class, both glycophytes and halophytes had the same model of best fit for explaining the response of biomass. The interaction between intensity and duration best explained the response of biomass in both glycophytes and halophytes.

Understanding the effects of stress on plant performance

The ability to survive in order to reproduce is the minimum prerequisite for plant performance and function, and as such the environmental variable(s) that impact survival dictate the baseline community structure of an ecosystem (Primack and Kang 1989). Identifying the component of a salinity stress regime that imposes the most severe environmental filter on the plant community could help inform important management implications when choosing mitigation strategies for coastal plant communities and agriculture (Yang et al. 2021). As intensity had the most explanatory power in describing patterns in survival of our native (*P. punctata*, and *P. maculosa*) and crop (*B. napus*, *G. max* and *S. bicolor*) glycophyte species, changes in the magnitude of pulse salinity disturbances could potentially threaten native species establishment as well as agricultural productivity in communities facing both increased salinity duration and intensity. Our findings suggest that investing resources into mitigating discrete pulse stresses of high intensity, such as storm events and surges, might reduce plant mortality

in glycophyte community assemblages and coastal agriculture, even as other aspects of plant physiology and higher salinity tolerance classes are impacted by additional components of salinity stress.

Differences in establishment among our native species can cascade into higher community organization and function. Li and Pennings (2019), in their factorial greenhouse experiment exposing mesocosm communities of six common freshwater plant species to three salinity intensity levels (3, 5, and 10 PSU) and five duration levels (5, 10, 15, 20, and 30 days per month), found that salt sensitive species were suppressed with increasing salinity exposure as both intensity and duration increased, resulting in shifts in species composition and decreased species richness. While these mesocosm communities were given 10 months in freshwater to recover, communities exposed to high salinity and duration were unable to recover species richness due to the loss of salt-sensitive species, highlighting the importance of survival for community development (Li and Pennings 2019). Species-specific effects of salinity stress on survival, observed in our study, describe how communities will develop in the face of salinity. Specifically, we expect the less tolerant glycophytes, such as *P. maculosa,* to be the first ones filtered from the community, while more tolerant species will persist as coastal salinity regimes shift.

While the same model of best fit best explained patterns in plant ecophysiology among glycophytes and halophytes, the interaction between salinity intensity and duration took a different shape for the response variable of biomass than that of chlorophyll. While the effect of intensity on biomass became more negative with increasing duration for the majority of species (glycophytes: native *P. maculosa,* crops *S.*

bicolor and *G. max*; halophytes: *P. amarum*, *P. virgatum*, *P. floridanum*, and *K. virginica*), the opposite relationship occurred in chlorophyll for two of the five higher-tolerance species (invasive *P. australis* and native *K. virginica*), where chlorophyll had an increasingly positive relationship with intensity as duration increased.

This difference in the interaction between intensity and duration to explain patterns of biomass and chlorophyll response might be best explained by differences in allocation of resources by plants, particularly those with halophytic adaptations, in response to stress. Biomass represents the total sum of the entire plants' production throughout its life (Younginger 2017), while plants are able to allocate and invest energy into chlorophyll and light harvesting as they develop. While chlorophyll concentrations most commonly decline with increased salinity (Netondo et al. 2004; Taïbi 2016), salinity stress can prompt a compensatory response in some species, in which individuals invest assimilated carbon into cell maintenance and organelle construction rather than somatic growth; this results in reductions in biomass even while chlorophyll concentration in remaining tissues remains constant or increases (Shoukat et al. 2019).

Compensation in photosynthetic activity with increased stress has been observed in response to other stressors. Caudle and Maricle (2012) tested the effect of flooding on plant physiology and found an increase in photosynthetic rates in the wetland grass *Phalaris arun*, *Zea mays*, and the brood bean *Vicia faba* after a three-day flooding treatment, suggesting that this short duration stress event increased the efficiency of light-harvesting processes in these species (Caudle and Maricle 2012). However, as in our study investigating the impacts of salinity, Caudle and Maricle (2012) also found inter-species variability in response to flooding, with two species in their study (the common

bean *Phaseolus vulgaris* and the pea *Pisum sativum*) experiencing declines in photosynthesis during the flooding treatment (Caudle and Maricle 2012). Li et al. (2007) also found no significant reductions in net photosynthetic rate or chlorophyll with long-term flooding (60 days) in *P. australis* and the wetland grass species *Hemarthria altissima*. These results suggest that certain plant species, including the persistent invasive *P. australis*, can respond to both short- and long-term stress events by maintaining or increasing their photosynthetic rates.

Species-specific mechanisms of plant tolerance to salinity

Given the range of halophytic adaptations found in the focal species of this study, physiological response curves were expected to vary between species and salinity tolerance class (Li and Pennings 2019; Table 1). The salt sensitivity analysis generally confirmed the expected tolerance levels of the tested species, with halophytes outperforming glycophytes (Table 6).

The comparison of the two glycophyte species in the genus *Persicaria* presents revealing differences. While we found *P. maculosa* to be one of the most salt-sensitive species of the study, *P. punctata*, was the most salt-tolerant. While there is little literature on the specific mechanisms of salt tolerance in *P. punctata*, Humphreys et al. (2021) noted finding this species in tidal marsh inventories dating back to 1980 in the low-salinity tidal marshes of Virginia. Our study suggests that the species *P. punctata*, or at least the source population of *P. punctata* sampled for this study, likely has saline adaptations that would allow it to persist in native community assemblages under increasing salinity.

We found the wetland species *P. australis* (an invasive) and *K. virginica* (a native) to be among the most tolerant to salinity stress of the species tested. *P. australis* is regarded to have broad salinity tolerance (Achenbach et al. 2013), and we found that it was able to maintain biomass production at every level of salinity intensity and duration tested. These findings lend further support to why *P. australis* is such a successful invasive species dominating coastal wetlands along the East Coast US, able to outperform native plant species and take over entire communities (Meyerson et al. 2016). *K. virginica*, one such native wetland species to this region, has previously been identified as a possible species for cultivation in salt-affected coastal areas (Voutsina et al. 2015). In identifying *K. virginica* as a species of high salinity stress tolerance in both pulse and press events, our study supports this application. We also found evidence for non-linear effects of salinity on plant biomass in *K. virginica*, where mean total biomass peaked at 6 PPT, then significantly decreased at higher salinity (24 PPT). This apparent stimulation of biomass production at low levels of salinity further supports the use of *K. virginica* for restoration projects in fields experiencing elevated salinity.

For most species across both halophytes and glycophytes, the relationship between biomass and intensity became more negative with increasing duration. An acclimation period, such as would occur in an incremental or ramped stress event, may change this response. This may be particularly important for glycophyte species that lack tolerance adaptations. For example, in a previous study, crop species *S. bicolor* was able to acclimate to salinity in a pre-treatment period followed by moderate salinity exposure at 300 mol m^{-3} NaCl (Amzallag et al. 1990). As our experiment lacked any sort of pre-treatment, there was no opportunity for acclimation in *S. bicolor* or any other species.

Pre-treatment of salinity could potentially be applied as an agricultural best-practice as coastal farmland experiences elevated salinity regimes.

The soybean *G. max* was the most sensitive species to salt stress of those included in this study, based on the strong, negative effect of salinity intensity and duration on biomass. *G. max* has previously been demonstrated to decrease aboveground biomass, as well as leaf area, when exposed to salinity; this has been suggested as an adaptation to salt exposure by limiting transpiration and improving the plants' hydration (Queiroz et al. 2012). The variety of *G. max* used was a chloride-excluding variety, but nonetheless, it did not perform well in this experiment or in field trials with the same variety in salt-stressed fields (de la Reguera et al. 2020). Based on our findings, *S. bicolor*, and perhaps *B. napus*, would be a better choice of crop for salt-affected coastal areas than *G. max*.

Conclusion.

This study identified salinity intensity to best explain patterns in plant survival in the tested glycophytes, while the interaction between intensity and duration best helped explain variation in survival of tested halophytes, as well as biomass production for both glycophytes and halophytes. As these models outperformed the SSE index, combining duration and intensity into a single variable oversimplified the effect of salinity stress on plant fitness. We found important interactive effects of salinity intensity and duration on plant ecophysiology. Most species experienced more negative effects of intensity on total biomass with increasing duration. However, in chlorophyll production, some halophytes exhibited a positive response to salinity in which chlorophyll production increased with salinity intensity and this effect was more pronounced at higher duration, suggesting a compensatory resource allocation. Understanding interspecies variation in tolerance to

salinity intensity and duration helps to identify management recommendations for crop and restoration species, as well as predict shifts in plant community composition as sensitive species are filtered by shifts in coastal salinity and hydrology.

Tables and Figures.

Table 1. Known halophytic adaptations of the focal species in this study.

Species	Tolerance class	Halophytic adaptations
Kosteletzkya virginica	higher tolerance, native	Accumulates inorganic compounds and synthesizes organic compounds to maintain osmotic balance (Blits 1993), including proline (Blits 1993; Guo et al. 2009). Mucilage in shoot, stems, and roots assists in regulating water ascent and ion transport (Ghanem et al. 2010).
Panicum amarum	higher tolerance, native	Adapted to the harsh environment of dune systems, where plants are subjected to salt spray, storm surges, inundation, high temperatures, low soil moisture, low nutrient availability, and sand burial; can withstand periods of extended drought (NRCS Plant Guide; Lonard and Judd 2011).
Panicum virgatum	higher tolerance, native	Salt excretion glands (Kobayashi 2008); synthesizes organic compounds (proline) to maintain osmotic balance (Guan et al. 2018, Liu et al. 2014).
Panicum floridanum	higher tolerance, native	Found growing in saline ditches adjacent to farm fields (pers. comm., Chris Miller).
Phragmites australis	higher tolerance, invasive	Salt excretion glands (Burke et al. 2000); organic and inorganic osmotica to supplement osmotic pressure (Zhao et al. 1999); oxidoreductase activity and glutathione metabolism (Zhang et al. 2020); uses free amino acids and sugars as osmolytes (namely proline

		and glutamine) (Hartzendorf and Rolletschek 2001); ion distribution in vascular bundle sheath and mesophyll cells (K+, Na+, Ca2+, Mg2+ and Cl-) (Wen-Ju et al. 2002)
Brassica napus	lower tolerance, native	Accumulates proteins associated with protein metabolism, damage repair, and defense response when grown at 200 mM NaCl; these adaptations were suggested to alleviate adverse effects on plant physiology and function (Jia et al. 2015).
Glycine max	lower tolerance, crop	When grown at 50 mM (~3 PPT), 100 mM (~6 PPT) and 200 mM (~12 PPT), root length was unaffected and root water content increased (diminishing salt concentration), though shoot height and leaf area decreased (limiting transpiration) (Queiroz et al. 2012).
Persicaria punctata	lower tolerance, native	Found in low-salinity tidal marshes (~5 ppt salinity) (Humphreys et al. 2021).
Persicaria maculosa	lower tolerance, native	
Sorghum bicolor	lower tolerance, crop	After 20 days of acclimation at 75 or 150 mol m^{-3}, *S. bicolor* was able to grow at 300 mol m^{-3} by modulating genome expression to regulate internal Na+ and Cl− concentrations during extended exposure to non-lethal salt concentrations (Amzallag et al. 1990).

Table 2. AIC values for all models including all species. R^2m = R^2 marginal. R^2c = R^2 conditional.

