Effects of diversity and coalescence of species assemblages on ecosystem function at the margins of an environmental shift

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Sea level rise is mixing formerly isolated freshwater communities with saltwater communities. The structure of these new aquatic communities is jointly controlled by pre- and post-colonization processes. Thus, changes in dispersal, a pre-colonization process, may change these communities. Similarly, since salinity is a strong abiotic determinant of post-colonization survival in coastal systems changes in salinity will likely impact community composition. In this study, we examine how a strong abiotic gradient affects the diversity and structure of zooplankton and bacterioplankton communities and associated ecosystem functions (decomposition and carbon mineralization). We ran a six week dispersal experiment using mesocosm ponds with four distinct salinity profiles (0, 5, 9, and 13 psu). We find that salinity is the primary driver of both bacterial and zooplankton community composition. We find evidence that as bacterial richness increases so do both the amount of decomposition and mineralized carbon. Understanding how salinization changes community structure and ecosystem function may be paramount for managing and conserving coastal plain ecosystems where salinity is increasing due to sea level rise, saltwater intrusion, storm surges, and drought

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ABSTRACT

Sea level rise is mixing formerly isolated freshwater communities with saltwater communities. The structure of these new aquatic communities is jointly controlled by pre- and post-colonization processes. Thus, changes in dispersal, a pre-colonization process, may change these communities. Similarly, since salinity is a strong abiotic determinant of post-colonization survival in coastal systems changes in salinity will likely impact community composition. In this study, we examine how a strong abiotic gradient affects the diversity and structure of zooplankton and bacterioplankton communities and associated ecosystem functions (decomposition and carbon mineralization). We ran a six week dispersal experiment using mesocosm ponds with four distinct salinity profiles (0, 5, 9, and 13 psu). We find that salinity is the primary driver of both bacterial and zooplankton community composition. We find evidence that as bacterial richness increases so do both the amount of decomposition and mineralized carbon. Understanding how salinization changes community structure and ecosystem function may be paramount for managing and conserving coastal plain ecosystems where salinity is increasing due to sea level rise, saltwater intrusion, storm surges, and drought.

Keywords dispersal, ecosystem function, abiotic filter, salinization, climate change

INTRODUCTION

Salinity is an abiotic filter for almost all aquatic organisms, and therefore strongly influences their distribution and abundance. Thus, changes in salinity can alter the distribution of organisms (Gutiérrez-Cánovas et al., 2015), community assembly processes (Zhang et al., 2014; Mayfield et al., 2010), and associated ecosystem functions (Mayfield et al., 2010). Understanding how communities are altered following changes in habitat quality is critical for predicting the consequences of environmental changes. In this experiment, we investigate the impacts of salinization on biodiversity in coastal shallow ponds to advance our understanding of how environmental and ecological filters affect species diversity, community structure and associated ecosystem functions.

Changes in salinity due to climate change associated sea level rise (SLR), coastal storm surges, ditching and dredging, over-extraction of aquifers, and increased input of salts from upstream sources greatly affect coastal wetlands (Nicholls and Cazenave, 2010; Craft et al., 2009). Specifically, SLR and ocean over-wash from storm surges change the chemical make up of coastal freshwater bodies and increase the movement of organisms between salt and freshwater habitat types creating new species assemblages by merging communities that were historically allopatric. Furthermore, the increases in salinity, alkalinity, pH, and ion concentrations from salt water incursions into freshwater habitats is toxic to many freshwater organisms (e.g. Albecker and McCoy, 2017; Hintz and Relyea, 2017), creating a physiological barrier that

affects the composition of freshwater communities. Indeed, changes in abiotic conditions, disturbance regime, and dispersal dynamics in coastal ponds likely affects both the compositions of species and their ecological functions which can ultimately jeopardize the important socio-economic services that these ecosystems provide (de Groot et al., 2002; Kirwan and Megonigal, 2013).

In this study we examined how secondary salinization events along with mixing of freshwater and saltwater taxa affects the diversity and composition of bacterioplankton and zooplankton communities and downstream ecosystem functions. Zooplankton communities are models for testing questions about biodiversity-ecosystem function relationships because they can strongly influence primary production (Arnér et al., 1998) and bacterial diversity (Berga et al., 2015). Zooplankaton and microbes are also widely recognized for their roles in essential biogeochemical processes responsible for controlling flows of carbon, nitrogen and phosphorus (Hébert et al., 2016b) in wetland systems (Schimel and Schaeffer, 2012; Herbert et al., 2015). In addition, salinity is well recognized as a primary determinate of both zooplankton (Bate et al., 2002; Kimmel, 2011; Breckenridge et al., 2015) and bacterial community structure (Stagg et al., 2017). Salinization might have particularly strong affects on these systems because osmotic tolerance is not easily gained or lost in most taxa. Therefore, changes in environmental salinity due to sea level rise, storm surge, or incursions are expected to cause major shifts in community composition, diversity, and associated ecosystem functions (Rath et al., 2016; Trivedi et al., 2016; Paver et al., 2018; Stagg et al., 2017). Therefore, to test the effects of salinization on diversity and ecosystem function we performed a semi-natural mesocosm experiment in which we simulated wetlands with a range of salinity observed in natural coastal ponds (0, 5, 9, and 13 psu) (Albecker and McCoy in press). Each experimental pond was seeded with bacterioplankton and zooplankton from natural wetlands with matching salinity. We then simulated the effects of salt water incursions and the mixing of salt and freshwater communities by imposing two treatments: one that included a sample of both fresh and 13 psu plankton, and a second that was a sample of salt-only plankton communities. We quantified changes in zooplankton and bacterioplankton communities and measured two representative ecosystem functions (carbon mineralization and litter decomposition). We expected differences in species identities and diversity among patches would translate into differences in aggregate ecosystem functions (Staddon et al., 2010; Symons and Arnott, 2013; Dodson, 1992). Our results highlight the need to better understand how changes in the abiotic environment and mixing of novel communities interact to affect how ecosystems (such as coastal ponds) respond to the rapid environmental changes and accelerating rates of global change.

METHODS

Experimental Set-Up

We created 39 experimental ponds using 567 L stock watering tanks. Tanks were filled with 378 L of water. Instant Ocean sea salt was used to generate salinity treatments that closely matched the salinity of local coastal ponds (0, 5, 9 and 13 psu). The tanks were randomly assigned to receive one of the four salinity treatments (0, 5, 9 and 13 psu), and each was initially seeded with zooplankton and bacterioplankton from five natural ponds with matching salinity (samples from two different ponds were mixed for the highest salinity treatment) located along the inner and outer banks, of North Carolina, USA on May 3, 2015 (supplementary Table S1).

We maintained ”source” experimental ponds at 0 and 13 psu that were used to provide the colonists that would be added to the other experimental ponds. These species mixing treatments consisted of a ”salt-only” plankton community which only received water from the 13 psu source tanks or ”mixed” plankton treatment which received an aliquot of water and plankton consisting of equal volumes (each 50% of the total aliquot) sampled from the zero and 13 psu source tanks (Figure 1). Species mixing treatments were applied every nine days for a total of five species introductions over the course of the experiment. Plankton communities in all experimental ponds were sampled prior to each new introduction event. We chose this mixing regime to mimic the effects of saltwater over-wash and intrusion on freshwater wetlands since salinization events may be common in coastal ponds (Albecker and McCoy, 2019) and likely represent the unidirectional movement of saltwater species into freshwater communities. Each treatment combination was replicated four times, except for the 5 psu/mixed mixing treatment which only had three replicates due to a leak in the experimental mesocosm.

