

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. 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 bacterial and zooplankton 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 does the amount of decomposition. A phenomenological model suggests carbon mineralization may decrease at mid-salinities; this warrants future work into possible mechanisms for this apparent loss of function. 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. 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 bacterial and zooplankton 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 does the amount of decomposition. A phenomenological model suggests carbon mineralization may decrease at mid-salinities; this warrants future work into possible mechanisms for this apparent loss of function. 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, decomposition, carbon mineralization, 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 (Hall and Burns, 2002), community assembly processes (Jones and McMahon, 2009), and associated ecosystem functions (Schäfer et al., 2012; Wieski et al., 2010). Understanding how communities are altered following changes in habitat quality is critical for predicting the consequences of environmental change. 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 composition of species and thus the ecological

functions of the system, which can ultimately jeopardize the important socio-economic services that these ecosystems provide (de Groot et al., 2002; Kirwan and Megonigal, 2013).

Zooplankton and microbes are widely recognized for their essential role in biogeochemical processes that control flows of carbon, nitrogen and phosphorus (Hébert et al., 2016b) in wetland systems (Schimel and Schaeffer, 2012; Herbert et al., 2015). Salinization of wetlands might therefore be expected to have particularly strong effects because osmotic tolerance is not easily gained or lost in most taxa. Indeed, salinity is recognized as a primary determinant of both zooplankton (Bate et al., 2002; Kimmel, 2011; Breckenridge et al., 2015) and bacterial communities.

Despite the likely widespread dispersal of most microorganisms, a large review of fresh and marine species found little overlap between habitats, reinforcing that salinity acts as a large abiotic barrier for most microorganisms (Logares et al., 2009). Microbial functional groups also change along a salinity gradient, for example, the relative abundance of microbes that perform different core metabolic processes (e.g. glycolysis and osmolyte transport Dupont et al. (2014); ATP dependent amino acid transfer is greater in marine habitats and greater phosphorus uptake in freshwater habitats (Eiler et al., 2014)). Experimentally, Coci et al. (2005) showed that increasing salinity of freshwater habitats leads to shifts in which groups of ammonia-oxidising bacteria are present, likely changing key aspects of nutrient cycling. This finding is similar to those of Langenheder et al. (2003), who showed that changes in the composition of bacterial communities across salinities led to changes in carbon respiration, growth rate and growth efficiency, as well as decreases in overall abundance in salinities above that of their natural habitat. These lines of evidence suggest that increases in salinity in freshwater ponds could lead to decreases in the abundance, richness and functional processes of bacterial communities that are critical in all ecosystems.

Like bacterial response to salinity, zooplankton abundance and diversity also decreases as salinity increases (Nielsen et al., 2008; Helenius et al., 2017; Schallenberg et al., 2003). Additionally, changes in zooplankton diversity and abundance are also associated with changes in function. For example, grazing is lower in more estuarine habitats (Zervoudaki et al., 2009), and different taxa differentially affect respiration and nutrient cycling (Makarewicz and Likens, 1979). Further, changes in zooplankton communities can change their migration patterns and downstream functions such as carbon export (Isla et al., 2015).

Coastal wetlands are economically and ecologically important ecosystems (Kirwan and Megonigal, 2013). Coastal wetlands are also highly impacted by humans, making them even more vulnerable to climate change than other less intruded habitats (Kirwan and Megonigal, 2013). Current coastal wetlands have been formed from historical storm regimes and hydrological dynamics. Climate change alters the frequency and intensity of coastal storms which will cause coastal wetlands to experience rapid and large changes in water chemistry, hydrology, biotic structure and nutrient cycling (Michener et al., 1997). However, the potential effects of SLR and salinity changes on important ecosystem functions such as litter decomposition and nutrient mobilization are not well understood. Rates of decomposition may differ as a function of salinity, the type of litter, micro- and macro-fauna present in the community, and the time since decomposition began. For instance, the home field advantage hypothesis (Hunt et al., 1988; Gholz et al., 2000) suggests that decomposition rate is most efficient when leaf litter is being decomposed in its natural habitat. That is, terrestrial species (e.g. *Acer sp.*) will decompose best in freshwater, while marine species (e.g. *Fucus sp.*) will decompose faster in marine systems than in estuarine or freshwater habitats. The home-field advantage is supported by Franzitta et al. (2015) and partially by Lettice et al. (2011) who found this was true for a terrestrial species (*Alder sp.*) but not for a marine species (*Fucus sp.*). Indeed, evidence is mixed for the home-field advantage hypothesis. Long term (60 day) decomposition seems to differ little between salinities for *Phragmites australis*, but shorter term (30 day) decomposition is faster in more natural environments (Quintino et al., 2009). Alternatively Reice and Herbst (1982) found that *Phragmites* decomposed more slowly as salinity increased, while Lopes et al. (2011) found that *Phragmites* decomposed most quickly in mid-salinities (*Phragmites* natural environment). Alternatively Connolly et al. (2014) were unable to detect an effect of salinity on decomposition rate and Stagg et al. (2018) suggest that rather than salinity initial nitrogen and lignin content are better indicators of decomposition rate. To gain more clarity on decomposition changes across salinities we test the home field advantage hypothesis by measuring decomposition of three species that have different natural habitats over 6 weeks along a salinity gradient. Additionally, we hypothesize that differences in decomposition will be correlated to the associated microbial and zooplankton communities.

Estuarine wetlands rapidly sequester carbon, accounting for approximately 30% of carbon sequestra-

tion in the lower USA (Bridgham et al., 2006), and hold onto carbon storage longer than other ecosystems (McLeod et al., 2011). Yet coastal wetlands are being lost rapidly (Hopkinson et al., 2012) and are vulnerable to biogeochemical changes due to SLR. Though carbon budgets in wetlands are complicated because wetlands release methane gas in addition to sequestering carbon, wetlands are a net carbon sink in the long term (Mitsch et al., 2013). Understanding how carbon budgets may change as wetlands change is critical for understanding and mitigating impacts of climate change. Most evidence suggests that higher salinity soils have lower levels of carbon mineralization and methane gas release (Setia et al., 2011; Weston et al., 2006; Al-Busaidi et al., 2014; Poffenbarger et al., 2011). However, Chambers et al. (2011) found short-term salt water intrusion increased carbon mineralization and carbon mineralization was generally higher in sea-water than freshwater. To help further enhance our understanding of how SLR and salinization might affect the carbon cycle in the face of ongoing impacts from climate change, we examine how salinity and the underlying zooplankton and bacterial communities correlate with carbon mineralization.

Specifically, this study focuses on the impacts of salinization on coastal shallow freshwater wetlands to advance our understanding of environmental change on species diversity, community structure and associated ecosystem functions. Specifically, we examined how overwash events along with mixing of freshwater and saltwater taxa affects the diversity and composition of bacteria and zooplankton communities and downstream ecosystem functions. To test the effects of salinization on diversity and ecosystem function we performed a semi-natural mesocosm experiment in which we simulated wetlands with different salinity. We 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 microbes, and a second that was a sample of salt-only plankton and microbe communities. We quantified changes in zooplankton and bacteria 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). We find that salinity is a key determinant of both bacterial and zooplankton communities. Additionally, decomposition decreased with higher salinity and increased with greater bacterial diversity. Carbon mineralization results were more interesting, with different models suggesting different underlying relationships. These results suggest that ecosystem functions in coastal areas are likely to change as wetlands become more saline and changing bacterial communities are likely playing a significant role in determining the outcome.

METHODS

Experimental Set-Up

Our experiment took place in North Carolina, USA. North Carolina is a good place for studying the affects of salinity because wetlands there are particularly vulnerable to changes due to higher SLR than other regions on the US Atlantic coast (Kemp et al., 2009; Kopp et al., 2015).

We created 39 experimental ponds using 567 L stock watering tanks. Tanks were filled with 378 L of water from a hose; we recognize that by not sterilizing the water it is possible that bacteria were introduced in such a way that bacterial richness was disproportionately increased in freshwater communities. 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)(Albecker and McCoy, 2019). Tanks were randomly assigned to receive one of the four salinity treatments (0, 5, 9 and 13 psu), and each tank was initially seeded with zooplankton and bacteria from a natural pond with matching salinity (e.g. at 5 psu treatment was seeded with a community from a natural pond at 5 psu) located along the inner and outer banks of North Carolina on May 3, 2015 (supplementary Table S1). (N.B. samples from two different ponds were mixed for the highest salinity treatment).

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 one experimental mesocosm.