Experiment	**Response Variable**	**Fixed and Random Predictor Variables**	**AIC**	R^2m	R^2c
halophytes	survival	species * DPM * PPT + (0 + PPT | tray) + (0 + DPM | tray)	**335.07**	0.951	0.951
halophytes	survival	species * sse + (0 + sse | tray)	343.89	0.968	0.968
halophytes	survival	species * PPT + (0 + PPT | tray)	340.79	0.958	0.958
halophytes	survival	species * DPM + (0 + DPM | tray)	405.04	0.961	0.961
glycophytes	survival	species * DPM * PPT + (0 + PPT | tray) + (0 + DPM | tray)	238.51	0.961	0.961
glycophytes	survival	species * sse + (0 + sse | tray)	224.89	0.962	0.962
glycophytes	survival	species * PPT + (0 + PPT | tray)	**219.45**	0.962	0.962
glycophytes	survival	species * DPM + (0 + DPM | tray)	225.26	0.962	0.962
halophytes	standardized total biomass	species * PPT * DPM + (0 + PPT | tray) + (0 + DPM | tray)	**1409.7**	0.123	0.123
halophytes	standardized total biomass	species * sse + (0 + sse | tray)	1439.6	0.036	0.036
halophytes	standardized total biomass	species * PPT + (0 + PPT | tray)	1436	0.043	0.043

halophytes	standardized total biomass	species * DPM + (0 + DPM | tray)	1453.1	0.011	0.011
glycophytes	standardized total biomass	species * PPT * DPM + (0 + PPT | tray) + (0 + DPM | tray)	**1559.2**	0.185	0.185
glycophytes	standardized total biomass	species * sse + (0 + sse | tray)	1588.1	0.111	0.111
glycophytes	standardized total biomass	species * PPT + (0 + PPT | tray)	1617.6	0.066	0.066
glycophytes	standardized total biomass	species * DPM + (0 + DPM | tray)	1623.5	0.056	0.056
halophytes	standardized aboveground biomass	species * PPT * DPM + (0 + PPT | tray) + (0 + DPM | tray)	**1424.2**	0.098	0.098
halophytes	standardized aboveground biomass	species * sse + (0 + sse | tray)	1455.4	0.006	0.006
halophytes	standardized aboveground biomass	species * PPT + (0 + PPT | tray)	1453.8	0.009	0.009

halophytes	standardized aboveground biomass	species * DPM + (0 + DPM | tray)	1442.4	0.031	0.031
glycophytes	standardized aboveground biomass	species * PPT * DPM + (0 + PPT | tray)	**1572.7**	0.173	0.173
glycophytes	standardized aboveground biomass	species * sse + (0 + sse | tray)	1602.5	0.098	0.098
glycophytes	standardized aboveground biomass	species * PPT + (0 + PPT | tray)	1621.2	0.069	0.069
glycophytes	standardized aboveground biomass	species * DPM + (0 + DPM | tray)	1632.7	0.050	0.050
halophytes	log chlorophyll	species * PPT * DPM + (0 + PPT | tray) + (0 + DPM | tray)	**-15.57**	0.596	0.596
halophytes	log chlorophyll	species * sse + (0 + sse | tray)	46.748	0.530	0.530
halophytes	log chlorophyll	species * PPT + (0 + PPT | tray)	67.648	0.511	0.511
halophytes	log chlorophyll	species * DPM + (0 + DPM | tray)	13.062	0.561	0.561

Table 3. Significant relationships found in the models of best fit that explained variation in **survival** for each species by PPT or PPT * DPM. * denotes $p < 0.05$. ** denotes $p < 0.01$. *** denotes $p < 0.001$. Each row denotes a species and the p value for any significant relationships between survival and PPT or PPT * DPM. Glycophytes were analyzed using the PPT model, and halophytes were analyzed using the full model with PPT*DPM, as the AIC model selection found these to be the models of best fit to explain patterns in survival by experiment. Each cell contains the coefficient value followed in parentheses by the p value.

species	**model of best fit**	**PPT**	**DPM**	**PPT*DPM**
Kosteletzkya virginica	PPT*DPM	p = 0.00314 **	n.s.	n.s.
Panicum virgatum	PPT*DPM	n.s.	n.s.	n.s.
Panicum amarum	PPT*DPM	n.s.	n.s.	n.s.
Paspalum floridanum	PPT*DPM	n.s.	n.s.	p = 0.061
Phragmites australis	PPT*DPM	n.s.	n.s.	n.s.
Brassica napus	PPT	n.s.		
Glycine max	PPT	n.s.		
Persicaria maculosa	PPT	n.s.		
Persicaria punctata	PPT	n.s.		
Sorghum bicolor	PPT	n.s.		

Table 4. P-values of all significant relationships or interactions where salinity (PPT and/or DPM) significantly explained patterns in **total biomass**, transformed to meet normality, by species. * denotes $p < 0.05$. ** denotes $p < 0.01$. *** denotes $p < 0.001$. Each row defines a species, the transformation necessary on the biomass data for that species to meet the assumption of normality, and the p-value for significant relationships between biomass and PPT, DPM, or their interaction, as denoted by the column. Both glycophytes and halophytes were analyzed using the full model, as the AIC model selection found this to be the model of best fit to explain patterns in biomass responses to salinity intensity and duration. Each cell contains the coefficient value followed in parentheses by the p value.

species	**Data transf.**	**PPT**	**DPM**	**PPT*DPM**
Brassica napus	square root	-0.038 (p=0.137)	0.053 (p=0.276)	0.00009 (p=0.946)
Glycine max	standardized	0.132 (***p=0.029***)	0.021 (p=0.081)	-0.022 (***p<0.0001****)
Kosteletzkya virginica	standardized	-0.030 (p=0.101)	0.042 (***p=0.004***)	-0.003 (***p=0.0252****)
Panicum amarum	standardized	0.043 (***p=0.004***)	0.039 (***p=0.002***)	-0.004 (***p<0.0001****)
Panicum virgatum	log	-0.001 (p=0.953)	-0.030 (***p=0.007***)	0.003 (***p=0.0468****)
Paspalum floridanum	log	-0.013 (p=0.413)	0.054 (***p=0.0001***)	-0.003 (***p=0.0092****)
Persicaria maculosa	standardized	0.020 (p=0.756)	0.041 (***p=0.002***)	-0.016 (***p<0.0001****)
Persicaria punctata	standardized	-0.076 (p=0.308)	-0.022 (p=0.137)	0.003 (p=0.563)
Phragmites australis	log	-0.010 (p=0.467)	0.021 (p=0.091)	-0.0003 (p=0.7413)
Sorghum bicolor	standardized	0.281 (***p<0.0001***)	0.068 (***p<0.0001***)	-0.017 (***p<0.0001****)

Table 5. Significant relationships found in the models of best fit that explained chlorophyll for each species by DPM, the interaction between PPT*DPM, or SSE. * denotes p < 0.05. ** denotes p < 0.01. *** denotes p < 0.001. Each row denotes a species and the p value for any significant relationships between the biomass and predictor variable, or interaction, as denoted by the column name. Chlorophyll for all species was log-transformed to meet the assumption of normality. All species were analyzed using the full model, as the AIC model selection found this to be the model of best fit to explain patterns in chlorophyll responses to salinity intensity and duration. Each cell contains the coefficient value followed in parentheses by the p value.

species	**PPT**	**DPM**	**PPT*DPM**
Kosteletzkya virginica	-0.016 (***p=0.0002***)	-0.007 (***p=0.017***)	0.001 (***p<0.0001****)
Panicum amarum	0.002 (p=0.496)	0.008 (***p=0.013****)	0.000008 (p=0.975)
Panicum virgatum	-0.007 (p=0.131)	0.004 (p=0.363)	0.0001 (p=0.834)
Paspalum floridanum	-0.006 (p=0.289)	0.024 (***p<0.0001***)	-0.0008 (***p=0.045****)
Phragmites australis	-0.004 (p=0.164)	-0.0003 (p=0.911)	0.0004518 (***p=0.023****)

Table 6. Salt sensitivity score of the 10 species, quantified as the slope of the relationship between total biomass and PPT using only data from the highest duration treatment (30 DPM) to avoid interactive effects between intensity and duration. These regression models used the raw total biomass data, so the slope of the line (i.e. the Salt Sensitivity Score) is directly interpretable as loss in biomass (g) with each increase in one PPT. Species are ordered from most to least sensitive; a more negative slope means a more sensitive species.

Species	Tolerance class	Salt Sensitivity Score (g/PPT)
Persicaria punctata	lower	0.042
Panicum virgatum	higher	0.024
Phragmites australis	higher	-0.007
Panicum amarum	higher	-0.022
Kosteletzkya virginica	higher	-0.040
Paspalum floridanum	higher	-0.059
Brassica napus	lower	-0.092
Sorghum bicolor	lower	-0.131
Persicaria maculosa	lower	-0.530
Glycine max	lower	-0.599

Table 7. Proportion survival by intensity and duration in the halophyte experiment. Darker red refers to lower survival percentage. 100% means no mortality.

Species	Duration (DPM)	0 ppt	6 ppt	12 ppt	24 ppt
Panicum virgatum	0	100%			
Panicum virgatum	5		100%	90%	80%
Panicum virgatum	15		100%	80%	50%
Panicum virgatum	30		100%	80%	**0%**
Panicum amarum	0	100%			
Panicum amarum	5		100%	100%	100%
Panicum amarum	15		100%	100%	100%
Panicum amarum	30		100%	100%	90%
Paspalum floridanum	0	96%			
Paspalum floridanum	5		100%	89%	89%
Paspalum floridanum	15		100%	89%	56%
Paspalum floridanum	30		100%	100%	44%
Phragmites australis	0	93%			
Phragmites australis	5		90%	90%	90%
Phragmites australis	15		90%	100%	70%
Phragmites australis	30		89%	70%	70%
Kosteletzkya virginica	0	100%			
Kosteletzkya virginica	5		91%	90%	50%
Kosteletzkya virginica	15		100%	90%	40%
Kosteletzkya virginica	30		80%	90%	40%

Table 8. Proportion survival by intensity and duration in the glycophyte experiment. Darker red refers to lower survival percentage. 100% means no mortality.

Species	Duration (DPM)	0 ppt	1 ppt	2 ppt	4 ppt	6 ppt
Brassica napus	0	92%				
Brassica napus	5		100%	100%	100%	67%
Brassica napus	10		100%	100%	100%	100%
Brassica napus	20		100%	100%	80%	100%
Brassica napus	30		100%	100%	100%	100%
Glycine max	0	100%				
Glycine max	5		100%	100%	100%	80%
Glycine max	10		67%	100%	100%	100%
Glycine max	20		100%	100%	100%	83%
Glycine max	30		100%	100%	100%	80%
Panicum virgatum	0	91%				
Panicum virgatum	5		50%	100%	**17%**	50%
Panicum virgatum	10		83%	83%	67%	67%
Panicum virgatum	20		83%	100%	50%	67%
Panicum virgatum	30		83%	50%	67%	67%
Persicaria maculosa	0	100%				
Persicaria maculosa	5		100%	100%	100%	100%
Persicaria maculosa	10		100%	100%	100%	100%
Persicaria maculosa	20		100%	100%	100%	100%
Persicaria maculosa	30		100%	100%	100%	100%
Persicaria punctata	0	100%				
Persicaria punctata	5		100%	100%	100%	100%
Persicaria punctata	10		100%	100%	100%	100%

Persicaria punctata	20		100%	100%	100%	100%
Persicaria punctata	30		100%	100%	100%	100%
Sorghum bicolor	0	100%				
Sorghum bicolor	5		100%	100%	100%	100%
Sorghum bicolor	10		100%	100%	100%	100%
Sorghum bicolor	20		100%	100%	100%	100%
Sorghum bicolor	30		100%	100%	100%	100%