To collect our source zooplankton and bacterioplankton from coastal ponds, we sampled along a single 100 m transect at each pond taking twenty 1 L samples of water and straining them through a 62.5

μm mesh filter. If a pond was too small to complete a full 100 meter transect, a second transect was used. These samples served as the starting communities for the experiment. In addition to samples from coastal ponds, the experimental tanks were seeded with peat moss to provide a nutrient pulse and the tank bottoms were covered with sand as a benthic substrate. Mesocosms were covered with 60% shade cloth to prevent macroinvertebrates and other higher trophic level organisms from colonizing.

Species mixing consisted of a 2 L aliquot of water from the source tanks, therefore due to natural dynamics in these tanks the actual abundances varied for each mixing event (Supplementary Table S1). On June 1, 2015, prior to beginning the experiment, we detected very low zooplankton abundance from the first seeding in the 13 psu tanks, so we re-seeded with a new wild sample of zooplankton. To allow populations to stabilize, the experiment began 6 weeks after initial seeding. For 45 days, we sampled all experimental ponds every 9 days. We had a 9 day sampling regime because for most zooplankton species this is long enough to complete at least one-generation cycle (Thompson and Shurin, 2012). Prior to sampling, we mixed each tank by stirring them in a circular motion around the perimeter five times. Twenty liters (approximately 5% of total volume) of water was sampled from the water column at 20 random locations using an integrated tube sampler. The samples were condensed through a 62.5 μm filter into 25 mL containers. Zooplankton was preserved in 10% formalin. For each tank at the time of sampling, we measured DO, NH_4, temperature and pH with a YSI Professional Plus multiparameter meter (Xylem, Inc., Yellow Springs, OH).

Zooplankton were counted in three 5 mL subsamples and identified to the lowest taxonomic level possible (order, family, or genus when feasible using Johnson and Allen (2012) and Pennak (1953)); however, for all analyses either family or order were used. Based on known functional redundancy within zooplankon orders and family level taxonmic groupings (e.g. Barnett et al., 2007) we expected this level of resolution to be sufficient to capture major impacts of changes in assemblages on ecosystem functions.

0.1 Bacterial sampling

Bacterial sampling was concurrent to zooplankton sampling. At each sampling event we collected 1 L of water from each tank. Each 1 L bottle of water was homogenized and 200 mL of the water sample was concentrated onto 0.22 μm filters within 24 hours of field sampling (Supor-200; Pall Gelman, East Hills, NY). Filters were transferred into 2 mL sterile tubes and stored at -80 $^\circ$C until molecular analyses could be completed.

0.1.1 Bacterial Community Sequencing

To examine shifts in bacterial community composition and diversity, bacterioplankton in each mesocosm were characterized using paired-end targeted Illumina sequencing of the 16S rRNA gene (bacteria, archaea) (Caporaso et al., 2011). We extracted DNA from filters collected at 3 of the 6 time points representing the initial, middle, and final sampling days (Days 0, 18, 45). We extracted and purified the DNA concentrated onto 0.22 μm supor filters from each mesocosm using the PowerWater DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) . We used this DNA as a template in PCR reactions. To characterize bacterial communities, we used barcoded primers (515FB/806RB) originally developed by the Earth Microbiome Project (Caporaso et al., 2012) to target the V4-V5 region of the bacterial 16S subunit of the ribosomal RNA gene (Apprill et al., 2015; Parada et al., 2016). We used Earth Microbiome Project primer set (515FB/806R), which targets Bacteria and Archaea. For this study, we focused on the bacterioplankton. PCR products were combined in equimolar concentrations and sequenced using paired-end (2 x 250 bp) approach using the Illumina MiSeq platform at the Indiana University Center for Genomics and Bioinformatics.

Raw bacterial sequences were processed using the Mothur pipeline (version 1.39.4 Kozich et al., 2013; Schloss et al., 2009). Contigs from the paired end reads were assembled and quality trimmed using an average quality score, sequences were aligned to the Silva Database (version 123), and chimeric sequences were removed using the VSEARCH algorithm (Rognes et al., 2016). Next, we created operational taxonomic units (OTUs) by splitting sequences based on taxonomic class and then binning these OTUs by 97% sequence similarity. To estimate observed bacterial richness, we rarefied abundances to the minimum sequence depth of 13,000 reads. The original sequence data set had 12 million total sequences with 95,000 sequences per sample on average. After initial filtering there were 8.1 million sequences with 58,000 sequences on average per sample.

0.2 Statistical Analyses

0.2.1 Alpha Diversity

We used richness to explore alpha diversity. Zooplankton taxonomic order richness was evaluated using a generalized linear model with a quasi-Poisson error distribution; a quasi-Poisson distribution was used because data were under-dispersed. For all Poisson distributed models, we determined under/over dispersion of our error distribution by looking at the ratio of Pearson's residuals and the residual degrees of freedom (Bolker, 2008). We defined observed bacterial richness by the number of different OTUs in a community. Over-dispersed observed bacterial richness was modeled using a negative binomial error distribution. Analyses were conducted using the `lme4` (Bates et al., 2015) and `MASS` (Venables and Ripley, 2013) packages, respectively, in the R programming environment (R Core Team, 2016). Richness was modeled as a function of salinity, mixing treatment, time, and the interactions between time and salinity and salinity and mixing. We included a random effect of replicate over time to account for repeated measures. For analysis, parameter-specific p-values in a fully parameterized model were used to determine the significance of predictors. Additionally we include results for Shannon Diversity in the supplement section 9.3.3.

0.2.2 Testing for effects on community composition

Community structure of both bacterial and zooplankton communities, including visualizing community turnover over time and turnover between treatments, was evaluated using Principle Coordinates Analysis (PCoA) with Bray-Curtis dissimilarity. Variation explained by mixing, salinity, and time was analyzed using a permutational multivariate analysis of variance (PERMANOVA). These analyses were conducted in the R Statistical Programming Environment using the `Vegan 2.3.3` package (Oksanen et al., 2016). We used indicator species analysis to identify which bacterial taxa were most representative of each salinity treatment (Dufrêne and Legendre, 1997). We used the `Labdsv` package in R to run the analysis (Roberts, 2016). For the indicator species analysis, we only included bacterial taxa with a relative abundance greater than 0.05 when summed across all tanks.

0.3 Ecosystem Function

We assessed the effects of salinity, zooplankton, bacterioplankton, and species mixing on ecosystem functions using two different proxies for ecosystem function: decomposition amount and carbon mineralization.