To collect our initial zooplankton and bacteria from coastal ponds, we sampled along a single 100 m transect at each pond taking twenty 1 L samples of water from within a foot of the surface (most ponds were less than 2 feet deep at the time of sampling). We strained each sample them through a 62.5 μ m 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. Peat moss and sand were purchased. 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; due to natural dynamics in these tanks the actual abundances varied for each mixing event (Table 1). 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 this is long enough for most zooplankton species 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. After mixing we sampled from the center of the water column; we don't expect our tanks to stratified due to their depth (< 0.6 m) (Snucins and John, 2000). Samples were condensed through a 62.5 μ m filter into 25 mL containers. Zooplankton from each tank at the time of sampling were preserved in 10% formalin.

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 some known functional redundancy within zooplankton orders and family level taxonomic 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 by scooping a bottle several times in the tank until we had 1 L. Each 1 L bottle of water was homogenized and 200 mL of the water sample was concentrated onto 0.22 μ m 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 °C until molecular analyses was completed.

0.1.1 Bacterial Community Sequencing

To examine shifts in bacterial community composition and diversity, bacteria 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 from 0.22 μ m supor filters from each mesocosm using the PowerWater DNA Isolation Kit (MO BIO Laboratories, Inc CA). We used this DNA as a template in PCR reactions. To characterize particle and free-living organism 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). This primer set targets Bacteria and Archaea. For this study, we focused on the bacteria. 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 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) (Quast et al., 2012), 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 clustering 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 evaluated 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 which allows the intercept and slope of each replicate to vary which takes into account the grouping of repeated measures within each tank. 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. The PCoA graphs (Figures 2, 3) are generated based on a single ordination. 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, bacteria, and species mixing on ecosystem functions using two different proxies for ecosystem function: decomposition amount and carbon mineralization of the final communities.

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, 2015. Each tank received standardized amounts of leaf litter (*Acer rubrum*: 4.00 g; stdev ± 0.01 ; *Spartina alterniflora*: 6.99 g stdev ± 0.03 ; *Phragmites australis*: 10.01 g stdev ± 0.03). *Phragmites australis* and *Acer rubrum* were housed in 24 inch mesh mariculture bags, while *Spartina alterniflora* was housed in window screen 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 CO₂ 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 CO₂ concentration readings were determined using an 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 CO₂ 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 CO₂ respiration rates over time. To determine the CO₂ 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 CO₂ 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 sample. To calculate the CO₂ respiration rate, the concentration of CO₂ calculated from the calibration curve was converted to volume units (ppm) using the following equation:

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

where Cm is carbon mineralization, Cv is the volume (ppm) of CO₂, 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 CO₂-C gram⁻¹ water. To get the rate, this number is divided by the number of days incubated to get μg CO₂-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.

After seeing the data we ran an *a posteriori* exploratory analysis where we used the same model as above but included a squared (quadratic) term for salinity to examine evidence of an intermediate minimum. We used AIC to compare the two models.

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 (all $p > 0.05$, Figure 4); for model parameter estimates see supplementary Table S2. We find similar results 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 5). 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 5 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 2). 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 from 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 $p < 0.05$, except the interaction of dispersal and salinity $p > 0.05$), the total amount of variation explained is quite small ($R^2 < 0.01$ in all cases). For source tanks alone and source tanks in relation to all other tanks see Supplement Figures S3 and S4.

0.5.2 Bacterial Community

A mantel test revealed that zooplankton and bacterial communities were positively 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: $R^2 = 0.052, p < 0.001$). 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 3). 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 Figures S5 and S6.

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 zero salinity (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 the zero salinity 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). Bacteria representing Salinity 9 and 13 psu environments were less clear. In the more saline treatments, 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 a large fraction of the variation (pseudo $R^2 = 0.66$).

0.6.2 Carbon mineralization

In our first *a priori* model we found that 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 $p > 0.5$).

However, in our exploratory model we found that carbon mineralization decreased in the mid-salinity treatments (Figure 8) (poly(salinity,2): estimate: 8.2, standard error: 1.4, $t = 5.9$, $p < 0.001$) and that carbon mineralization increased with zooplankton richness (estimate: 0.5, standard error: 0.16, $t = 3.1$, $p = 0.003$). This model explained more variation than our *a priori* model (adjusted $R^2 = 0.4$, $F - statistic = 14.4$ on 5 and 84 DF). We were unable to detect an effect of microbial richness, mixing treatment, or the main effect of salinity on carbon mineralization (all $p > 0.5$). Based on AIC the second model, with the squared salinity term, has more support (Delta AIC = 30).

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).

Our results for zooplankton diversity and observed microbial richness patterns are consistent with communities being determined by strong abiotic filters (Figures 5, 4) (Leibold et al., 2017). Indeed, we found a clear delineation between freshwater and brackish water in our experiment (Figures 2, 3) 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 effect of species mixing in this study was likely masked by the strong effect 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 S7). 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 might be further resistant to invasion by new taxa (Symons and Arnott, 2013, 2014) because 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 2). 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.

Different microbial taxa were representative of each of the four different salinity levels, supporting previous work that suggests salinity tolerance is a specialized trait that determines bacterial community composition (Martiny et al., 2015). For salinity 0, the top three taxa determined to be representative were OTUs that were matched to taxa at the level of Proteobacteria (phylum), Rhodospirillales (order), Polynucleobacter (genus), and Spartobacteria (class). Taxa that are assigned to the phylum level were unable to be resolved to finer taxonomic resolutions; however, all OTUs were clustered into a group if sequences were 97% similar. The Proteobacteria phylum is considered the most diverse phylum of bacteria both in terms of taxonomic and functional diversity. The order Rhodospirillales is within the Proteobacteria phylum and is most often found in fresh water habitats. Many species within this order contain photosynthetic pigments and function as photoheterotrophs. Polynucleobacter is a cosmopolitan bacterial genus; sequences and strains of this genus are globally found in a wide variety of freshwater ecosystems. Consistent with our experiment, each of these three bacterial taxa are typical of freshwater environments. The top three taxa in the 5 salinity environment were Betaproteobacteria (class), Flavobacterium (genus), and Alcaligenaceae (family). The Betaproteobacteria class consists of aerobic or facultative bacteria that are the most common within Proteobacteria (phylum) found in freshwater lakes. The genus Flavobacterium is widely distributed in soil and freshwater habitats and the family

Alcaligenaceae are found in a wide variety of environments, including soil, water, and mammals (Newton et al., 2011). The taxa found in salinity 5 are not characterized as existing in any one specific salinity. This may be attributed to the bacteria in the salinity 5 tanks being able to persist through the salinity change from fresh to salinity 5. For the salinity 9 environment, 3 of the top 4 OTUs remained unclassified up to the phylum level, while Phycisphaera (genus) was identified as an indicator taxa. The presence of this phylum in salinity 9 tanks represents a slight shift in community dominance from fresh to salt-tolerant taxa; however, the other top 3 indicator taxa of salinity 9 tanks were unclassified, so conclusions regarding key bacterial taxa involved remain elusive. Salinity 13 also had unclassified taxa identified in the top five indicators species; there were 2 classified and 2 unclassified taxa. The 2 classified taxa were Haliea (genus) and Alphaproteobacteria (class). Genus Haliea is a Gammaproteobacteria (class) with species isolated from aquatic marine environments. Class Alphaproteobacteria are oligotrophs; organisms in this class can live in environments with low nutrients (Newton et al., 2011).

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. The observed changes in richness across salinity, in part, led to changes in ecosystem function. 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 5). The smaller difference in richness across salinities from the beginning to the end of the experiment (Figure 5: 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 3), it is unlikely that the increase in observed bacterial richness is due to mixing of species pools via the mixed treatments. Instead it is likely that rare taxa, which we didn't detect at the beginning, become dominant in intermediate salinities (Rocca et al., 2019) and that there was higher immigration from natural sources to freshwater treatments than other treatments. We, in fact, do expect passive dispersal via wind (Nemergut et al., 2013). 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. 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 types as a function of salinity. In fact there is very mixed evidence for the home-field advantage hypothesis generally, so it comes as no surprise that we also were unable to find conclusive results. Instead, the relationship between habitat and decomposition may be better described along a continuum of decomposer-litter interactions (Freschet et al., 2012).