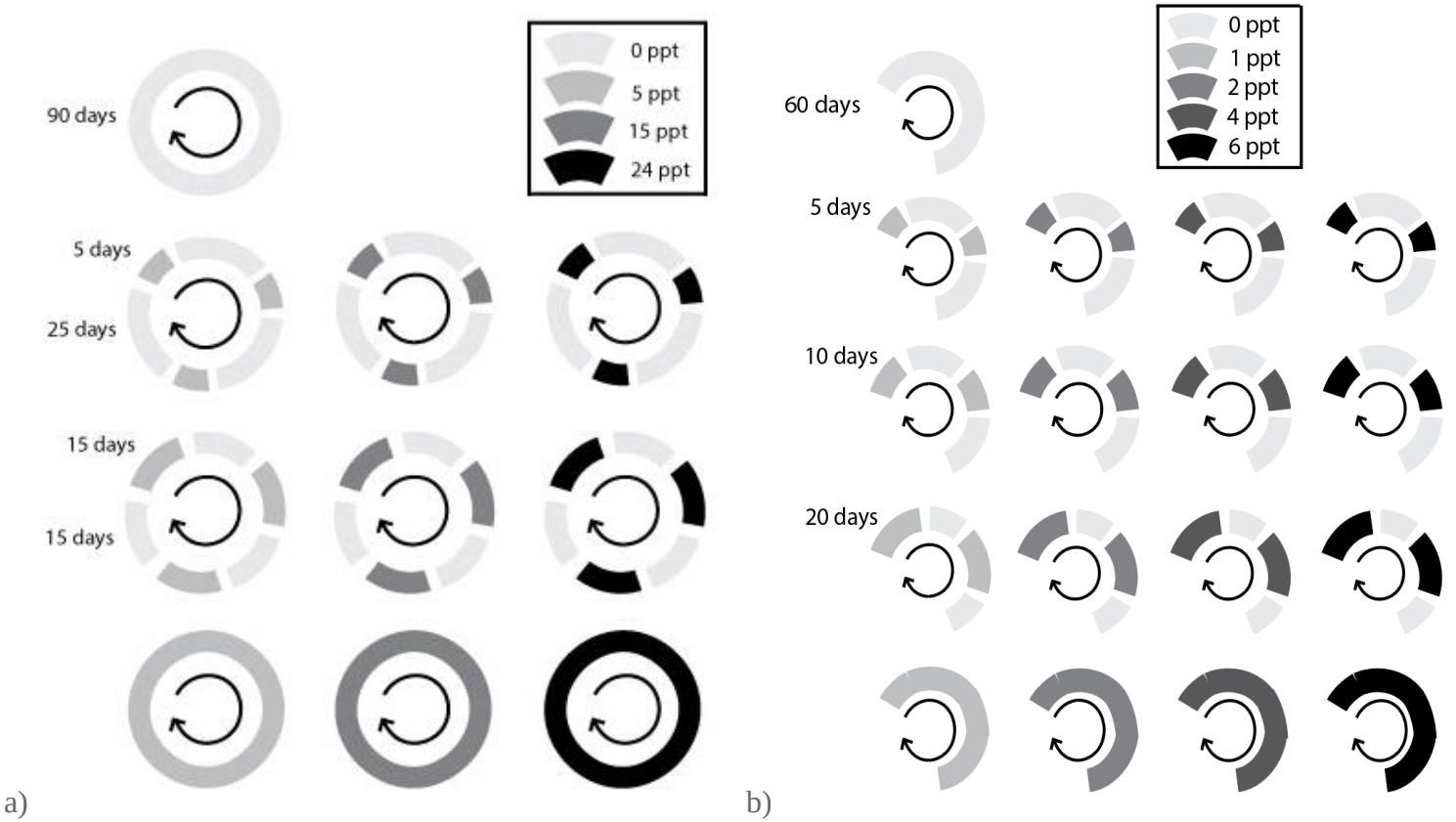

Figure 1. Diagram of the a) halophyte experiment and b) glycophyte experiment incomplete factorial design of salinity intensity x duration, labeled by color for salinity intensity treatment and diagrammed for salinity duration treatment. Each circle represents an experimental unit. Salinity intensity treatments were a) 0 PPT (control), 6, 12, and 24 PPT and b) 0 PPT (control), 1, 2, 4, and 6 PPT; salinity duration treatments were a) 0 days per month (control), 5 days/month, 15 days/month, and 30 days/month and b) 0 days per month (control), 5 days/month, 10 days/month, 20 days/month, and 30 days/month. All plants were watered bottom-up; trays were filled with either salt or freshwater for the duration of the experiment. Experiment concluded after a) 90 days and b) 60 days.

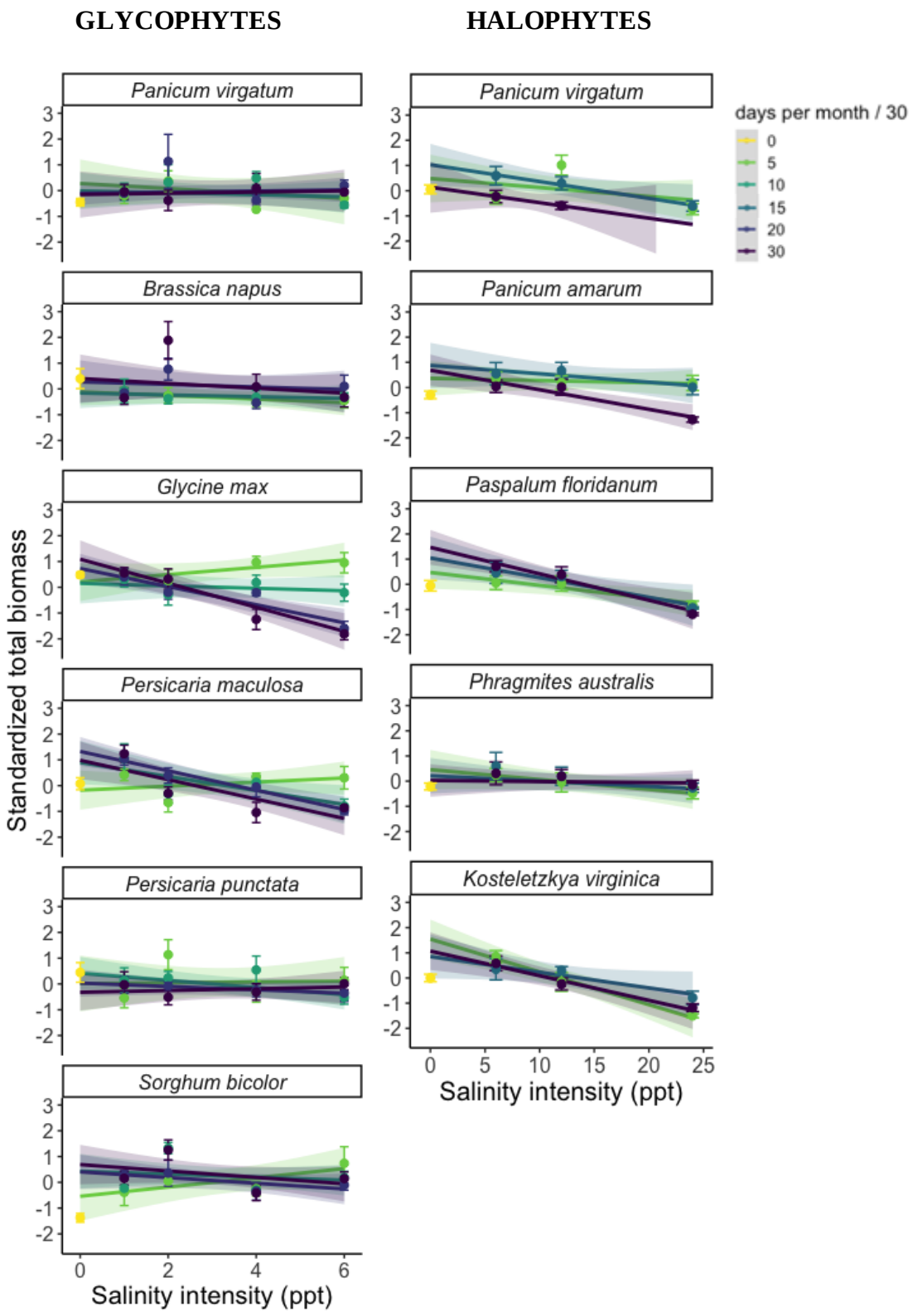

Figure 2. Standardized total biomass by salinity intensity (PPT), grouped and colored by salinity duration. Faceted by species.

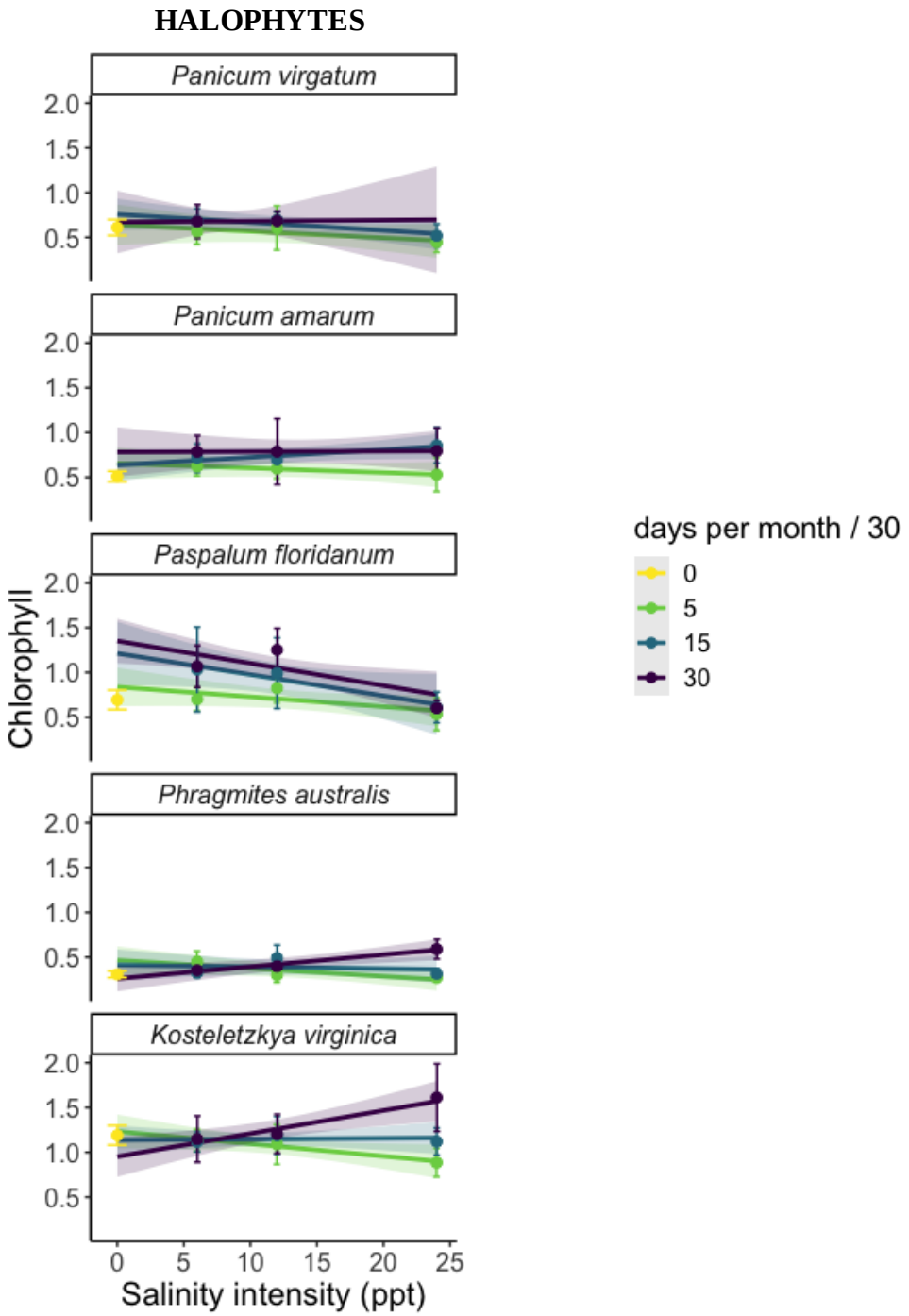

Figure 3. Log transformed chlorophyll by salinity intensity (PPT), grouped and colored by salinity duration. Faceted by species.