0.3.1 Decomposition

Leaf litter from three plant species were used in each tank to represent different habitat types: *Spartina alterniflora* found in salt marshes, *Acer rubrum* found in freshwater wetlands, and *Phragmites australis* found in both fresh and saltwater wetlands. We wanted to represent the three natural habitats along our gradient to understand the potential for differential effects of mixing on ecosystems along this salinity gradient. Leaves were harvested and air-dried in late May. Each tank received standardized amounts of leaf litter (*Acer rubrum*: 4.00 g; stdev $\pm$0.01; *Spartina alterniflora*: 6.99 g stdev $\pm$0.03; *Phragmites australis*: 10.01 g stdev $\pm$0.03). *Phragmites australis* and *Acer rubrum* were housed in 24 inch mesh mariculture bags, while *Spartina alterniflora* was housed in windowscreen bags with smaller holes since *Spartina alterniflora* was not securely retained within the mesh mariculture bags. Leaf litter remained in the tanks for the duration of the experiment. On day 45, the bags were removed, air-dried, oven dried for 48 hours, and then weighed. Decomposition was quantified as the proportion of leaf dry weight loss (housed in decomposition bags) from the beginning to end of the experiment. To determine the relationship between proportional change in dry weight and the predictor variables; observed bacterial richness, zooplankton richness, salinity, mixing treatment, leaf litter type, and the interaction of salinity and leaf litter type, we used a beta regression `betareg` (Grün et al., 2012) (because the response is continuous and bounded between 0 to 1). We included the interaction between salinity and leaf litter type because we expected leaf litter would decompose differently in its native vs non-native abiotic conditions (e.g. *Acer rubrum* in freshwater verses the 13 psu water).

0.3.2 Carbon Mineralization

On the final sampling day (day 45), we measured the amount of CO_2 respired from the aquatic communities using a laboratory-based bottle assay. Wheaton bottles (125 mL) fitted with septa were filled with water samples (25 mL) from each mesocosm tank. The CO_2 concentration readings were determined using a LI-7000 Infrared Gas Analyzer (IRGA). On the day of collection (the final day of the experiment),

bottles were filled with 25 mL of mesocosm tank water, and the gas samples were collected and analyzed immediately using the IRGA to determine the baseline CO_2 concentration. A syringe was inserted into the septa and the headspace gas was mixed 3 times before pulling a sample and beginning analysis using the IRGA. This process was repeated on days 1, 3, and 7 following collection in order to determine CO_2 respiration rates over time. To determine the CO_2 production of each aquatic sample, the initial reading was subtracted from the analyzed day's reading. We made a calibration curve with a known concentration of CO_2 over a set of known volumes to get the calibration curve. Then, the unknown gas samples from our sample set was compared to the known. To calculate the CO_2 respiration rate, the concentration of CO_2 calculated from the calibration curve was converted to volume units (ppm) using the following equation:

$$Cm\left(CO_2^{-C}L^{-1}_{headspace}\right) = \frac{Cv \cdot M \cdot P}{R \cdot T}$$

where Cm is carbon mineralization Cv is the volume (ppm) of CO_2, M is the molecular weight of carbon, P is 1 atm, R is the universal gas constant (0.0820575 L atm K mole), and T is the incubation temperature in Kelvin. This value is then multiplied by the volume of the incubation chamber (L) and divided by the weight of water in the bottle used in the incubation to get μg CO_2-C gram^{-1} water. To get the rate, this number is divided by the number of days incubated to get μg CO_2-C gram water^{-1} day^{-1}.

We ran a linear model for carbon mineralization with zooplankton richness, microbial richness, mixing treatments, and salinity as predictors. In order to meet the assumptions of normality we log transformed the carbon mineralization data. There was a single replicate of a 9 psu tank that received the salt-only mixing treatment that was removed from the carbon mineralization analysis due to a missing data point.

RESULTS

0.4 Alpha Diversity

0.4.1 Zooplankton Community

Differences in zooplankton family richness was not well described by any of the predictors used in our analyses ($p > 0.05$, Figure 2), for model parameter estimates see supplementary Table S2. We find similar results with using Shannon Diversity (see Supplement section 9.3.3) For source tanks richness see supplementary Figure S1.

0.4.2 Bacterial Community

Observed species richness for the bacterial community increased as salinity increased (estimate (log scale) = 0.035, standard error (log scale) = 0.008, $z = 4.0, p = 4.97e-05$), and over time (estimate (log scale) = 0.008, standard error (log scale) = 0.002, $z = 4.07, p = 4.51e-05$) (Figure 3). However, the observed increase in richness over salinity reversed by the end of the experiment (Salinity:time: estimate (log scale) = -0.001, standard error (log scale) = 0.0003, $z = -4.2, p = 2.33e-05$) (Figure 3 panel 3). There were no clear differences as a result of the mixing treatments nor the interaction between salinity and mixing treatment ($p > 0.05$, see Supplementary Table S2 for coefficients). For source tanks richness, see supplementary Figure S2. We find similar results when using Shannon Diversity (see Supplement section 9.3.3).

0.5 Community Composition

0.5.1 Zooplankton Community

Zooplankton communities initially aggregated into two distinct groups: a freshwater group and a group consisting of all other salinities (Figure 4). However, by the final day, the low salinity (5 psu) ponds receiving the mixed species treatment, were more similar in composition to the freshwater community. The 9 and 13 psu salinity treatments remained distinct freshwater treatments with regards to their community structure. PCoA one explained 31% of variation and PCoA two explained 14%. PERMANOVA results suggest that salinity contributed most to variation in zooplankton communities ($R^2 = 0.23, p < 0.0001$). In contrast, the effects of the mixing treatment ($R^2 = 0.03, p < 0.0001$), time ($R^2 = 0.029, p < 0.0001$) and the interaction between time and salinity ($R^2 = 0.019, p < 0.0001$) on community variance were relatively more modest. While we observe an effect of the two and three way interactions between salinity, mixing, and time (all $p < 0.05$), the total amount of variation explained is quite small ($R^2 < 0.01$ in all cases).

0.5.2 Bacterial Community

A mantel test revealed that zooplankton and bacterial communities were correlated (mantel test: $r = 0.409, p = 0.001$). For the bacterial community the main effects of salinity and time account for the most variation (PERMANOVA, salinity: $R^2 = 0.115, p = 0.001$, time: $R^2 = 0.052, p < 0.001$), while the different mixing treatments did not have a clear differential effect on bacterial community structure (PERMANOVA, mixing: $R^2 = 0.007, p = 0.786$). The bacterial communities in the treatment tanks separated into salt vs. freshwater environments along the primary axis, which explained 17.3% of the variation among communities (Figure 5). Distinct bacterial communities grouped according to increasing salinity (5, 9, 13 psu) and separated along the secondary axis, which explained 7.3% of the variation in bacterial community composition. For information on the source tanks see the supplement Figure S4.