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 our *a priori* model. 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 first model 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, in our exploratory model, when we considered a quadratic term, we are able to detect a positive relationship with zooplankton richness and carbon mineralization. We also see a decrease in carbon mineralization at mid-salinity compared to either extreme in our exploratory model. This result leaves room for more specific experiments to determine if this is repeatable and what mechanisms could cause a unimodal response. This highlights the need for future work on biodiversity-ecosystem function to clearly identify mechanisms for the relationships and possibly the importance of exploring multiple trophic levels.

1 CONCLUSIONS

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). 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.

FIGURES AND TABLES

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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

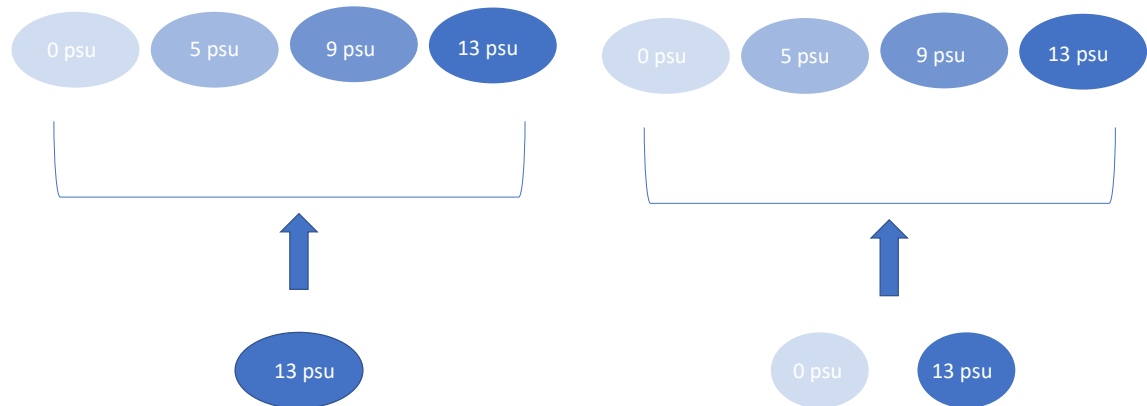


Figure 2

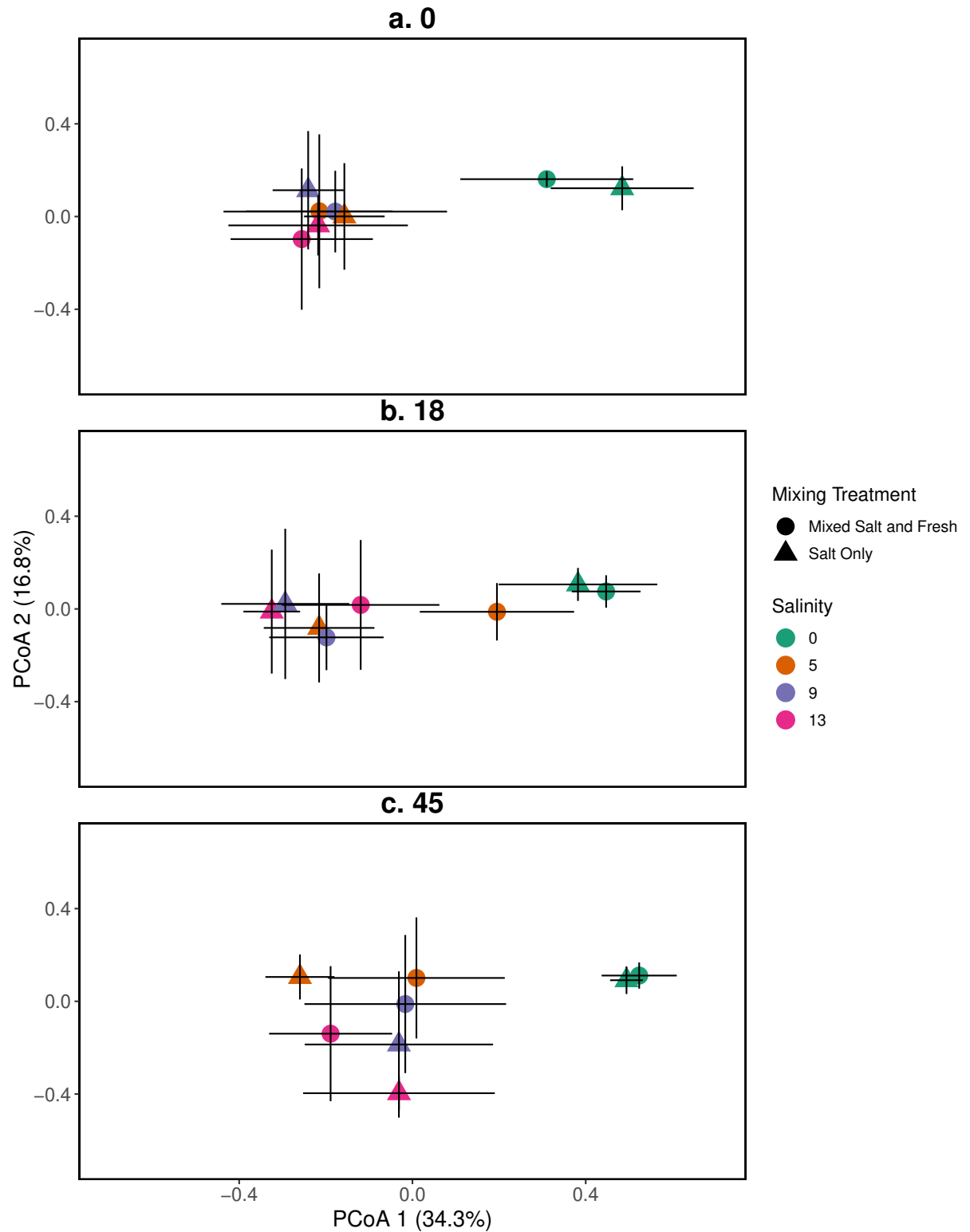


Figure 3

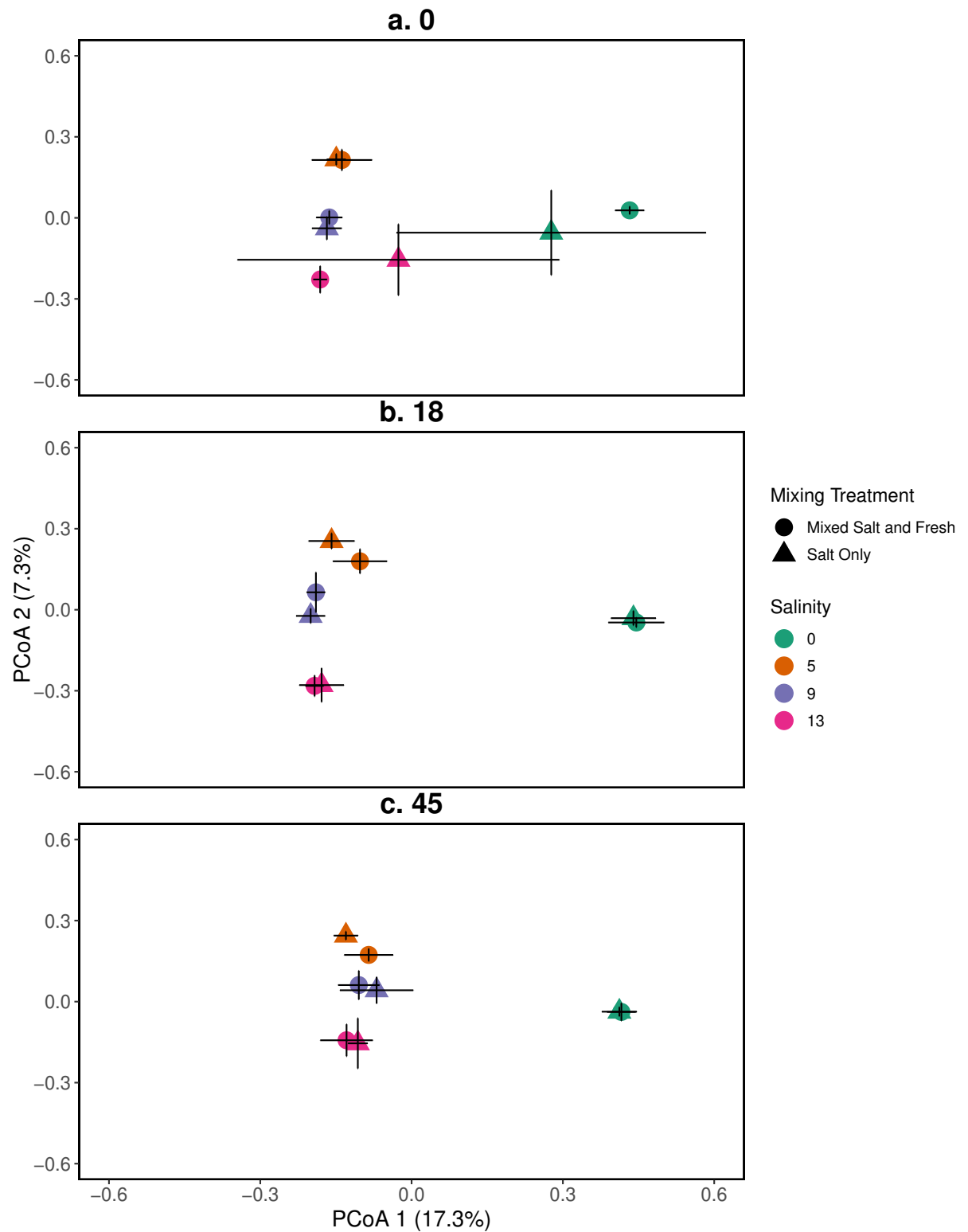


Figure 4

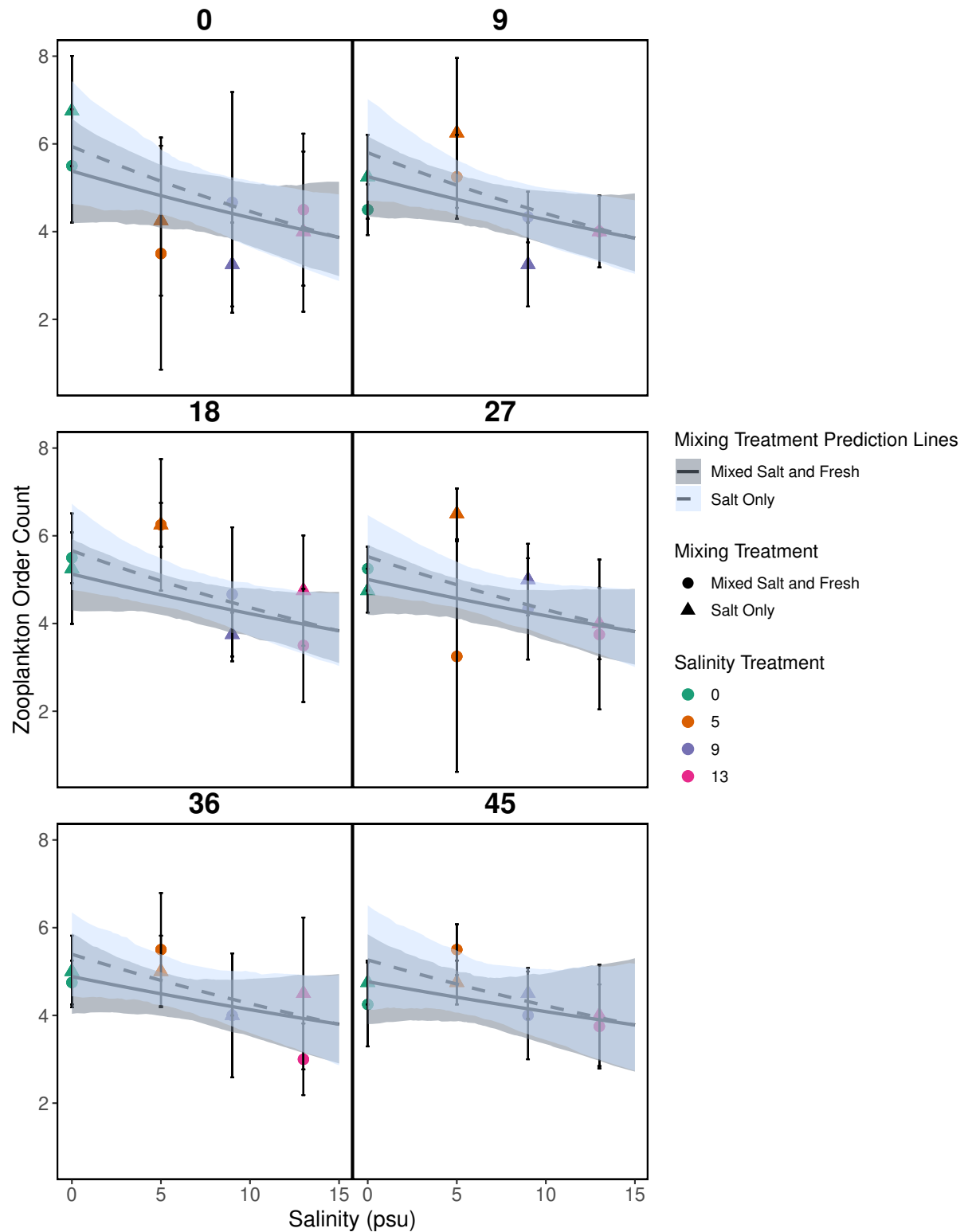


Figure 5

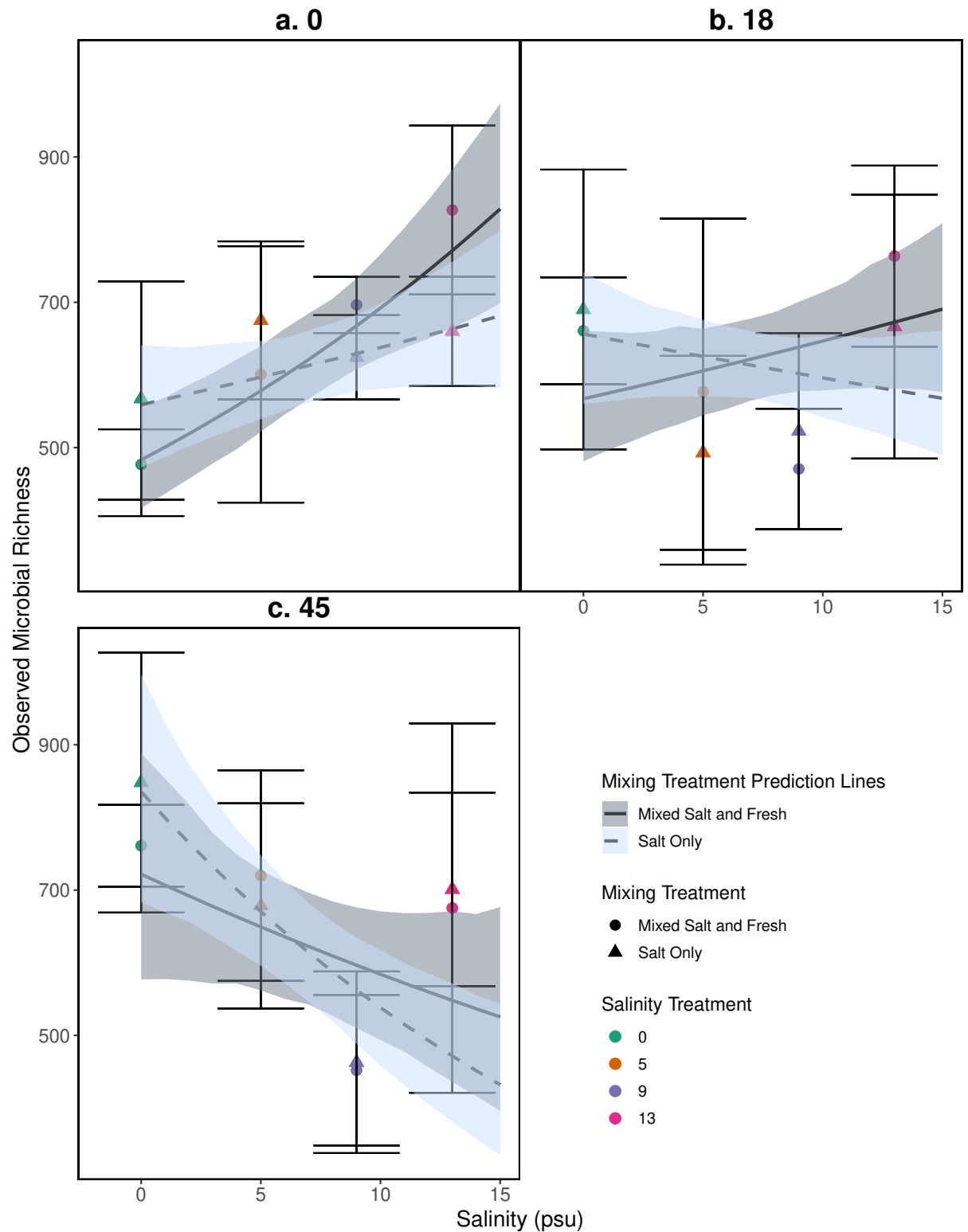


Figure 6

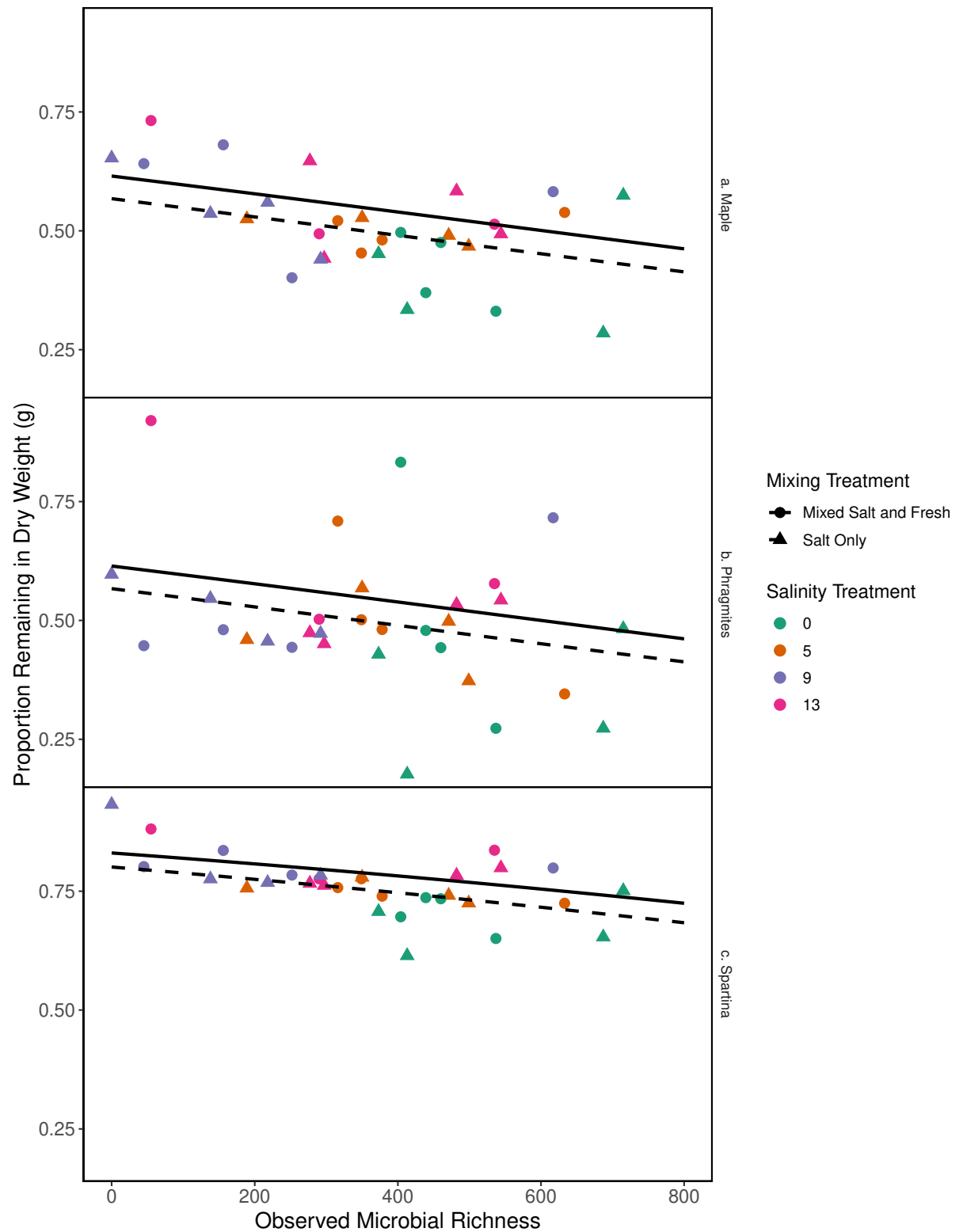


Figure 7

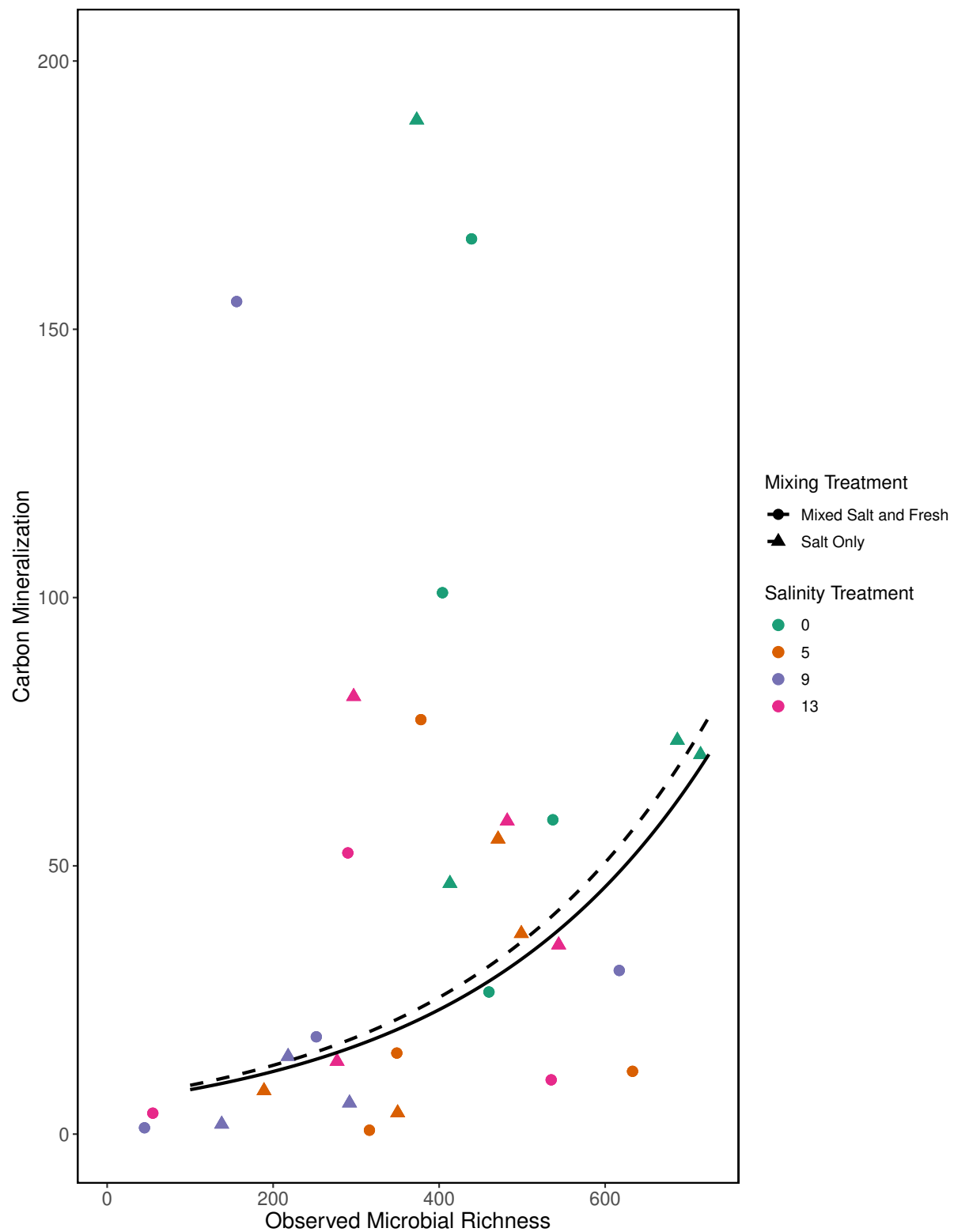
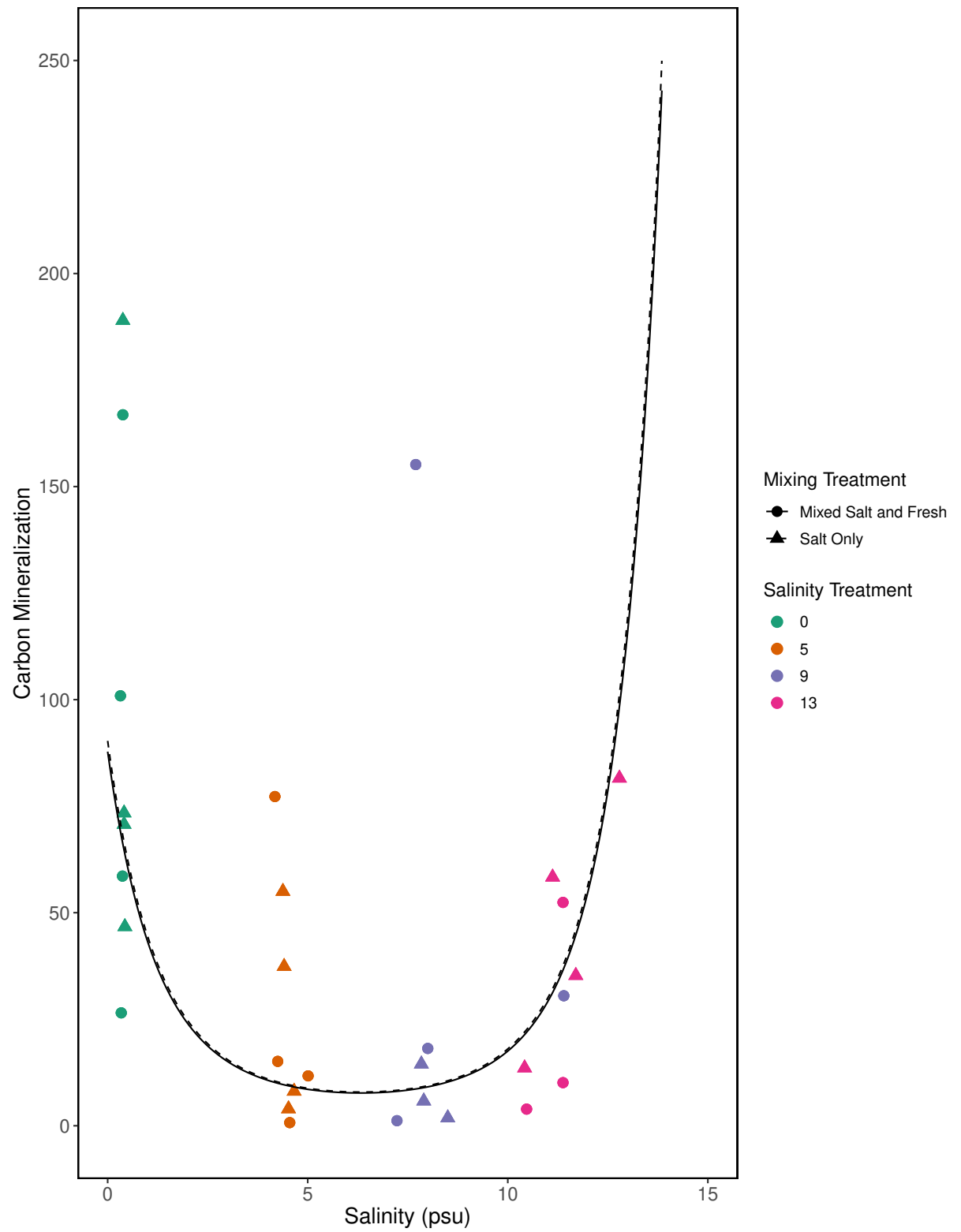