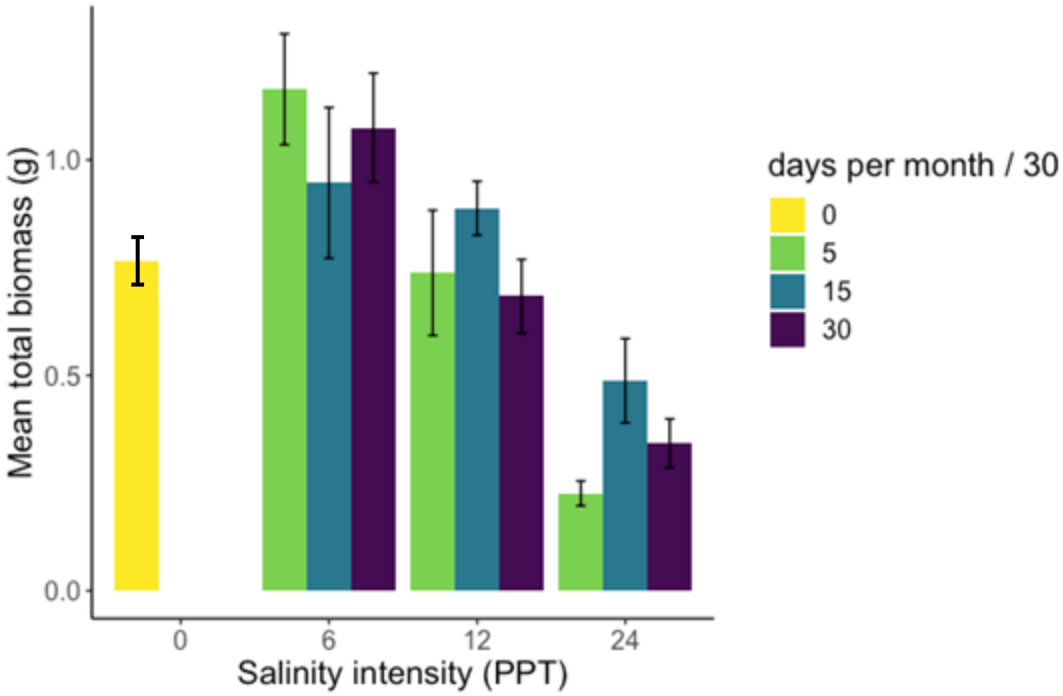

Figure 4. Mean total biomass (g) of *K. virginica* of all plants in each intensity and duration treatment.

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Supplementary materials.

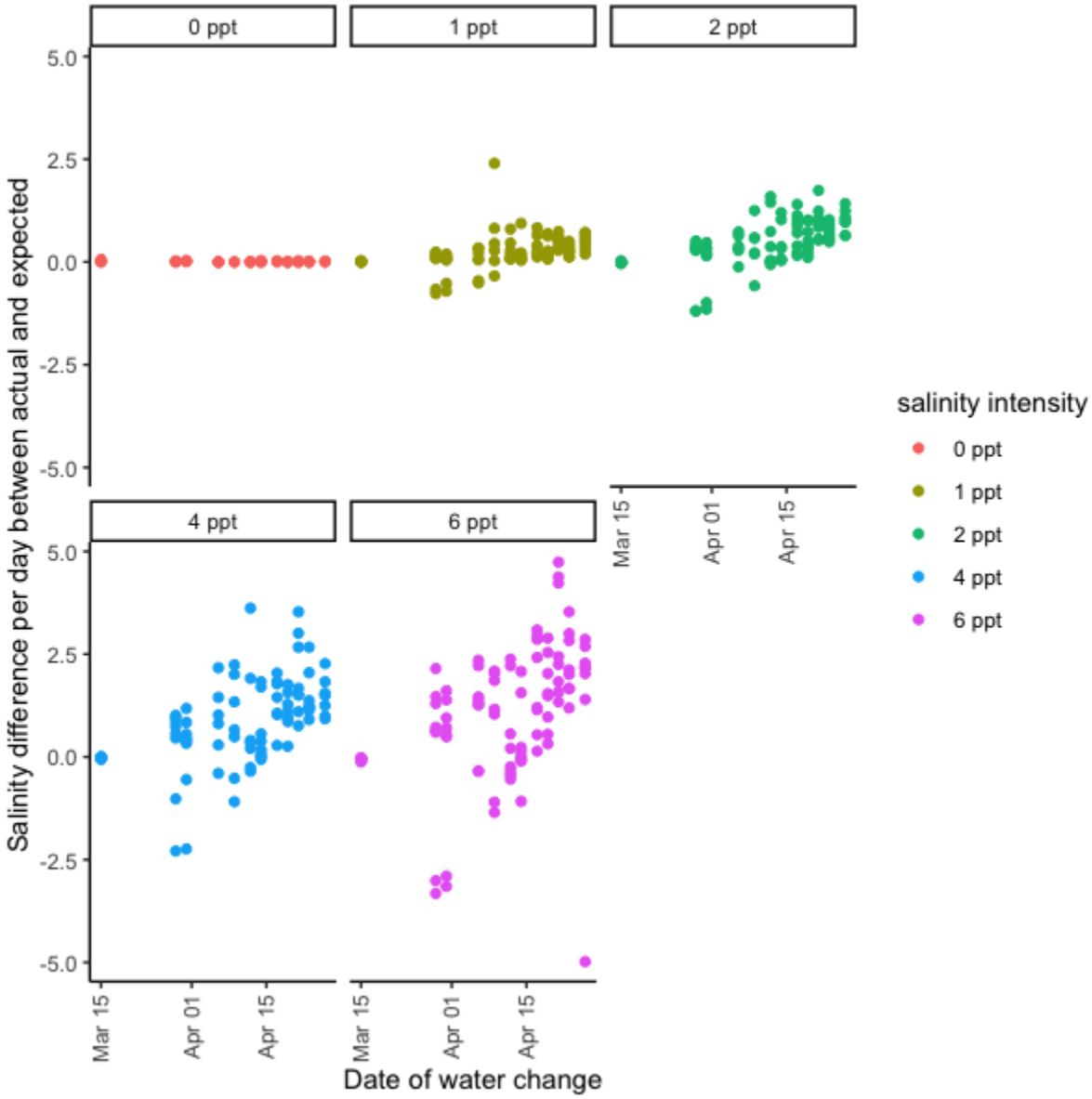

Figure S1. Salinity difference per day for glycophyte experiment. Each point represents one tray.

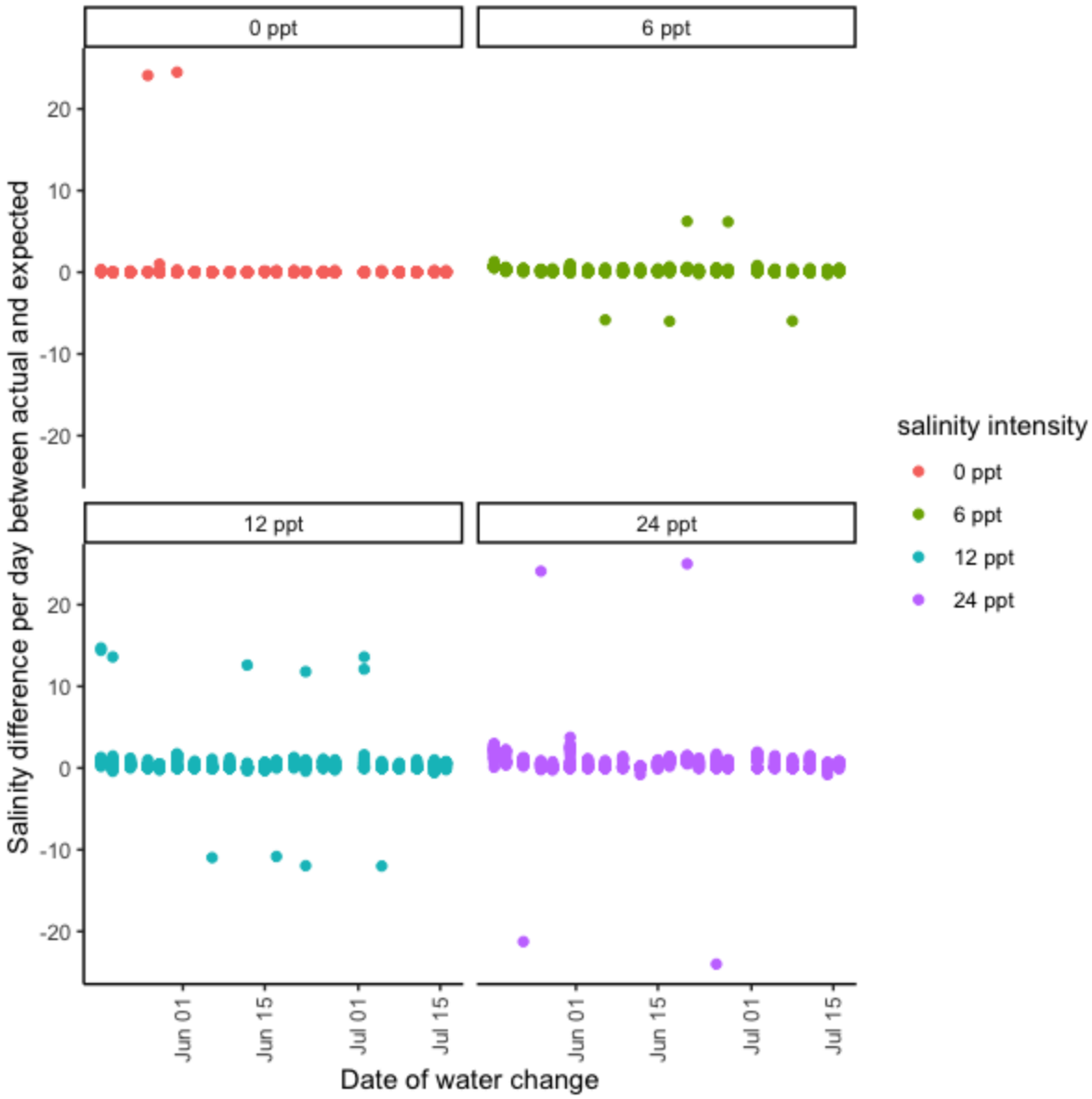

Figure S2. Salinity difference per day for halophyte experiment. Each point represents one tupperware.

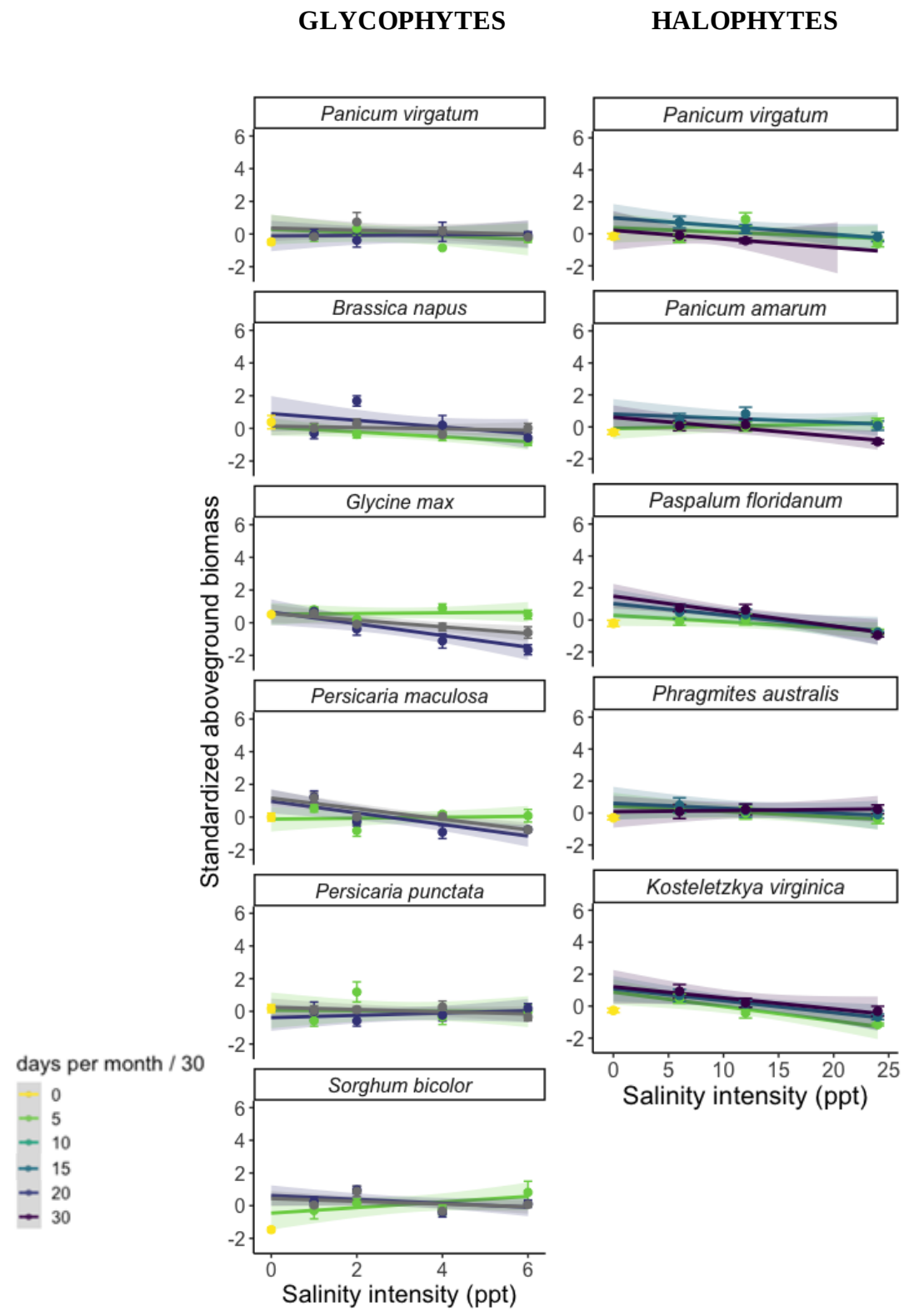

Figure S3. Standardized aboveground biomass by salinity intensity (PPT), grouped and colored by salinity duration. Faceted by species.