Indicator species analysis identified 225 bacterial taxa (OTUs) that were representative of salinity treatment (Supplementary Table S3). Associating these organisms with a salinity level can identify key taxa contributing to shifts in bacterial community structure. Due to the great diversity of bacterial communities, many bacterial sequences were unresolved to the 'species' (i.e. operationally defined at 97% sequence similarity) level but instead were classified according to the closest known sequence match. Proteobacteria (phylum) was the strongest indicator of salinity 0 (IndVal = 0.991) with Rhodospirillales (class) being the second highest indicator taxon (IndVal = 0.990). *Polynucleobacter* (genus) was the next highest indicator (IndVal = 0.983) of salinity 0 treatment. Unclassified Betaproteobacteria (class; IndVal = 0.936) represented the salinity 5 psu environments, followed by *Flavobacterium* (genus; IndVal = 0.889) and Alcaligenaceae (family; IndVal = 0.852). Bacterioplankton representing Salinity 9 and 13 psu environments were less clear. In the more saline treatments of salinity 9 and 13 psu, 5 of 8 OTUs were unclassified and were unresolved beyond the Bacterial domain (Supplementary Table S3). For salinity 9 psu, Planctomycetes had the third highest indicator value, and was only 1 of 4 classified OTUs indicative of the salinity 9 environment (phylum; IndVal = 0.804). At the most saline end, Alphaproteobacteria (class; IndVal = 0.928) and *Haliea* (genus; IndVal = 0.869) were representative of salinity 13 psu tanks.

0.6 Ecosystem Function

0.6.1 Decomposition

As bacterial richness increased the proportion of leaf mass remaining decreased, representing an increase in decomposition (estimate (log-odds scale) = -0.0007, standard error(log-odds scale) = 0.0002, $z = -3.04, p = 0.002$). As salinity increased, mass change decreased (estimate(log-odds scale) = 0.043, standard error(log-odds scales) = 0.018, $z = 2.38, p = 0.017$). The salt-only mixing treatment had lower overall decomposition (less mass lost) than the mixed mixing treatment (estimate (log-odds scale) = -0.19, standard error(log-odds scale) = 0.086, $z = -2.26, p = 0.02$). *Spartina alterniflora* loss less material than *Acer rubrum* leaves (estimate:log link 1.1, standard error:log link 0.18, $z = 5.9, p << .001$) (Figure 6). In contrast, we were unable to detect an affect of zooplankton richness or any of the interaction terms with leaf type (all $p > 0.05$). Overall the model accounted for large fraction of the variaton (pseudo $R^2 = 0.66$).

0.6.2 Carbon mineralization

Carbon mineralization increased with observed bacterial richness (estimate: 0.003, standard error: 0.001, $t = 2.78, p = 0.008$) (Figure 7). Overall model fit was moderate (adjusted $R^2 = 0.31$, $F - statistic = 4.4$ on 5 and 32 DF). We were unable to detect an effect of zooplankton richness, mixing treatment, or salinity on carbon mineralization (all $p > 0.5$).

DISCUSSION

Understanding how extreme environmental gradients and changing patterns of connectivity can influence community structure and ecosystem functions is becoming increasingly important as species assemblages shift to keep pace with climate change (Root et al., 2015). While the mixing of previously distinct communities from environmental change may have dire consequences for some species (Cahill et al., 2012), an increased capacity to maintain ecosystem functions in the face of those same environmental perturbations may also be expected due to introduction of redundant or tolerant species (e.g. Thompson and Shurin, 2012; de Boer et al., 2014; Mansour et al., 2018).

While we hypothesized that zooplankton diversity and observed bacterial richness would be highest in mid-salinities, we found a pattern more consistent with communities being deterministically limited by strong abiotic filters (Figures 3, 2) (Leibold et al., 2017). Indeed, we found a clear delineation between

freshwater and brackish water in our experiment (Figures 4,5) which suggests that abiotic filters are a strong and critical delineating force that regulates the composition of zoo- and bacterio- planktonic communities at the fresh-brackish water interface. While an increase in species richness may have been expected in low to mid salinity pools due to sampling from a more diverse species pool (mixed salinity), the affects of species mixing in this study was likely masked by the strong affects of salinity on community composition (Mouquet and Loreau, 2003). Additionally, our experimental protocol permitted salinities and biotic communities to stabilize which may have further buffered experimental pools against invasion (Supplementary Figure S5) Although a larger regional species pool (fresh and salt water species) might be expected to positively influence local diversity and function, fresh or salt water systems that have low levels of disturbance (Symons and Arnott, 2013, 2014) might be further resistant to invasion by new taxa as a result of strong priority effects and competitive dominance hierarchies (e.g. Geange and Stier, 2009). Interestingly, we only observed changes in community structure in the 5 psu zooplankton community. Specifically, this community became more similar to a freshwater community in the mixed-salinity mixing treatment (Figure 4). In contrast, the 13 psu or 0 psu salinity communities did not change over time, suggesting that new species are unable to easily colonize and establish in these highly filtered and stable environments.

While it is not surprising that abiotic filtering had strong effects on community structure in our study, this study expands our understanding about how coastal systems may be affected by changes in salinity and species mixing. We can expect to see shifts in both richness and community composition as salinity increases and these likely lead to changes in ecosystem functions. Indeed, in contrast to the responses of zooplankton we found that bacterial richness increased with salinity and this increase in species richness was correlated with rates of decomposition (Cotner and Biddanda, 2002; Kennedy and El-Sabaawi, 2017). Interestingly, this result lends support to the hypothesis that changes in biodiversity can affect ecosystem function (Mouquet and Loreau, 2003). But, this effect is even more interesting because it acts inversely to the effect of salinity; as salinity increased, decomposition decreased overall (Figure 6). That bacterial richness increased with increased salinity in our system suggests there is some small compensation by bacteria that is mitigating the effect of salinity, though the effect may be temporary because the increase in richness over salinity is reduced over time (Figure 3). The smaller difference in richness across salinities from the beginning to the end of the experiment (Figure 3: Day 0 and 45) is driven by larger increases in richness in the freshwater treatments compared to the other treatments. However, because the freshwater communities did not become more similar to the salt communities over time (Figure 5), it is unlikely that the increase in observed bacterial richness is due to mixing of species pools via the mixed treatments. This pattern also does not support horizontal gene transfer allowing for osmotic tolerance as a viable explanation. Instead it is likely that rare taxa become dominant in the intermediate salinities (Rocca et al., 2019). Another line of evidence supporting the idea that influxes from high saline environments can change ecosystem function is that the salt-only mixing treatments had lower decomposition than the other mixing scenarios. The correlation between bacterial richness and higher decomposition rates (Figure 6) is consistent with an earlier study by Stagg et al. (2017) that also found that salinity induced changes on microbial communities that directly changed decomposition rates. We also expected differential leaf litter decomposition based on the leaf litter's native habitat (e.g. *Acer rubrum* in freshwater), but there were no detectable differences in decomposition among different leaf litter tyoes as a function of salinity. Although *Spartina alterniflora* experienced lower decomposition rates overall across treatments.

Bacterial communities are known to be important in linking terrestrial, fresh and marine carbon cycles through transport, mineralization, and storage of carbon (Ardón et al., 2016). Consistent with this expectation we found a positive correlation between bacterial communities and carbon mineralization in this study. While zooplankton communities have also been directly linked to carbon mineralization (Jonsson et al., 2001) and carbon cycling (Six and Maier-Reimer, 1996), they may only account for a small proportion of total mineralization (Jonsson et al., 2001). In our study, we did not find a direct link between zooplankton richness and carbon mineralization, this is likely a consequence of small sample sizes and small expected direct effect of zooplankton on total carbon mineralization. However, zooplankton and bacterial communities were correlated, suggesting a link to the bacterial community, which are the ultimate actors for both decomposition and carbon mineralization. This highlights the need for future work on biodiversity-ecosystem function to clearly identify connections between the taxonomic or functional group and the ecosystem function of interest, because indirect links through food webs may not be readily detected.