Figure 8



2 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 mixing treatment was exclusively freshwater, instead of half freshwater and half 13 psu.

Figure 1: This experimental design was replicated 4 times, except for 5 psu with mixed dispersal which was replicated 3 times. Arrows indicate mixing treatment.

Figure 4: This figure shows zooplankton richness across salinities. Each panel represents zooplankton richness at a single sampling day. Points are mean richness, color indicates the salinity treatment, error bars are standard deviation. Shape indicates mixing treatment: circles show salt and fresh water community mixing and triangles show salt-only mixing. 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 mixing treatment. Envelopes represent 95% on the prediction. Predicted lines are transformed back to original scale.

Figure 5: 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 4 and are on the original count scale.

Figure 2: 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 mixing. Colors represent salinity treatment. Axis are PCoA 1 (x-axis) and PCoA 2 (y-axis).

Figure 3: 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 2.

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 alterniflora* 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 mixing 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 mixing treatment. Estimates were obtained using average zooplankton richness (4.5) and mean salinity (6).

Figure 8: In an exploratory analysis carbon mineralization decreased at mid-salinities. Points are colored by salinity treatments and shapes are mixing treatment. The graph represents both mean zooplankton richness (4.5) and mean observed bacterial richness (380.4).

3 CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

- Al-Busaidi, K. T., Buerkert, A., and Joergensen, R. G. (2014). Carbon and nitrogen mineralization at different salinity levels in omani low organic matter soils. *Journal of Arid Environments*, 100:106–110.
- Albecker, M. A. and McCoy, M. W. (2017). Adaptive responses to salinity stress across multiple life stages in anuran amphibians. *Frontiers in zoology*, 14(1):40.
- Albecker, M. A. and McCoy, M. W. (2019). Local adaptation for enhanced salt tolerance reduces non-adaptive plasticity caused by osmotic stress. *Evolution*.
- Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to v4 region ssu rRNA 806r gene primer greatly increases detection of sar11 bacterioplankton. *Aquatic Microbial Ecology*, 75(2):129–137.
- Ardón, M., Helton, A. M., and Bernhardt, E. S. (2016). Drought and saltwater incursion synergistically reduce dissolved organic carbon export from coastal freshwater wetlands. *Biogeochemistry*, 127(2–3):411–426.
- Barnett, A. J., Finlay, K., and Beisner, B. E. (2007). Functional diversity of crustacean zooplankton communities: towards a trait-based classification. *Freshwater Biology*, 52(5):796–813.
- Bate, G. C., Whitfield, A. K., Adams, J. B., Huizinga, P., and Wooldridge, T. H. (2002). The importance of the river-estuary interface (REI) zone in estuaries. *Water SA*, 28(3):271–280.
- Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67(1):1–48.
- Bolker, B. M. (2008). *Ecological models and data in R*. Princeton University Press.
- Breckenridge, J., Bollens, S., Rollwagen-Bollens, G., and Roegner, G. C. (2015). Plankton assemblage variability in a river-dominated temperate estuary during late spring (high-flow) and late summer (low-flow) periods. *Estuaries and coasts*, 38(1):93–103.
- Bridgman, S. D., Megonigal, J. P., Keller, J. K., Bliss, N. B., and Trettin, C. (2006). The carbon balance of north american wetlands. *Wetlands*, 26(4):889–916.
- Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Ryu, H. Y., Sbeglia, G. C., Spagnolo, F., Waldron, J. B., Warsi, O., et al. (2012). How does climate change cause extinction? *Proc. R. Soc. B*, page rspb20121890.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., et al. (2012). Ultra-high-throughput microbial community analysis on the illumina hiseq and miseq platforms. *The ISME journal*, 6(8):1621.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., and Knight, R. (2011). Global patterns of 16s rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, 108(Supplement 1):4516–4522.
- Chambers, L. G., Reddy, K. R., and Osborne, T. Z. (2011). Short-term response of carbon cycling to salinity pulses in a freshwater wetland. *Soil Science Society of America Journal*, 75(5):2000–2007.
- Coci, M., Riechmann, D., Bodelier, P. L., Stefani, S., Zwart, G., and Laanbroek, H. J. (2005). Effect of salinity on temporal and spatial dynamics of ammonia-oxidising bacteria from intertidal freshwater sediment. *FEMS microbiology ecology*, 53(3):359–368.
- Connolly, C. T., Sobczak, W. V., and Findlay, S. E. (2014). Salinity effects on phragmites decomposition dynamics among the hudson river’s freshwater tidal wetlands. *Wetlands*, 34(3):575–582.
- Cotner, J. B. and Biddanda, B. A. (2002). Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. *Ecosystems*, 5(2):105–121.
- Craft, C., Clough, J., Ehman, J., Jove, S., Park, R., Pennings, S., Guo, H., and Machmuller, M. (2009). Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. *Frontiers in Ecology and the Environment*, 7(2):73–78.
- de Boer, M. K., Moor, H., Matthiessen, B., Hillebrand, H., and Eriksson, B. K. (2014). Dispersal