Figure S4. Photos demonstrating the setup of the glycophyte experiment of lower tolerant species.

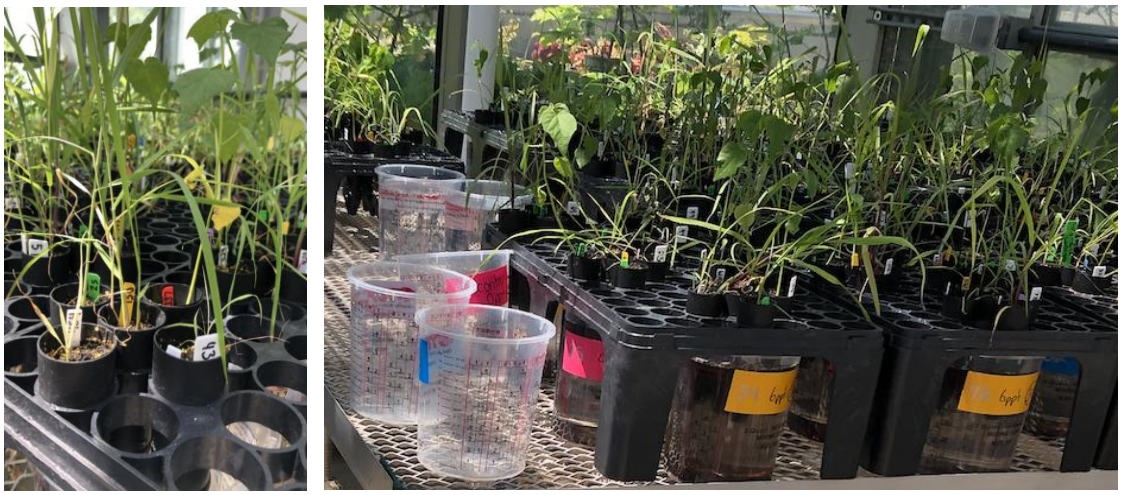

Figure S5. Photos demonstrating the setup of the halophyte experiment of higher tolerant species.

Chapter 2: Perennial grass species for restoration of salinized agricultural land to facilitate marsh transition

Introduction.

Coastal communities are particularly vulnerable to climate change as they face sea level rise (SLR), warming air and water temperatures, and intensifying storm events (Pörtner et al. 2022). The native plant communities of these coastal systems are ecologically and economically important to these regions because of the ecosystem services they provide, including buffering storm surges, maintaining water quality, stabilizing shorelines, and providing habitat (Duarte et al. 2013; Franklin et al. 2016; Barbier et al. 2011).

Restoration plantings are a management tool used to recover and re-establish degraded habitat, and have been administered with success in both marine and terrestrial systems (van Katwijk et al. 2015; Stanturf 2014). In coastal projects, the spacing between plants in a restoration effort has been shown to influence establishment success and growth, as measured by survival and biomass production. For example, Silliman et al. (2015) found that planting *Spartina alterniflora* in small clumps in a tidal marsh restoration produced greater biomass returns and had higher survival rates than when individuals were planted evenly spaced apart (Silliman et al. 2015). The clumped planting design provides an opportunity for facilitation between conspecifics, which can be especially important in environmentally stressful restoration contexts (Gedan and Silliman 2009), where facilitation can provide local stress amelioration, greater yield through niche complementarity, and emergent traits or structure that are not observed in

spaced plantings (Temmink et al. 2020). In this example, neighboring plants of *S. alterniflora* alleviated anoxia stress via oxygen exchange in the root layer (Silliman et al. 2015). Planting design and configuration therefore have important implications for restoration success in achieving maximum returns on investment (McIntire and Fajardo 2014). These principles have most often been applied in restorations in intertidal and shallow marine settings (Temmink et al. 2020) and semi-arid lands (Gomez-Aparicio et al. 2004), but may also apply to coastal uplands and other terrestrial settings where environmental stress is high (McIntire and Fajardo 2014).

Species selection is also an important decision in establishing an effective restoration project (Butterfield et al. 2016). Species vary in their stress tolerance, interactions with other species (e.g. competitive, facilitative, and trophic interactions), and effects on ecosystem function. The selection of species for a restoration planting should be closely aligned to the specific project goals (Knowles et al. 1995). Testing to identify a group of candidate species that could be useful in specific restoration contexts can also provide useful support to restoration practitioners (Knowles et al. 1995; Padilla et al. 2009).

To test six species of perennial grasses for use in coastal restoration, our study established a restoration planting in a coastal system experiencing elevated salt stress due to both SLR and salt water intrusion (SWI). Before land is fully inundated, salt can permeate inland into the soil and porewater, horizontally (through flooding) and vertically (both down into the soil after flooding events and up into the soil from salinized groundwater) (Barlow and Reichard 2010). The increase in salinity stresses coastal and upland plant communities that assembled under historic environmental

conditions where flooding and salinity stress was less common (Barlow and Reichard 2010; Tully et al. 2019). SWI is increasing with rates of SLR, both further exacerbated by groundwater extraction, land subsidence, coastal slope, storm surges, and ditches and canals which promote hydrologic connectivity between upland and tidal regions (Gedan et al. 2020; Tully et al. 2019).

The former farm sites chosen for our project, located in Somerset County on the Eastern Shore of Maryland, are too saline to support production of conventional grain crops such as corn and soy. These crops are sensitive to saline and saturated soil conditions, with salinity thresholds of 0.9 parts per thousand (PPT) and 2.7 ppt for corn and soybean respectively (de la Reguera et al. 2020). The study region is experiencing considerable losses in viable agricultural fields; a detailed land cover change analysis determined a 2% loss of farmland in Somerset County between 2009 and 2017, primarily due to transition of farmland to marsh habitat (Gedan et al. 2020). These saline fields provide opportune locations for restoration activities to recover species such as the black duck (*Anas rubripes*), as well as others that use tidal marsh and coastal habitat imperiled by SLR and SWI into our study region in Somerset County on the Eastern Shore of Maryland.

Species selected for this restoration planting experiment are perennial halophytes that are facultative or obligate wetland species, which are well-adapted to survive in saline and anoxic soils. The goals of the restoration project are to allow the transition of salinized, previously farmed land to quality habitat for native plants and wildlife. A particular species of conservation concern is the black duck, which has been in steep population decline in the Chesapeake Bay region (Wilson et al. 2007) and which is

known to feed on coastal and upland plant species, including agricultural species (English et al. 2017), in addition to aquatic plants and invertebrates.

Several of the selected species are dominant in brackish and salt marshes of the Chesapeake Bay, habitats which have an ambitious restoration target in the region to counterbalance historic regional decline, as well as predicted impacts of SLR (Spencer et al. 2016; Chesapeake Bay Program 2014; Maryland Department of the Environment 2019). Historic drainage and land reclamation diminished the remaining habitat of tidal wetlands, along with many of the species that rely on them as habitat during the last century (Costanzo and Hindman 2007; Deegan et al. 2012; Hughes 2004).

Beyond the need to grow and conserve tidal wetland areas, planting activities as a restoration tool for saline fields are warranted based on the fate of transitioning saline fields that go unrestored. Natural marsh migration into saline fields results in novel community assemblages composed of agricultural weeds and opportunistic facultative wetlands species (Gedan and Fernández-Pascual 2019). Restoration plantings of native species into saline fields may be able to jumpstart a native wetland plant community. It is further likely that the establishment of plant cover, and particularly deep-rooting and perennial plant cover, can prevent the export of nutrients from agricultural soils that can be a particular problem for salinized land (Weissman and Tully 2020). Specifically, salinity changes the biogeochemistry of soils, and salt liberates legacy nutrients leftover from agricultural fertilizers. Tully et al. (2019) suggested that saltwater mobilizes transport of phosphorus (P), a nutrient of particular environmental concern in the Chesapeake Bay, to saline field edges and adjacent waterways. Vegetated fields experienced less P mobilization according to Tully et al. (2019), supporting our study's

efforts to revegetate these saline fields with native perennial grasses that could potentially buffer nutrient runoff into adjacent water sources while rebuilding native habitat and quality forage (Rein et al. 2007).

In this study, we planted six restoration candidate species in saline fields to facilitate transition of these abandoned fields left to fallow into native obligate and facultative wetland habitat. Our study identifies the species that could successfully establish and produce biomass in saline fields and investigates how adjusting planting methodology via the space between propagules can impact survival and biomass returns. Our specific research questions are as follows:

1. Which of the identified candidate restoration species are able to survive and be the most productive in this restoration context?
2. What is the effect of plant spacing in restoration success in this context? If clumped plant spacing helps to establish restoration species, intraspecific facilitation is likely to be an important ecological factor in restoration success.

Methods.

Study species and sourcing of plant material

This study identified six candidate restoration species for the saline fields restoration planting: (1) *Panicum amarum*; (2) *Tripsacum dactyloides*; (3) *Spartina pectinata*; (4) *Spartina patens*; (5) *Panicum virgatum*; and (6) *Paspalum floridanum* (Table 1). All of these species are perennial halophytes native to the eastern United States that are adapted to living in saline environments, making them ideal candidates for restoration projects in salinized agricultural fields. These species were also selected for

this study based on the variety of ecosystem services they provide, including quality forage for local wildlife (such as the black duck), shoreline stabilization, and erosion control (Table 2).

Species were selected in cooperation with the NRCS Plant Materials Centers in Cape May, NJ and Beltsville, MD. The specific lineages chosen have previously been released to landowners, nurseries and seed producers for restoration activities and propagation. Species were grown in monoculture plant production fields and harvested for experimental planting with a seedling undercutter. During harvest, as much of the root biomass was collected as possible and the standing aboveground biomass was kept intact as a protection to the bare root matter. Bare-root propagules were immediately loaded into trailers for transport to the planting site to preserve the quality of the plant material. Plants were driven from Cape May, NJ to our study sites on the Eastern Shore of Maryland.

Experimental design and field site description

The field experiment was established on two recently farmed fields experiencing SWI that will be referred to using the site names: Oriole Farm and Venton Farm.

On March 19-21, 2021, we planted the six candidate species in a randomized block design at each site. Each site was divided into four blocks (replicates), each with nine plots. One species was planted per plot, in either a dispersed or clumped spacing treatment. Within each block, all six of the species were planted in the dispersed (i.e. evenly spaced grid) spacing of 50 x 50 cm, and three of the six species were also planted in a clumped spacing treatment of four clustered plants that were spaced 1 m apart. At Oriole Farm, *S. pectinata*, *S. patens*, and *P. virgatum* were planted in the clumped

treatment; at Venton Farm, *T. dactyloides, P. amarum*, and *P. floridanum* were planted in the clumped treatment. Therefore, between both sites, all six candidate species were planted in clumped and dispersed treatments. Each plot was 3 m wide by x 15 m long, with six rows of 30 plants in the dispersed spacing, or three rows of 60 plants in the clumped spacing. Therefore, regardless of the density treatment, each plot was planted with 180 plants per plot) (Figure 1).

Data collection

Biomass harvests and survival counts

In August of each study year, quadrats of biomass were sampled from each plot at Oriole and Venton Farms (36 plots per farm, 72 plots total). Each quadrat was 50 x 50 cm and was haphazardly placed within the plots. In 2021, we collected four quadrat replicates per plot, while in 2022 we collected three quadrat replicates per plot. Haphazard sampling was performed by tossing the quadrat into each plot and collecting all aboveground biomass within the $0.25m^2$. Each biomass sample was placed into a labeled plastic bag and transported to the George Washington University for storage in a cold room (6 degrees C) for immediate sorting or a freezer (-4 degrees C) for stable storage until sorting.