This study provides an important step toward understanding how mixing of communities along an extreme salt gradient will affect local and regional patterns of diversity and ecosystem function. Future research should include perturbations such as variability in salinity within a single season, perhaps explicitly testing predictions made over changing heterogeneous landscapes as presented by Thompson and Gonzalez (2017). Additionally, our study further supports recent calls for experiments that explicitly use traits or taxonomic groups related to functions of interest to investigate links to ecosystem functions (e.g. Violle et al., 2007; Hébert et al., 2016a).

FIGURES AND TABLES

Table 1

Source	1st Dispersal	SD	2nd Dispersal	SD	3rd Dispersal	SD	4th Dispersal	SD	5th Dispersal	SD
13	1.2	1.7	2.35	2.5	1.8	3.3	1.1	1.5	1.6	2.2
0	3.4	7.1	7.24	9.9	4.1	6.1	11	18.8	4.6	6.9

Figure 1

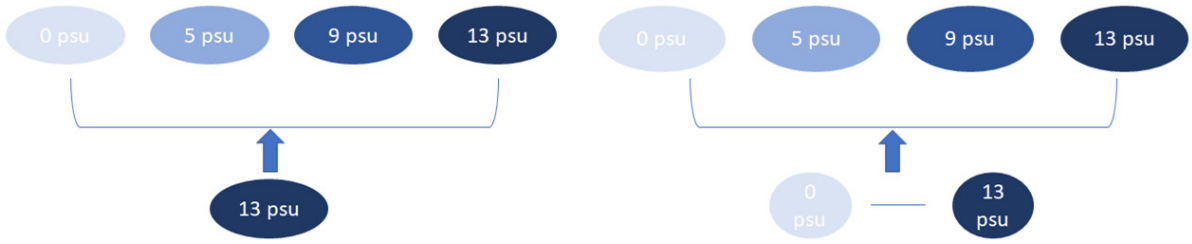


Figure 2

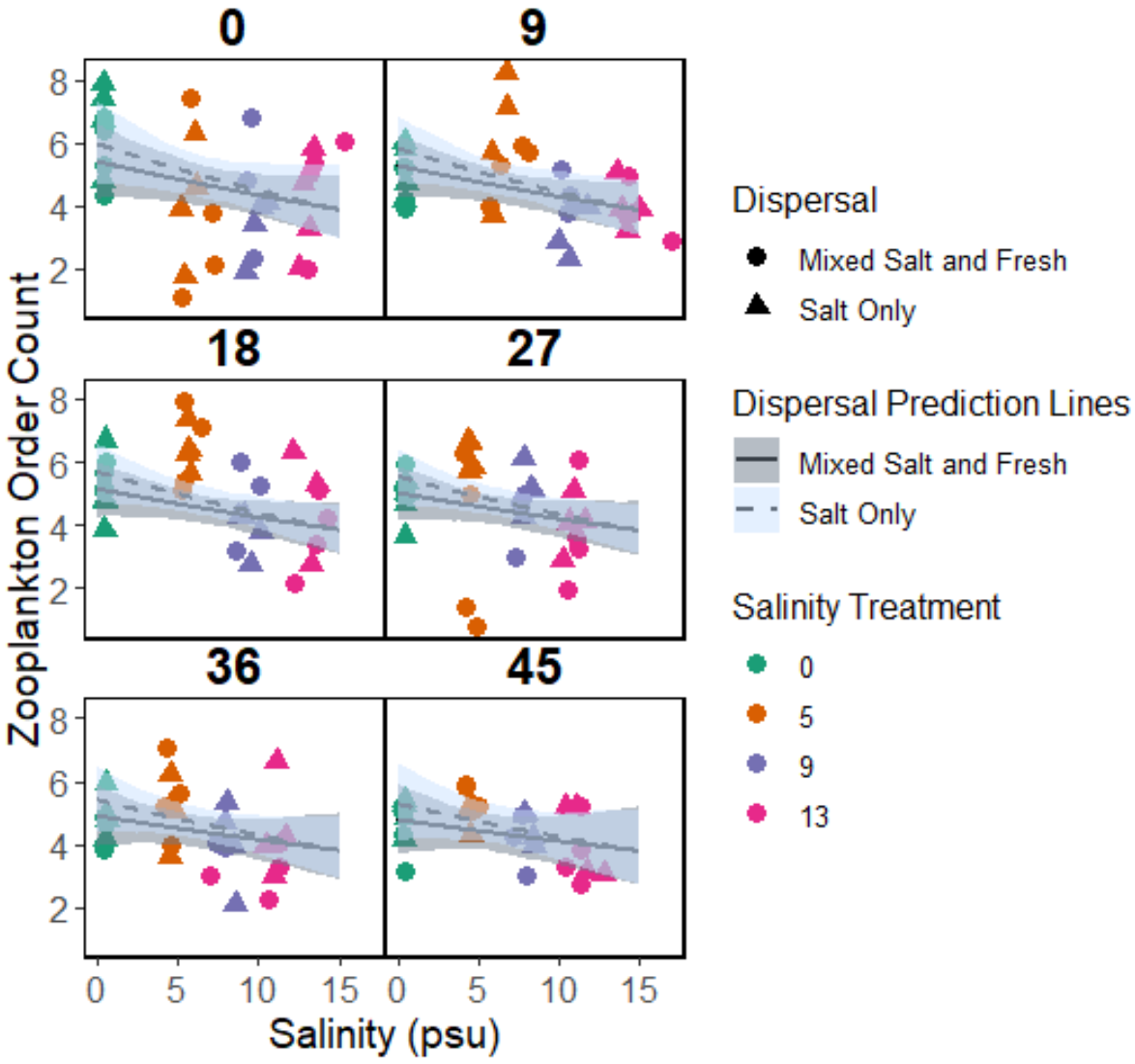

0
9
18
27
36
45
Zooplankton Order Count
Salinity (psu)
Dispersal
Mixed Salt and Fresh
Salt Only
Dispersal Prediction Lines
Mixed Salt and Fresh
Salt Only
Salinity Treatment
0
5
9
13