- 573 restricts local biomass but promotes the recovery of metacommunities after temperature stress. *Oikos*,
574 123(6):762–768.
- 575 de Groot, R. S., Wilson, M. a., and Boumans, R. M. (2002). A typology for the classification, description
576 and valuation of ecosystem functions, goods and services. *Ecological Economics*, 41(3):393–408.
- 577 Dodson, S. I. (1992). Predicting crustacean zooplankton species richness. *Limnology and Oceanography*,
578 37(4):848–856.
- 579 Dufrêne, M. and Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible
580 asymmetrical approach. *Ecological monographs*, 67(3):345–366.
- 581 Dupont, C. L., Larsson, J., Yooseph, S., Ininbergs, K., Goll, J., Asplund-Samuelsson, J., McCrow, J. P.,
582 Celepli, N., Allen, L. Z., Ekman, M., et al. (2014). Functional tradeoffs underpin salinity-driven
583 divergence in microbial community composition. *PloS one*, 9(2):e89549.
- 584 Eiler, A., Zaremba-Niedzwiedzka, K., Martínez-García, M., McMahon, K. D., Stepanauskas, R., An-
585 dersson, S. G., and Bertilsson, S. (2014). Productivity and salinity structuring of the microplankton
586 revealed by comparative freshwater metagenomics. *Environmental microbiology*, 16(9):2682–2698.
- 587 Franzitta, G., Hanley, M. E., Airoldi, L., Baggini, C., Bilton, D. T., Rundle, S. D., and Thompson, R. C.
588 (2015). Home advantage? decomposition across the freshwater-estuarine transition zone varies with
589 litter origin and local salinity. *Marine environmental research*, 110:1–7.
- 590 Freschet, G. T., Aerts, R., and Cornelissen, J. H. (2012). Multiple mechanisms for trait effects on litter
591 decomposition: moving beyond home-field advantage with a new hypothesis. *Journal of Ecology*,
592 100(3):619–630.
- 593 Geange, S. W. and Stier, A. C. (2009). Order of arrival affects competition in two reef fishes. *Ecology*,
594 90(10):2868–2878.
- 595 Gholz, H. L., Wedin, D. A., Smitherman, S. M., Harmon, M. E., and Parton, W. J. (2000). Long-
596 term dynamics of pine and hardwood litter in contrasting environments: toward a global model of
597 decomposition. *Global Change Biology*, 6(7):751–765.
- 598 Grün, B., Kosmidis, I., and Zeileis, A. (2012). Extended beta regression in R: Shaken, stirred, mixed, and
599 partitioned. *Journal of Statistical Software*, 48(11):1–25.
- 600 Hall, C. J. and Burns, C. W. (2002). Environmental gradients and zooplankton distribution in a shallow,
601 tidal lake. *Archiv für Hydrobiologie*, pages 485–497.
- 602 Hébert, M.-P., Beisner, B. E., and Maranger, R. (2016a). Linking zooplankton communities to ecosystem
603 functioning: toward an effect-trait framework. *Journal of Plankton Research*, 39(1):3–12.
- 604 Hébert, M.-P., Beisner, B. E., and Maranger, R. (2016b). A meta-analysis of zooplankton functional traits
605 influencing ecosystem function. *Ecology*, 97(4):1069–1080.
- 606 Helenius, L. K., Leskinen, E., Lehtonen, H., and Nurminen, L. (2017). Spatial patterns of littoral
607 zooplankton assemblages along a salinity gradient in a brackish sea: a functional diversity perspective.
608 *Estuarine, Coastal and Shelf Science*, 198:400–412.
- 609 Herbert, E. R., Boon, P., Burgin, A. J., Neubauer, S. C., Franklin, R. B., Ardón, M., Hopfensperger,
610 K. N., Lamers, L. P., and Gell, P. (2015). A global perspective on wetland salinization: ecological
611 consequences of a growing threat to freshwater wetlands. *Ecosphere*, 6(10):1–43.
- 612 Hintz, W. D. and Relyea, R. A. (2017). Impacts of road deicing salts on the early-life growth and
613 development of a stream salmonid: salt type matters. *Environmental pollution*, 223:409–415.
- 614 Hopkinson, C. S., Cai, W.-J., and Hu, X. (2012). Carbon sequestration in wetland dominated coastal
615 systems—a global sink of rapidly diminishing magnitude. *Current Opinion in Environmental Sustain-
616 ability*, 4(2):186–194.
- 617 Hunt, H., Ingham, E., Coleman, D., Elliott, E., and Reid, C. (1988). Nitrogen limitation of production and
618 decomposition in prairie, mountain meadow, and pine forest. *Ecology*, 69(4):1009–1016.
- 619 Isla, A., Scharek, R., and Latasa, M. (2015). Zooplankton diel vertical migration and contribution to deep
620 active carbon flux in the nw mediterranean. *Journal of Marine Systems*, 143:86–97.
- 621 Johnson, W. S. and Allen, D. M. (2012). *Zooplankton of the Atlantic and Gulf coasts: a guide to their
622 identification and ecology*. JHU Press.
- 623 Jones, S. E. and McMahon, K. D. (2009). Species-sorting may explain an apparent minimal effect of
624 immigration on freshwater bacterial community dynamics. *Environmental microbiology*, 11(4):905–
625 913.
- 626 Jonsson, A., Meili, M., Bergström, A.-K., and Jansson, M. (2001). Whole-lake mineralization of
627 allochthonous and autochthonous organic carbon in a large humic lake (örträsket, n. sweden). *Limnology*

- 628 *and oceanography*, 46(7):1691–1700.
- 629 Kemp, A. C., Horton, B. P., Culver, S. J., Corbett, D. R., van de Plassche, O., Gehrels, W. R., Douglas,
630 B. C., and Parnell, A. C. (2009). Timing and magnitude of recent accelerated sea-level rise (north
631 carolina, united states). *Geology*, 37(11):1035–1038.
- 632 Kennedy, K. and El-Sabaawi, R. W. (2017). A global meta-analysis of exotic versus native leaf decay in
633 stream ecosystems. *Freshwater biology*, 62(6):977–989.
- 634 Kimmel, D. (2011). Plankton consumer groups: Copepods.
- 635 Kirwan, M. L. and Megonigal, J. P. (2013). Tidal wetland stability in the face of human impacts and
636 sea-level rise. *Nature*, 504(7478):53–60.
- 637 Kopp, R. E., Horton, B. P., Kemp, A. C., and Tebaldi, C. (2015). Past and future sea-level rise along the
638 coast of north carolina, usa. *Climatic Change*, 132(4):693–707.
- 639 Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., and Schloss, P. D. (2013). Development of
640 a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the
641 miseq illumina sequencing platform. *Applied and environmental microbiology*, 79(17):5112–5120.
- 642 Langenheder, S., Kisand, V., Wikner, J., and Tranvik, L. J. (2003). Salinity as a structuring factor for
643 the composition and performance of bacterioplankton degrading riverine doc. *FEMS Microbiology
644 Ecology*, 45(2):189–202.
- 645 Leibold, M. A., Chase, J. M., and Ernest, S. M. (2017). Community assembly and the functioning of
646 ecosystems: how metacommunity processes alter ecosystems attributes. *Ecology*, 98(4):909–919.
- 647 Lettice, S., Jansen, M. A., and Chapman, D. V. (2011). Differential decomposition patterns of marine and
648 terrestrial biomass in a coastal lagoon. *GeoEcoMarina*, 17.
- 649 Logares, R., Bråte, J., Bertilsson, S., Clasen, J. L., Shalchian-Tabrizi, K., and Rengefors, K. (2009).
650 Infrequent marine–freshwater transitions in the microbial world. *Trends in microbiology*, 17(9):414–
651 422.
- 652 Lopes, M. L., Martins, P., Ricardo, F., Rodrigues, A. M., and Quintino, V. (2011). In situ experimental
653 decomposition studies in estuaries: A comparison of phragmites australis and fucus vesiculosus.
654 *Estuarine, Coastal and Shelf Science*, 92(4):573–580.
- 655 Makarewicz, J. C. and Likens, G. E. (1979). Structure and function of the zooplankton community of
656 mirror lake, new hampshire. *Ecological Monographs*, 49(1):109–127.
- 657 Mansour, I., Heppell, C. M., Ryo, M., and Rillig, M. C. (2018). Application of the microbial community
658 coalescence concept to riverine networks. *Biological Reviews*, 93(4):1832–1845.
- 659 Martiny, J. B., Jones, S. E., Lennon, J. T., and Martiny, A. C. (2015). Microbiomes in light of traits: a
660 phylogenetic perspective. *Science*, 350(6261):aac9323.
- 661 Mcleod, E., Chmura, G. L., Bouillon, S., Salm, R., Björk, M., Duarte, C. M., Lovelock, C. E., Schlesinger,
662 W. H., and Silliman, B. R. (2011). A blueprint for blue carbon: toward an improved understanding of
663 the role of vegetated coastal habitats in sequestering co2. *Frontiers in Ecology and the Environment*,
664 9(10):552–560.
- 665 Michener, W. K., Blood, E. R., Bildstein, K. L., Brinson, M. M., and Gardner, L. R. (1997). Climate
666 change, hurricanes and tropical storms, and rising sea level in coastal wetlands. *Ecological Applications*,
667 7(3):770–801.
- 668 Mitsch, W. J., Bernal, B., Nahlik, A. M., Mander, Ü., Zhang, L., Anderson, C. J., Jørgensen, S. E., and
669 Brix, H. (2013). Wetlands, carbon, and climate change. *Landscape Ecology*, 28(4):583–597.
- 670 Mouquet, N. and Loreau, M. (2003). Community Patterns in Source-Sink Metacommunities. *The
671 American Naturalist*, 162(5):544–557.
- 672 Nemergut, D. R., Schmidt, S. K., Fukami, T., O’Neill, S. P., Bilinski, T. M., Stanish, L. F., Knelman, J. E.,
673 Darcy, J. L., Lynch, R. C., Wickey, P., et al. (2013). Patterns and processes of microbial community
674 assembly. *Microbiol. Mol. Biol. Rev.*, 77(3):342–356.
- 675 Newton, R. J., Jones, S. E., Eiler, A., McMahon, K. D., and Bertilsson, S. (2011). A guide to the natural
676 history of freshwater lake bacteria. *Microbiology and Molecular Biology Reviews*, 75(1):14–49.
- 677 Nicholls, R. J. and Cazenave, A. (2010). Sea Level Rise and Its Impact on Coastal Zones. *Science*,
678 328(2010):1517–1520.
- 679 Nielsen, D. L., Brock, M. A., Vogel, M., and Petrie, R. (2008). From fresh to saline: a comparison
680 of zooplankton and plant communities developing under a gradient of salinity with communities
681 developing under constant salinity levels. *Marine and Freshwater Research*, 59(7):549–559.
- 682 Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O’Hara,