Each biomass sample was sorted to species based on morphological plant identification. Each species was placed into its own paper bag, dried at 60 degrees Celsius for 48-72 hours, and weighed. If the quadrat sample contained the planted species, approximately 5 g of dried plant material was archived for future nutrient analysis. Surviving transplants were regularly counted after planting. Plants with any green tissue were counted as alive, and the proportion surviving out of the 180 originally

planted in each plot was calculated. Counts were made once per month during April, May, and June of 2021, and May and June of 2022.

Data analysis

To examine the variation in biomass yield and survival rates of our candidate restoration species, I constructed mixed effect models of biomass and survival response variables with combinations of the predictor variables of species, year, site, block, and spacing treatment, and compared them to a full model containing all predictors. All models were built using the lmer function from the lme4 package in R. In all models, block was nested within site as a random effect. Year, species, and spacing treatment were fixed effects in the models. Biomass data was log-transformed to meet the assumption of normality; survival data was normally distributed. All analyses were conducted in R (Version 4.2.3).

I used the emmip() function from the emmeans package to visualize the interactions between our fixed predictors (species, spacing, and year), and used the function lsmeans(adjust = "tukey") for posthoc analysis to further tease apart these interactions.

I used the r.squaredGLMM() function in the MuMIn package to generate marginal and conditional R^2 values for all models. I identified the models of best fit using Akaike's Information Criterion (AIC) to determine the set of independent variables and their interactions that best explained patterns in biomass and survival.

Results.

Survival

The model that best explained patterns in plant survival included both the independent and interactive effects of species and year. This suggests that the effects of the spacing treatments on plant survival were minimal; however, the next best model included all three fixed predictor variables and allowed for two-way interactions but excluded the three-way interaction term (Model 3 in Table 3). Therefore, we conclude that spacing may have had a weak effect on survival, and we interpret this more complex model with posthoc analyses to test for differences between treatment combinations of year, spacing, and species.

There were significant differences in survival (averaged across all species) between 2021 and 2022 for both the clumped treatment ($p < 0.0001$) and the spaced treatment ($p < 0.0001$), where survival decreased from year one to year two (Table 3). However, within the same year, there was no significant difference in survival between the spaced and clumped planting treatments in 2021 ($p = 0.9959$) or 2022 ($p = 0.9664$) (Table 3).

In the first year of the experiment, *S. patens* had higher survivorship than several other candidate species (e.g. *P. virgatum* had lower survival than *S. patens* ($p = 0.0008$) and *S. pectinata* ($p = 0.0001$) in 2021), but this effect diminished in 2022 (n.s. contrasts between *S. patens* 2022 and other species). All species except *P. amarum* experienced a decline in survival from 2021 to 2022 (species*year effect, $p < 0.0001$ for *P. floridanum*, *P. virgatum*, *S. patens*, and *T. dactyloides*; $p = 0.0539$ for *S. pectinata*, n.s. for *P.*

amarum). However, *P. amarum* had lower survival than nearly all other species in 2021 (Fig. 2).

Biomass

The model that best explained patterns in biomass, included only the species effect (Table 4). Therefore, the strongest explanatory factor in biomass production was variation between species. However, models that included the other two predictor variables of spacing and year in various combinations of all three predictors also had high AIC values (within ± 2.5), suggesting that spacing and year and interactions of the factors do contribute more weakly to explain variation in biomass production. We therefore investigated these effects in post hoc analysis of a more complex model. For this, we selected the model that included all three of our fixed predictor variables and allowed for two way interactions but excluded the three-way interaction term (Model 7 in Table 4) to fully explore these relationships.

Biomass production in the first two years of this experiment was low overall (most treatments < 100 g/m^2) and highly variable across sites and species. Therefore, there were fewer treatment combinations that produced significant differences in biomass than in survival. *P. floridanum* produced more biomass than *P. virgatum* ($p = 0.0408$) and *S. patens* ($p = 0.0021$) in 2021; *T. dactyloides* also produced greater biomass than *S. patens*, though only in the clumped treatment ($p = 0.0713$), and *S. patens* produced greater biomass than *S. pectinata* in the spaced treatment ($p = 0.0135$).

Discussion.

All six of our restoration candidate species experienced some level of successful establishment and biomass production in our saline field sites across both the spaced and clumped planting treatments. However, there were interspecies differences in the degree of survival and magnitude of biomass production, suggesting that certain candidates might be better for restoration projects in these novel saline environments.

There was a notable drop in survival between year one and year two across all species except *P. amarum*; however, undercounting was likely a factor in the second summer due to intense weed pressure and competition. After a full year of disturbance from weather and wildlife, it was more difficult to follow the planting layout and locate the original propagules in the second year. Because of this confounding factor, we focus our discussion on comparison between species within the same year.

The two *Panicum* species experienced significantly lower levels of survival than several other restoration candidate species over both years, suggesting that *P. amarum* and *P. virgatum* were unable to handle transplant into our saline fields as well as other candidates. In 2021, both *P. amarum* and *P. virgatum* had lower survival than *S. patens*, while *P. amarum* also experienced lower survival than *T. dactyloides* and *P. virgatum*. However, *P. amarum* survival was not significantly lower than any candidate species in 2022, as *S. patens, T. dactyloides,* and *P. virgatum* survival declined in the second year to match *P. amarum* (which was the only species to not experience a significant drop in biomass from 2021 to 2022). This suggests that, while *P. amarum* did not handle transplant into saline fields as well as other species, the propagules that did survive also

survived into year two. Meanwhile, in 2022 *P. virgatum* had lower survival than *S. patens, S. pectinata,* and *T. dactyloides*; though all four of these species experienced lower survival in the second year, *P. virgatum* declined the most, having the lowest survival percentages of any species in any year. Overall, this suggests that of our restoration species, the two *Panicum* species are not the ideal candidates for rapid restoration projects in these saline field environments, as the effort invested in planting produced comparatively low survival returns. However, we note that the relatively low rate of survival in both *P. virgatum* and *P. amarum* may be at least in part contributed to error during planting; both of these species had the most standing aboveground biomass remaining from the previous growing season, making the propagules top heavy and prone to falling over and flushing out of their holes. While some were able to be salvaged and re-planted, this planting error may have contributed to the lower level of survival for the *Panicum* species.

S. patens, in addition to outperforming both *Panicum* species in either one or both 2021 and 2022, also had higher survival than *S. pectinata* in 2021, though this difference became insignificant in 2022 as *S. patens* had lower survival in the second year. *S. patens* consistently achieved higher survival rates than most other candidate species across both years (Figure 2). While previous restoration projects have found *S. patens* to be susceptible to transplant shock during restoration plantings (Anastasiou and Brooks 2003), our findings present a promising result for using *S. patens* as a restoration species in saline fields. As *S. patens* is rapidly disappearing from wetlands along the East Coast, this further supports the use of this foundational wetland species in restoration projects to rehabilitate degraded or abandoned habitat (Watson et al. 2016).

Because of the drop in survival in the second summer for 5 of the 6 restoration candidate species that was likely due to undercounting from weed pressure, biomass served as a better metric of comparison between year one and year two to assess species production. There were fewer interspecies differences found between treatment combinations in biomass than in survival. *P. floridanum* outperformed *P. virgatum* and *S. patens* in biomass production in 2021, but, similar to patterns we saw in survival, these differences did not appear in 2022 as *P. floridanum* biomass decreased to match *P. virgatum* and *S. patens*.

There were also no consistent patterns in the effect of spacing treatment on biomass by species, suggesting that there were no facilitative effects between conspecifics in the time scale of this experiment. Species performed equally well in the spaced and clumping treatment. As we have only yet collected data for the first two years of the restoration planting, there might yet be delayed impacts of spacing treatment on biomass production. We will be collecting biomass samples again in 2023 to further track the development of our candidate species. Overall, we note that the experiment needs another year for these species to more fully establish and perform and that it is early to draw conclusions about the success of each species as a restoration candidate.

Future work.

Biomass samples will be collected for a third and final year in August 2023, and processed as detailed in the Methods section. We will also be collecting seed heads of the restoration candidate species for nutritional analysis to approximate habitat quality for local wildlife, namely the black duck. Lastly, we will also process the biomass samples of our restoration candidate species for nutrient analysis.

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Figures and Tables.

Table 1. Restoration candidate species attribute table. National Wetland Plant List (NWPL) indicator values are for the Atlantic and Gulf Coastal Plain and can be found at https://wetland-plants.usace.army.mil/nwpl_static/v34/home/home.html. FACW = facultative wetland. FAC = facultative. OBL = obligate wetland.

Latin name	**Common name**	**Germplasm**	**NWPL**	**Soil salinity tolerance**	**Short term pulse tolerance**
P. floridanum	Florida paspalum	Mid-Atlantic	FACW	TBD	med.-high
P. amarum var. *amarulum*	Coastal panicgrass	Atlantic	FAC	medium. (10-15 ppt)	high (25-35 ppt)
P. virgatum	Switchgrass	High Tide	FAC	medium (10-15 ppt)	high (25-35 ppt)
S. pectinata	Prairie cordgrass	Southampton	OBL	medium (10-15)	medium (10-15)
S. patens	Saltmeadow cordgrass	Avalon	FACW	high (25-35 ppt)	high (25-35 ppt)
T. dactyloides	Eastern gamagrass		FAC	low/med. (TBD)	med. (10-15)

Table 2. Ecosystem services of the candidate restoration species. Information was gathered from the USDA-NRCS Plant Materials guides.

		SITE CONDITIONS					CONSERVATION PRATICE/USE							
COMMON NAME	**VARIETY**	**DRY SOILS**	**MESIC SOILS**	**WET SOILS**	**SALT TOLERANT**	**pH (Min. – Max.)**	**327- CONSERV. COVER**	**386 -FIELD BORDERS**	**390 -RIPARIAN HERB. COVER**	**420 -WILDLIFE HABITAT PLANTING**	**422 -HEDGEROW/ WIND BARRIER**	**512- PASTURE HAY**	**512 -BIOMASS**	**580 STREAM/SHORE STABILIZATION**
coastal panicgrass	'Atlantic'	★	★	✓	★	**4.5 - 7.5**	✓	✓	★	✓	★		✓	★
switchgrass	High Tide Germplasm	✓	★	★	★	**4.5 -8.0**	✓	✓	★	✓	✓			★
Florida paspalum	Mid-Atlantic Germplasm	✓	★	★	✓	**5.0 -7.5**	★	✓	★	★		★	✓	✓
saltmeadow cordgrass	'Avalon'	✓	✓	★	★	**4.8 - 8.0**			✓	★				★
prairie cordgrass	Southampton Germplasm	✓	★	★	✓	**6.0 - 8.5**			★	✓			✓	★
Eastern gamagrass	'Meadowcrest'	✓	★	✓	✓	**5.1 - 7.5**	★	★	★	★		★		★

(★) Optimal (✓) Acceptable

Table 3. Model comparison for assessing patterns in **survival** of the restoration candidate species. AIC values were generated by running an ANOVA between the full and reduced models. **Δ**AIC was calculated as the difference between the full and reduced models AIC values (full model AIC - reduced model AIC). R^2m = R^2 marginal. R^2c = R^2 conditional.

Model	Predictor variables	AIC	**Δ**AIC	R^2m	R^2c
1	species*spacing*year + (1|site:block)	-207.76		0.599	0.604
2	species*year + (1|site:block)	-219.79	12.03	0.608	0.608
3	species * spacing * year - species:spacing:year + (1 | site:block)	-211.33	3.57	0.619	0.620
4	species + spacing * year + (1 | site:block)	-193.36	-14.4	0.534	0.534
5	species * spacing + year + (1 | site:block)	-189.01	-18.75	0.543	0.543
6	year + (1|site:block)	-144.79	-62.97	0.350	0.350
7	spacing*year + (1|site:block)	-141.43	-66.33	0.352	0.352
8	species + (1|site:block)	-96.539	-111.221	0.195	0.195
9	species*spacing + (1|site:block)	-87.009	-120.751	0.206	0.206
10	spacing + (1|site:block)	-63.691	-144.069	0.0003	0.0003

Table 4. Model selection for assessing patterns in **biomass** yields of the restoration candidate species. AIC values were generated by running an ANOVA between the full and reduced model. **Δ**AIC was calculated as the difference between the full and reduced models AIC values (full model AIC - reduced model AIC). R^2m = R^2 marginal. R^2c = R^2 conditional.