Figure 3

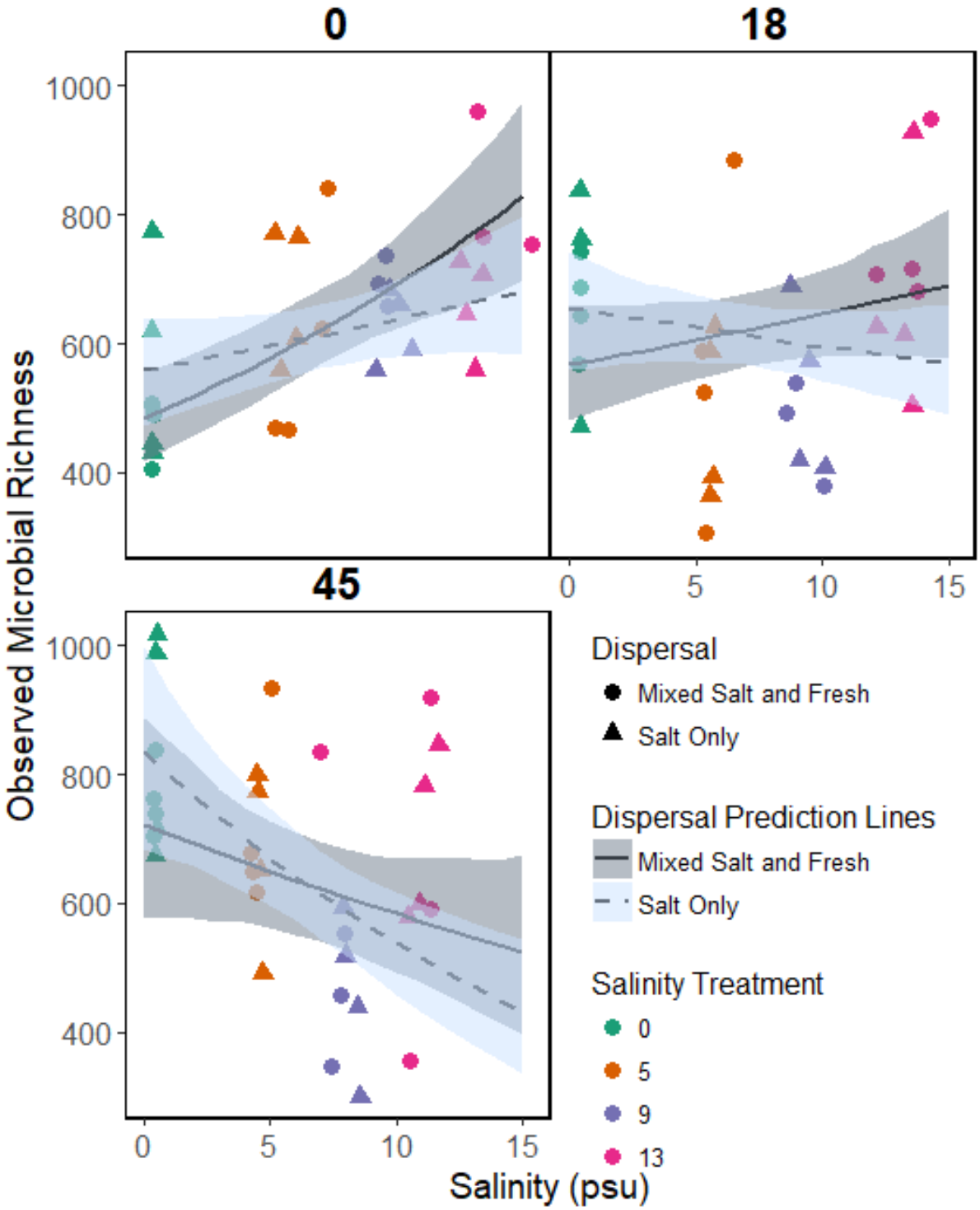

0
18
45
Observed Microbial Richness
1000
800
600
400
0
5
10
15
Salinity (psu)
Dispersal
Mixed Salt and Fresh
Salt Only
Dispersal Prediction Lines
Mixed Salt and Fresh
Salt Only
Salinity Treatment
0
5
9
13

Figure 4

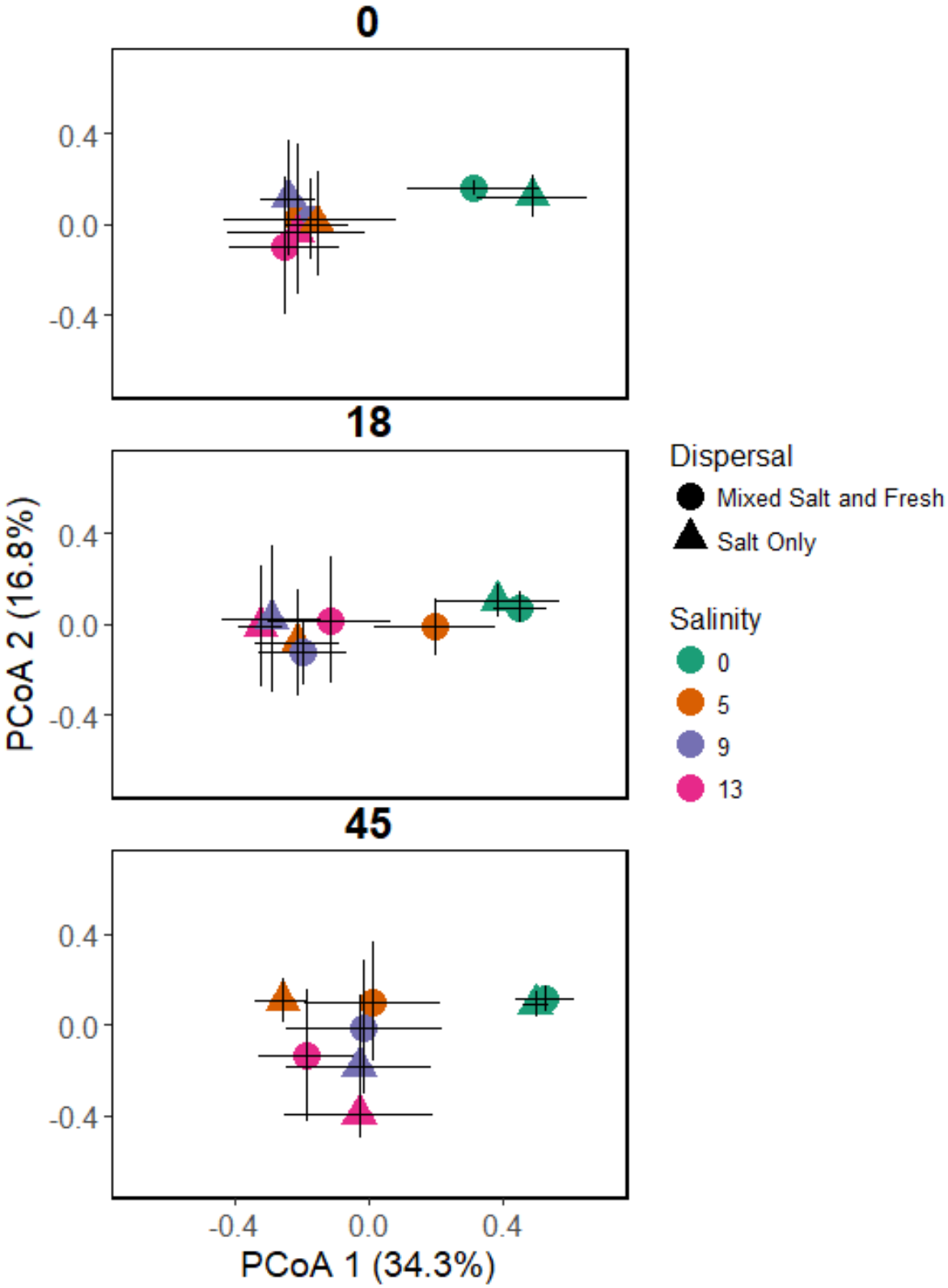

0
18
45
0.4
0.0
-0.4
PCoA 2 (16.8%)
PCoA 1 (34.3%)
Dispersal
Mixed Salt and Fresh
Salt Only
Salinity
0
5
9
13