- 683 R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., and Wagner, H. (2016). *vegan*:
684 *Community Ecology Package*. R package version 2.4-1.
- 685 Parada, A. E., Needham, D. M., and Fuhrman, J. A. (2016). Every base matters: assessing small subunit
686 rRNA primers for marine microbiomes with mock communities, time series and global field samples.
687 *Environmental microbiology*, 18(5):1403–1414.
- 688 Pennak, R. W. (1953). Fresh-water invertebrates of the United States. In *Fresh-water invertebrates of the*
689 *United States*. Ronald Press.
- 690 Poffenbarger, H. J., Needelman, B. A., and Megonigal, J. P. (2011). Salinity influence on methane
691 emissions from tidal marshes. *Wetlands*, 31(5):831–842.
- 692 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F. O.
693 (2012). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools.
694 *Nucleic acids research*, 41(D1):D590–D596.
- 695 Quintino, V., Sangiorgio, F., Ricardo, F., Mamede, R., Pires, A., Freitas, R., Rodrigues, A. M., and
696 Basset, A. (2009). In situ experimental study of reed leaf decomposition along a full salinity gradient.
697 *Estuarine, Coastal and Shelf Science*, 85(3):497–506.
- 698 R Core Team (2016). *R: a Language and Environment for Statistical Computing*. R Foundation for
699 Statistical Computing, Vienna, Austria.
- 700 Reice, S. R. and Herbst, G. (1982). The role of salinity in decomposition of leaves of *Phragmites australis*
701 in desert streams. *Journal of Arid Environments*, 5(4):361–368.
- 702 Roberts, D. W. (2016). *labdsv: Ordination and Multivariate Analysis for Ecology*. R package version
703 1.8-0.
- 704 Rocca, J. D., Simonin, M., Wright, J., Washburne, A., and Bernhardt, E. S. (2019). Rare microbial taxa
705 emerge when communities collide: Freshwater and marine microbiome responses to experimental
706 seawater intrusion. *bioRxiv*, page 550756.
- 707 Rognes, T., Flouri, T., Nichols, B., Quince, C., and Mahé, F. (2016). Vsearch: a versatile open source tool
708 for metagenomics. *PeerJ*, 4:e2584.
- 709 Root, T. L., Hall, K. R., Herzog, M. P., and Howell, C. A. (2015). *Biodiversity in a changing climate:*
710 *linking science and management in conservation*. Univ of California Press.
- 711 Schäfer, R. B., Bundschuh, M., Rouch, D. A., Szöcs, E., Peter, C., Pettigrove, V., Schulz, R., Nuggeoda,
712 D., and Kefford, B. J. (2012). Effects of pesticide toxicity, salinity and other environmental variables
713 on selected ecosystem functions in streams and the relevance for ecosystem services. *Science of the*
714 *Total Environment*, 415:69–78.
- 715 Schallenberg, M., Hall, C. J., and Burns, C. W. (2003). Consequences of climate-induced salinity increases
716 on zooplankton abundance and diversity in coastal lakes. *Marine ecology progress series*, 251:181–189.
- 717 Schimel, J. and Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in*
718 *microbiology*, 3:348.
- 719 Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A.,
720 Oakley, B. B., Parks, D. H., Robinson, C. J., et al. (2009). Introducing mothur: open-source, platform-
721 independent, community-supported software for describing and comparing microbial communities.
722 *Applied and environmental microbiology*, 75(23):7537–7541.
- 723 Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., and Smith, J. (2011). Salinity effects
724 on carbon mineralization in soils of varying texture. *Soil biology and biochemistry*, 43(9):1908–1916.
- 725 Six, K. D. and Maier-Reimer, E. (1996). Effects of plankton dynamics on seasonal carbon fluxes in an
726 ocean general circulation model. *Global Biogeochemical Cycles*, 10(4):559–583.
- 727 Snucins, E. and John, G. (2000). Interannual variation in the thermal structure of clear and colored lakes.
728 *Limnology and Oceanography*, 45(7):1639–1646.
- 729 Staddon, P., Lindo, Z., Crittenden, P. D., Gilbert, F., and Gonzalez, A. (2010). Connectivity, non-random
730 extinction and ecosystem function in experimental metacommunities. *Ecology Letters*, 13(5):543–552.
- 731 Stagg, C. L., Baustian, M. M., Perry, C. L., Carruthers, T. J., and Hall, C. T. (2018). Direct and indirect
732 controls on organic matter decomposition in four coastal wetland communities along a landscape
733 salinity gradient. *Journal of ecology*, 106(2):655–670.
- 734 Symons, C. C. and Arnott, S. E. (2013). Regional zooplankton dispersal provides spatial insurance for
735 ecosystem function. *Global change biology*, 19(5):1610–9.
- 736 Symons, C. C. and Arnott, S. E. (2014). Timing is everything: priority effects alter community invasibility
737 after disturbance. *Ecology and evolution*, 4(4):397–407.

- 738 Thompson, P. L. and Gonzalez, A. (2017). Dispersal governs the reorganization of ecological networks
739 under environmental change. *Nature ecology & evolution*, 1(6):0162.
- 740 Thompson, P. L. and Shurin, J. B. (2012). Regional zooplankton biodiversity provides limited buffering
741 of pond ecosystems against climate change. *The Journal of animal ecology*, 81(1):251–9.
- 742 Venables, W. N. and Ripley, B. D. (2013). *Modern applied statistics with S-PLUS*. Springer Science &
743 Business Media.
- 744 Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E. (2007). Let the
745 concept of trait be functional! *Oikos*, 116(5):882–892.
- 746 Weston, N. B., Dixon, R. E., and Joye, S. B. (2006). Ramifications of increased salinity in tidal
747 freshwater sediments: Geochemistry and microbial pathways of organic matter mineralization. *Journal*
748 *of Geophysical Research: Biogeosciences*, 111(G1).
- 749 Wieski, K., Guo, H., Craft, C. B., and Pennings, S. C. (2010). Ecosystem functions of tidal fresh, brackish,
750 and salt marshes on the georgia coast. *Estuaries and Coasts*, 33(1):161–169.
- 751 Zervoudaki, S., Nielsen, T. G., and Carstensen, J. (2009). Seasonal succession and composition of the
752 zooplankton community along an eutrophication and salinity gradient exemplified by danish waters.
753 *Journal of plankton research*, 31(12):1475–1492.