Model	Predictor variables	AIC	**Δ**AIC	R^2m	R^2c
1	species*spacing*year + (1|site:block)	629.98		0.242	0.242
2	species + (1|site:block)	616.44	13.54	0.171	0.171
3	species + spacing * year + (1 | site:block)	618.32	11.66	0.190	0.190
4	species*spacing + (1|site:block)	618.91	11.07	0.215	0.215
5	species*spacing + year + (1 | site:block)	619.77	10.21	0.220	0.220
6	species*year + (1|site:block)	624.18	5.8	0.191	0.191
7	species * spacing * year - species:spacing:year + (1 | site:block)	625.22	4.76	0.248	0.248
8	year + (1|site:block)	637.10	-7.12	0.008	0.060
9	spacing + (1|site:block)	638.50	-8.52	0.0002	0.049
10	spacing*year + (1|site:block)	638.93	-8.95	0.019	0.069

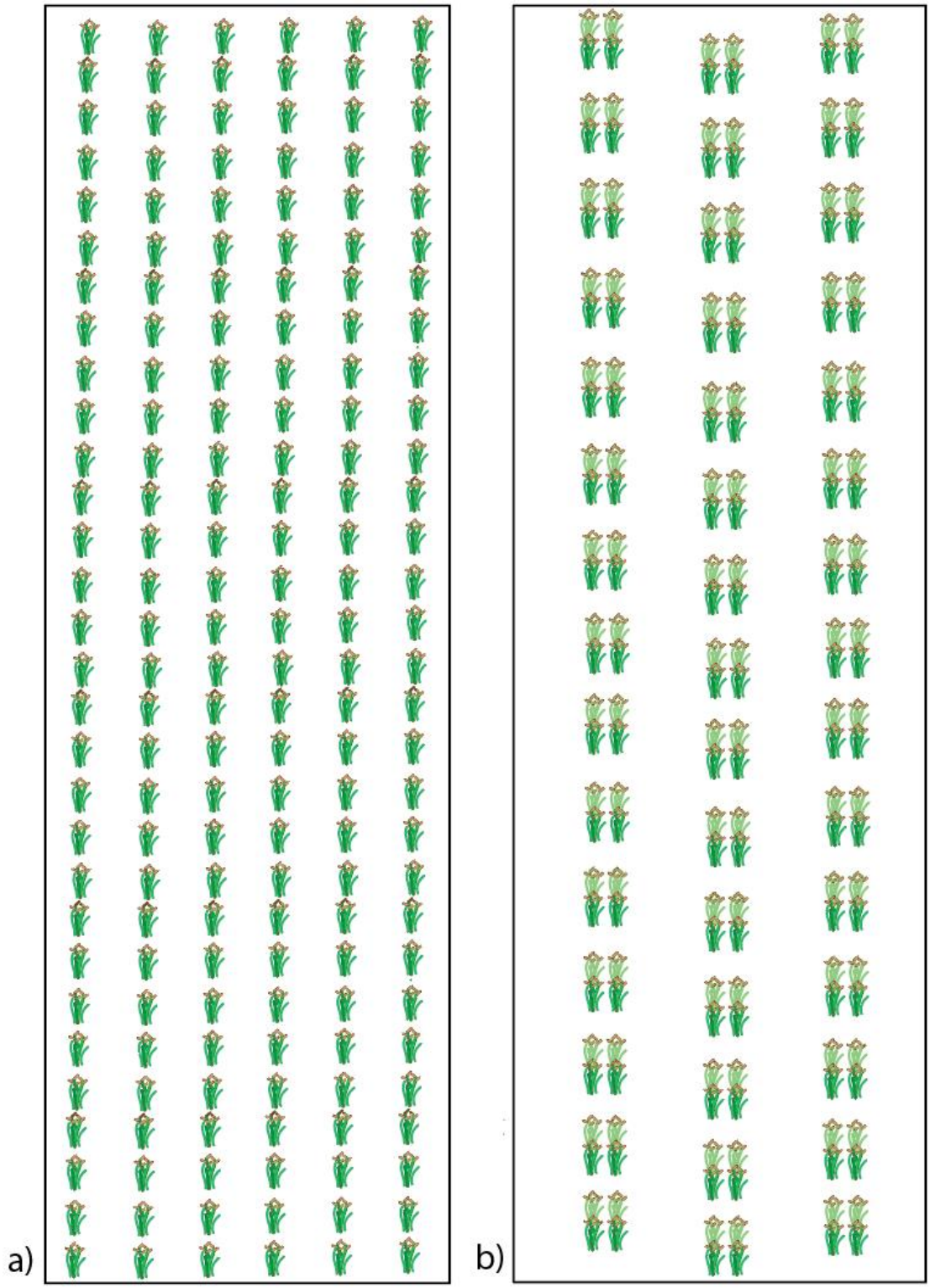

Figure 1. Diagram of a) spaced and b) clumped planting treatments. In the spaced treatment, 180 individuals were planted in 6 rows of 30. In the clumped treatment, 180 individuals were planted in 3 rows of 15 clumps of 4.

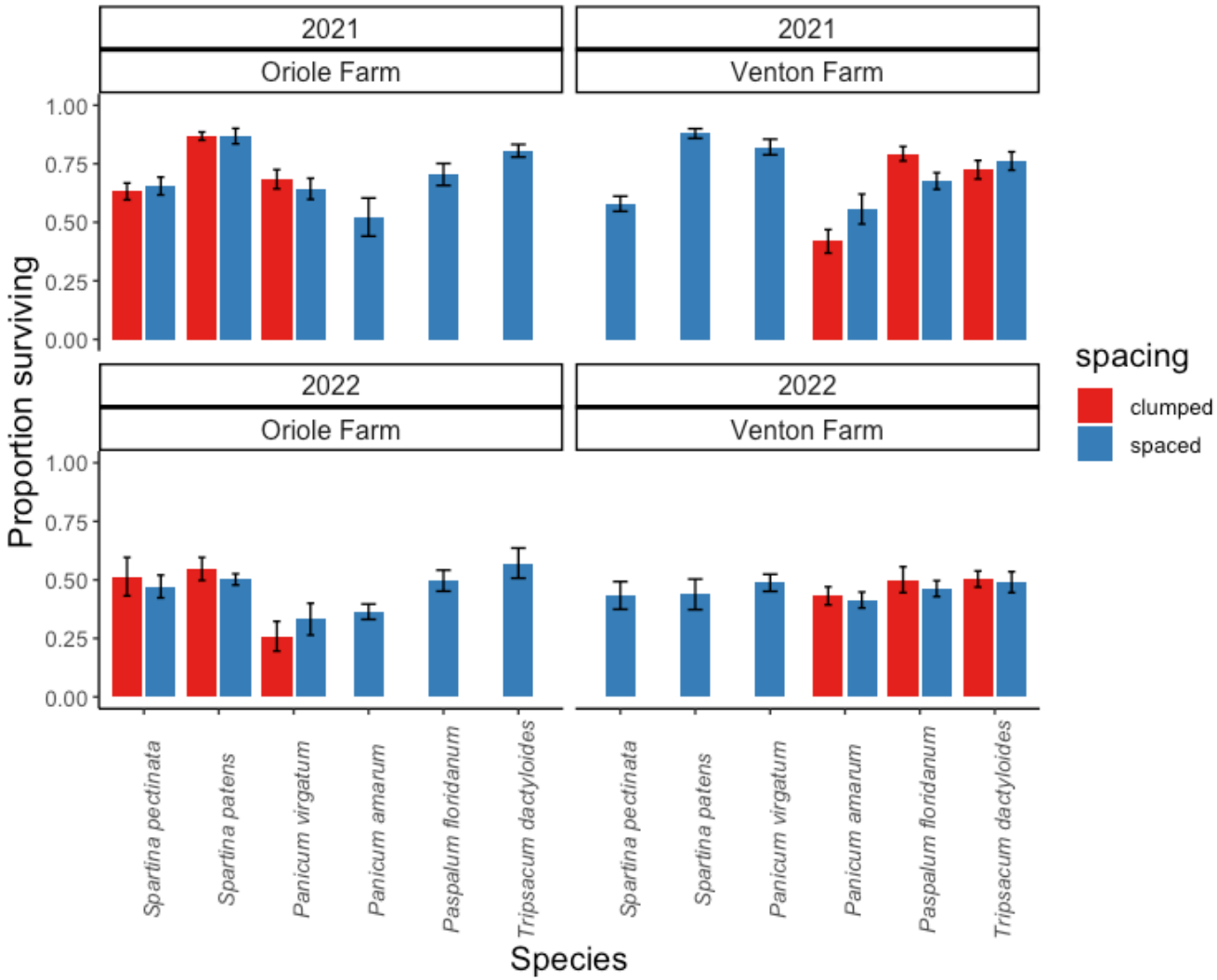

Figure 2. Proportion of 180 original plantings per plot surviving, faceted by site, year, species, and spacing treatment.

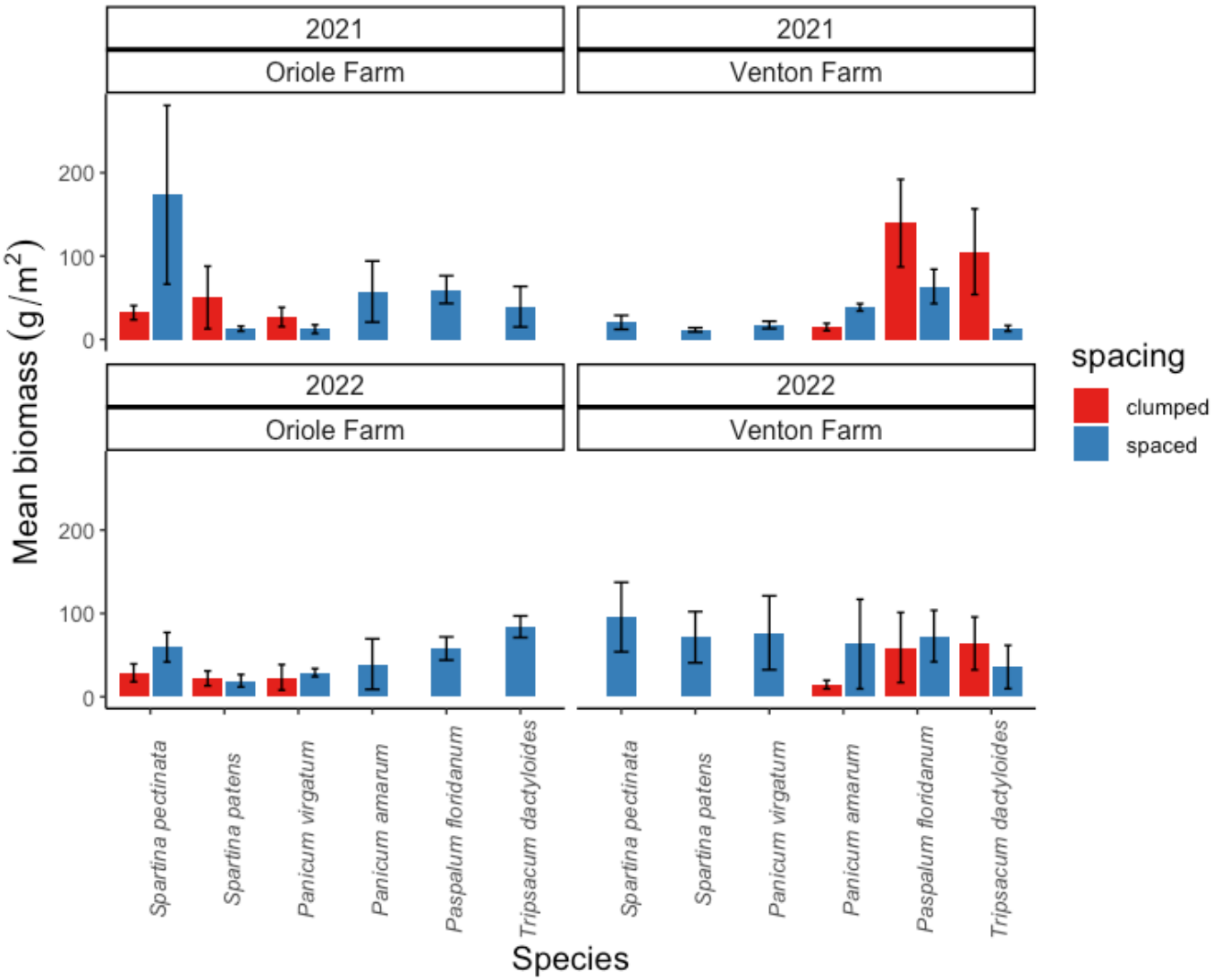

Figure 3. Mean biomass (g/m^2) of candidate restoration species, faceted by site, year, species, and spacing treatment.