Figure 5

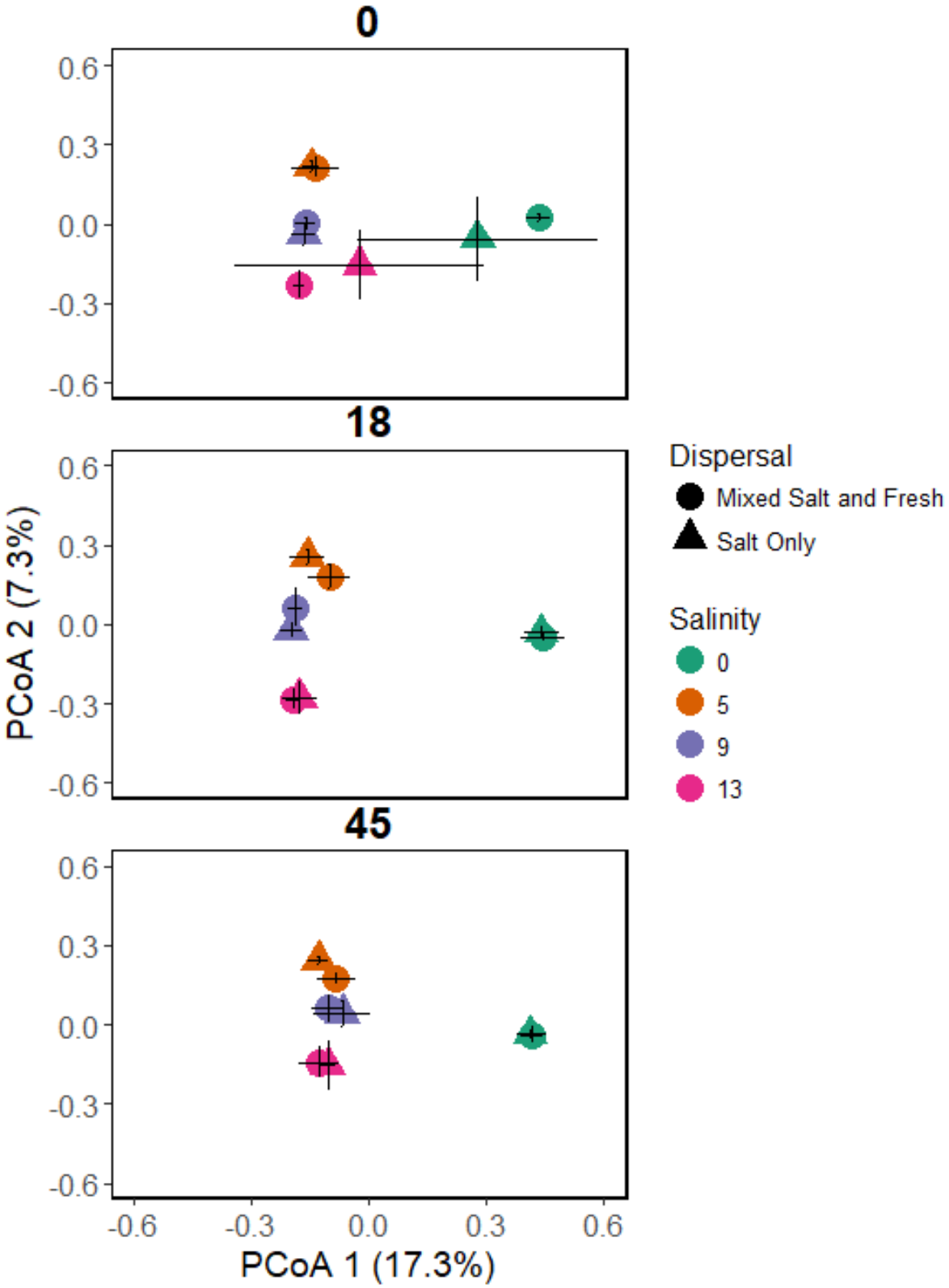

0
18
45
0.6
0.3
0.0
-0.3
-0.6
PCoA 2 (7.3%)
PCoA 1 (17.3%)
Dispersal
Mixed Salt and Fresh
Salt Only
Salinity
0
5
9
13

Figure 6

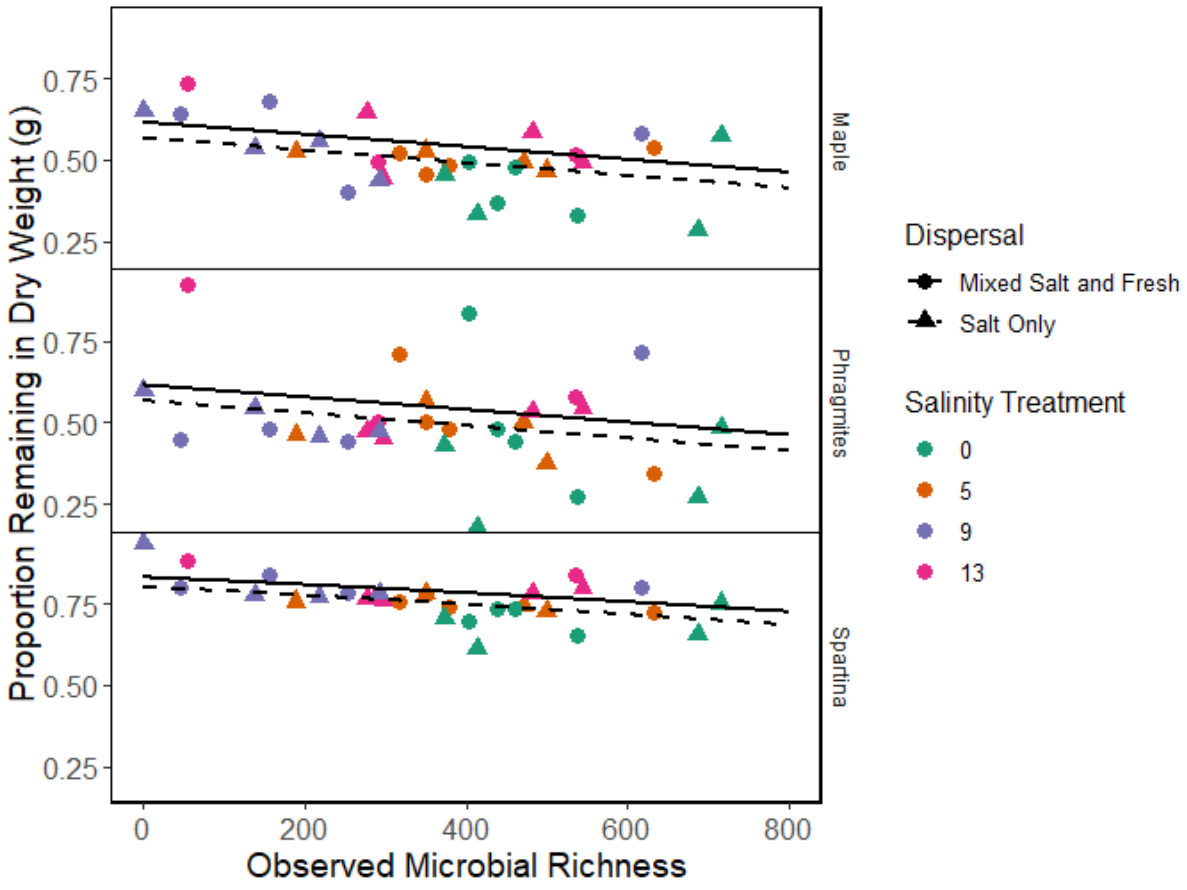
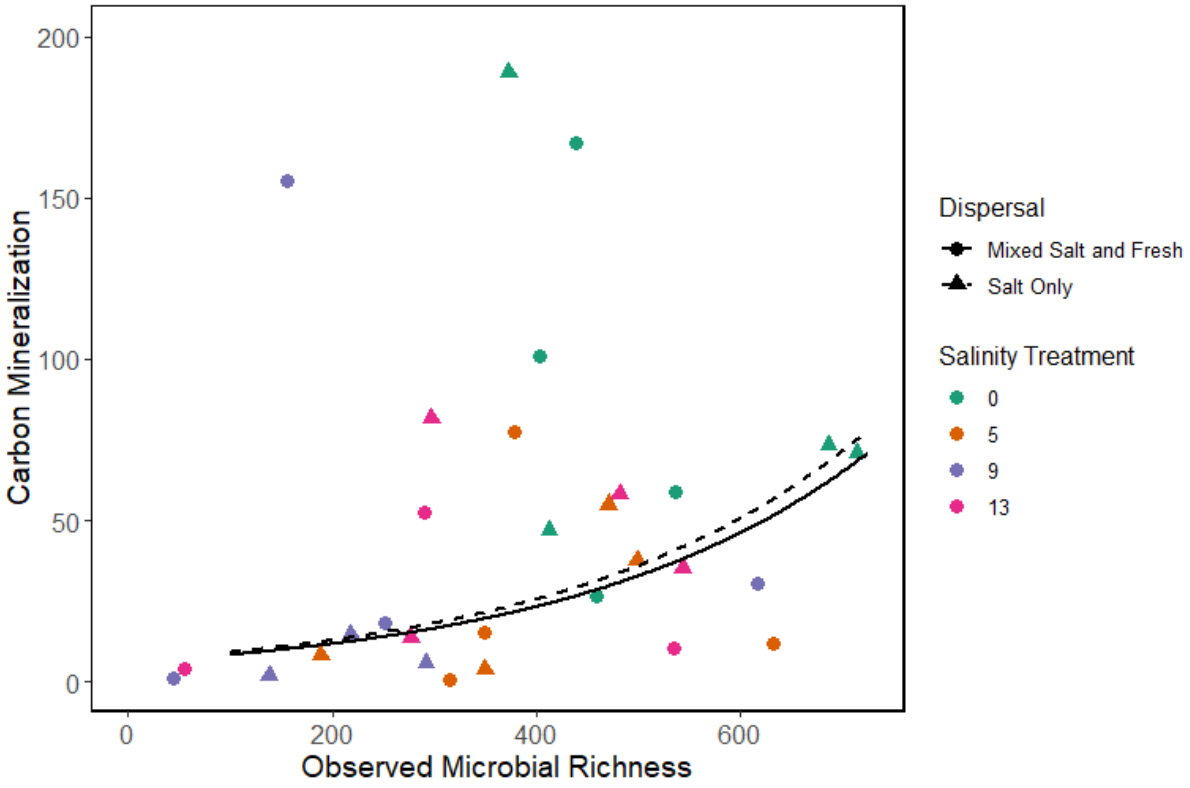

Proportion Remaining in Dry Weight (g)
0.75
0.50
0.25
Maple
Phragmites
Spartina
0
200
400
600
800
Observed Microbial Richness
Dispersal
Mixed Salt and Fresh
Salt Only
Salinity Treatment
0
5
9
13

Figure 7


Carbon Mineralization
200
150
100
50
0
0
200
400
600
Observed Microbial Richness
Dispersal
Mixed Salt and Fresh
Salt Only
Salinity Treatment
0
5
9
13

1 TABLE AND FIGURE LEGENDS

Table 1: Zooplankton abundance per liter for each dispersal source tank (13 psu or 0 psu). SD is standard deviation. No dispersal was exclusively freshwater, instead of half freshwater and half 13 psu.

Figure 1: This experimental design was replicated 4 times. Arrows indicate dispersal.

Figure 2: This figure shows zooplankton richness across salinities. Each panel represents zooplankton richness at a single sampling day. Points are colored by initial salinity treatment but are plotted on the x-axis using measured salinity on the sampling day. Shape indicates dispersal treatment: circles show salt and fresh water mixed dispersal and triangles show salt-only dispersal. Lines are model estimates: solid lines represent predictions for the mixed fresh and salt water treatment and dotted lines show predictions for the salt-only dispersal treatment. Envelopes represent 95% on the prediction. Predicted lines are transformed back to original scale.

Figure 3: Observed bacterial richness increased as salinity increased but this effect lessened over time, and reversed by the final time point. Each panel represents bacterial richness for a single sampling day for which bacteria were sequenced (0, 18, 45). All symbols and colors match Figure 2 and are on the original count scale.

Figure 4: These plots show zooplankton community aggregations at the different salinity treatments. Here zooplankton communities are represented by their centroid. Error bars show standard deviation. The top panel is the starting structure, the middle panel is day 18, and the bottom panel is the final day (day 45). Shapes indicate dispersal treatment: circles show mixed salt and freshwater, triangles show salt water only dispersal. Colors represent salinity treatment. Axis are PCoA 1 (x-axis) and PCoA 2 (y-axis).

Figure 5: Observed bacterial communities segregate into two groups, freshwater and salt communities. Points represent the centroid of the bacterial community structure. Error bars represent standard deviation. The top panel is the starting structure, the middle panel is day 18 and the bottom panel is the final day. All shapes and colors as Figure 4.

Figure 6: Decomposition increased as observed microbial richness increased. The y-axis shows the proportion of leaf litter remaining at the end of the experiment, the more leaf litter remaining the less decomposition occurred. From top to bottom the panels represent change in weight in *Acer rubrum*, *Phragmites australis*, and *Spartina alternaflora* respectively. Points are colored by salinity treatment and shaped by leaf litter type. Lines represent model predictions:solid lines represent predictions for the mixed fresh and salt water treatment and dotted lines show predictions for the salt-only dispersal treatment. Envelopes represent 95% on the prediction. Estimates were obtained using average zooplankton richness (4.5) and mean salinity (6).

Figure 7: Carbon mineralization increased with increasing observed bacterial richness. Points are colored by salinity treatment. Lines represent model predictions:solid lines represent predictions for the mixed fresh and salt water treatment and dotted lines show predictions for the salt-only dispersal treatment. Estimates were obtained using average zooplankton richness (4.5) and mean salinity (6).

2 CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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