Supplemental material.

Table S1. Differences in survival between species by year and spacing treatment. Color corresponds to model results: lsmeans(reduced_model_3way, pairwise ~ species*spacing, adjust="tukey"); lsmeans(reduced_model_3way, pairwise ~ species*summer, adjust="tukey"). Comparisons between different species in different years were not reported, as well as between different species in different spacing treatments, as these results are not relevant to our analysis (ex: P. virgatum 2021 and P. floridanum 2022).

	P. floridanum	*P. amarum*	*P. virgatum*	*S. pectinata*	*S. patens*	*T. dactyloides*
P. floridanum	2021 > 2022 ($p < 0.0001$)	*P. floridanum* clumped > *P. amarum* clumped ($p = 0.0001$) *P. floridanum* > *P. amarum* in 2021 ($p < 0.0001$)	*P. floridanum* clumped > *P. virgatum* clumped ($p = 0.0138$) *P. floridanum* > *P. virgatum* in 2022 ($p = 0.0062$)			
P. amarum			*P. virgatum* > *P. amarum* in 2021 ($p = 0.0081$)	*S. pectinata* clumped > *P. amarum* clumped ($p = 0.0553$) *S. pectinata* > *P. amarum* in 2021 ($p = 0.0328$)	*S. patens* clumped > *P. amarum* for both clumped ($p < 0.0001$) and spaced ($p = 0.0105$) *S. patens* 2021 > *P. amarum*	*T. dactyloides* clumped > *P. amarum* clumped ($p = 0.0015$) *T. dactyloides* > *P. amarum* in 2021 ($p < 0.0001$)

					2021 ($p < 0.0001$)	
P. virgatum			2021 > 2022 ($p < 0.0001$)	*S. pectinata* > *P. virgatum* and in 2022 ($p = 0.0016$)	*S. patens* clumped > *P. virgatum* for both clumped ($p = 0.0001$) and spaced ($p = 0.0027$) *S. patens* > *P. virgatum* in 2021 ($p = 0.0008$) and 2022 ($p = 0.0003$)	*T. dactyloides* > *P. virgatum* in 2022 ($p = 0.0019$)
S. pectinata				2021 > 2022 ($p = 0.0539$)	*S. patens* > *S. pectinata* in 2021 ($p = 0.0001$)	
S. patens					2021 > 2022 ($p < 0.0001$)	
T. dactyloides						2021 > 2022 ($p < 0.0001$)

Table S2. Differences in biomass between species by year and spacing treatment. lsmeans(reduced_model_3way, pairwise ~ species*spacing, adjust="tukey"). lsmeans(reduced_model_3way, pairwise ~ species*year, adjust="tukey"). Comparisons between different species in different years were not reported, as well as between different species in different spacing treatments, as these results are not relevant to our analysis (ex: P. virgatum 2021 and P. floridanum 2022).

	P. floridanum	*P. amarum*	*P. virgatum*	*S. pectinata*	*S. patens*	*T. dactyloides*
P. floridanum			P. floridanum > P. virgatum in 2021 (p = 0.0408)		*P. floridanum* clumped > *S. patens* both clumped (p = 0.0254) and spaced (p = 0.0276) P. floridanum > S. patens in 2021 (p = 0.0021)	
P. amarum						
P. virgatum						
S. pectinata					*S. patens* spaced > *S. pectinata* spaced (p = 0.0135)	
S. patens						*T. dactyloides* clumped > *S. patens* clumped (p = 0.0713)
T. dactyloides						

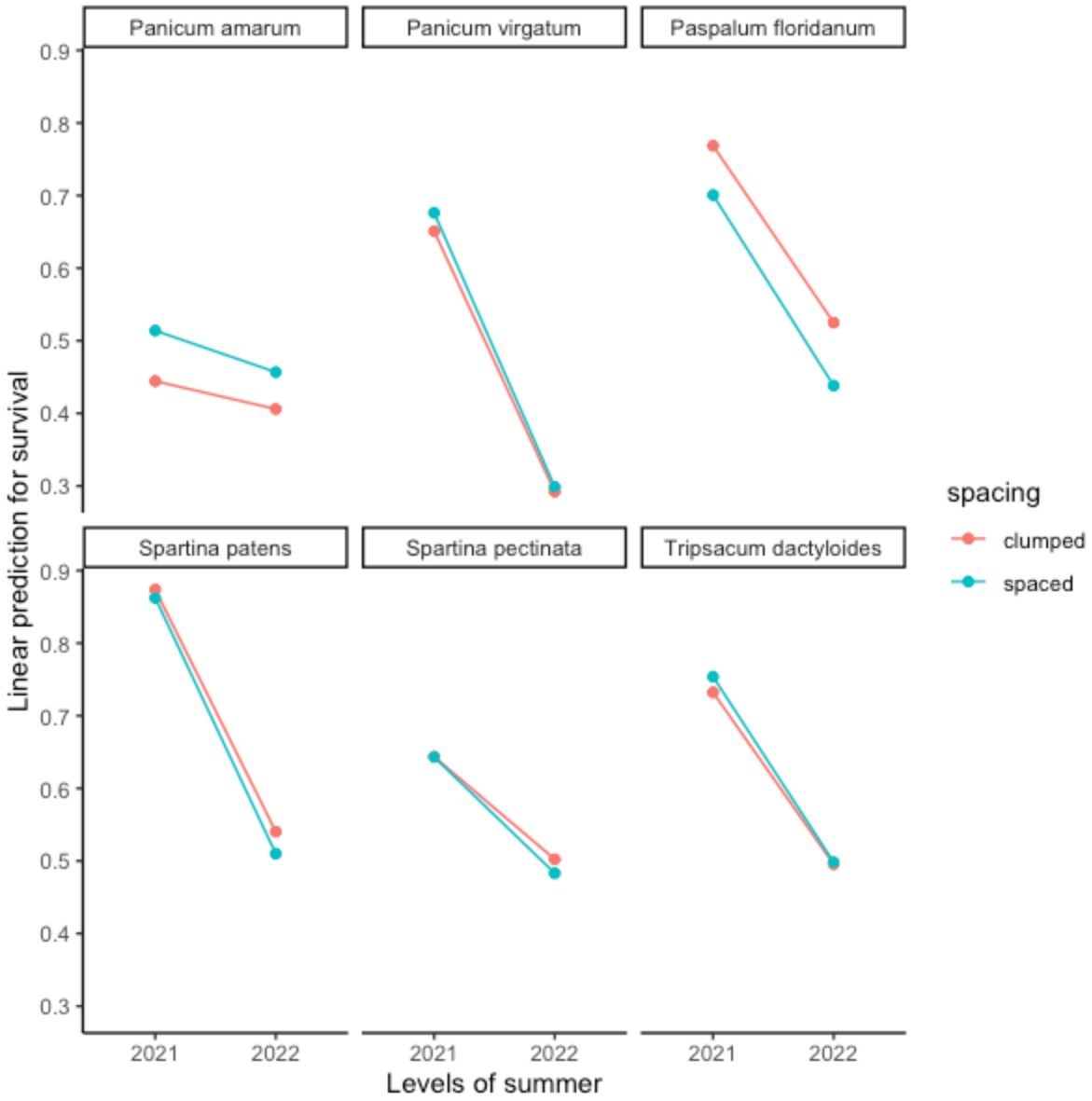

Figure S1. Interaction plot for survival by species, year, and spacing.

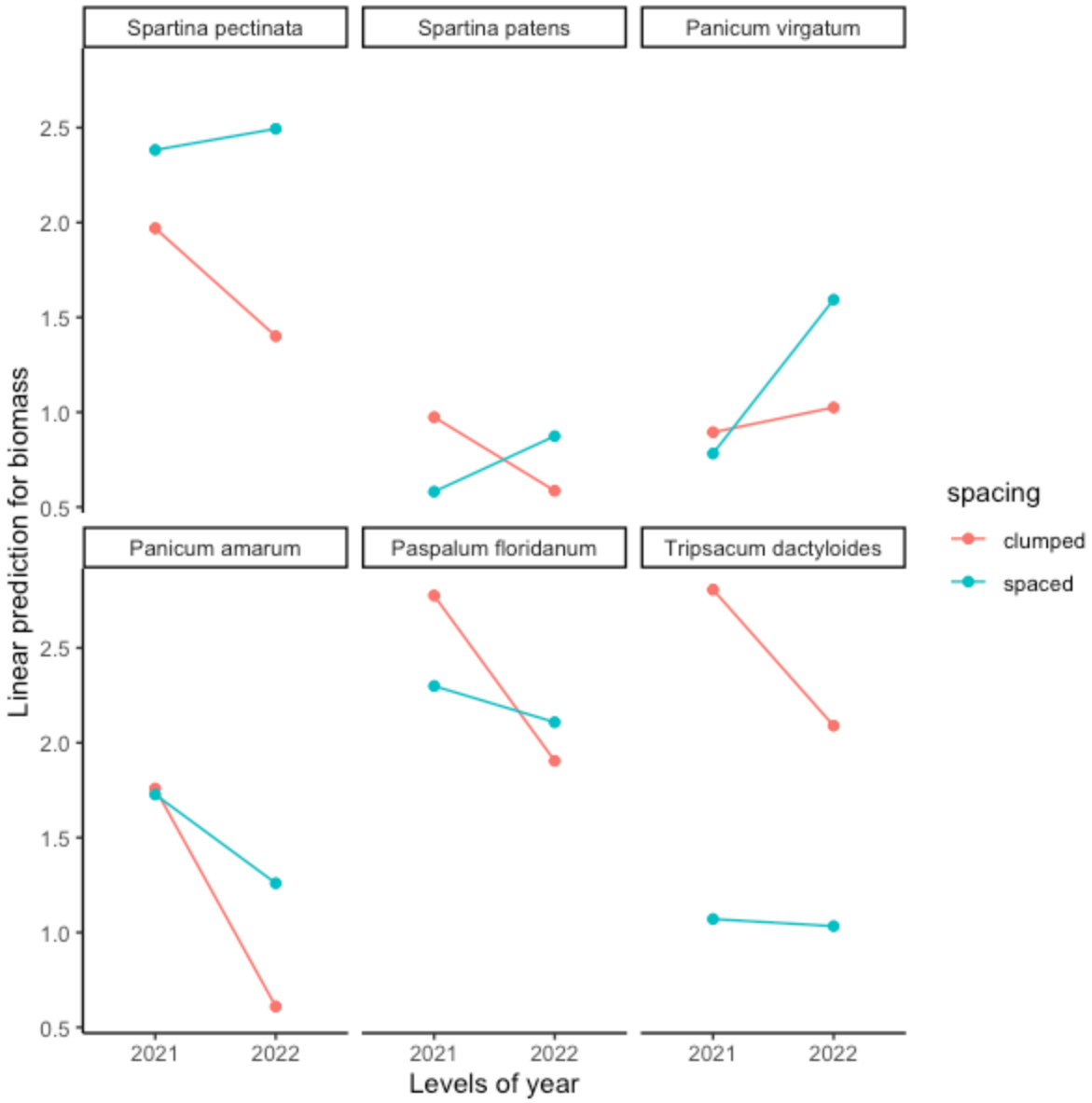

Figure S2. Interaction plot for biomass by species, year, and spacing.