SALINIZATION IMPACTS ON HISTORICALLY FRESHWATER BACTERIAL COMMUNITIES

by

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Microorganisms regulate the movement of energy and nutrients through ecosystems. When environmental changes influence the composition of microbial communities, associated biogeochemical cycles can change in potentially unpredictable ways. Salinization is a widespread environmental concern for both inland and coastal wetlands. Sea level rise is a long-term problem, with salinization occurring gradually. Storm surge, drought, and geomorphology alterations can also cause salinization events. This saltwater intrusion is known to reduce freshwater wetland ecosystem functions such as decreased inorganic nitrogen removal and carbon storage. Though both fresh and salt water microbial communities have been studied, it is unclear how historically freshwater wetland microbial communities will respond to increased salinization and to the influx of saltwater microbial communities. Salinization is a strong environmental filter and with increased salinity, microbial communities will shift due to the inability of freshwater species to quickly adapt to saline conditions and compete with saltwater microbes. I hypothesized that bacterial communities will decrease in taxonomic and phylogenetic diversity as salinity is increased, and microbial communities will differ in composition according to salinity and colonization of saline communities. Salinity-induced shifts in microbial community composition

will directly influence rates of decomposition and CO_2 respiration. I used a combination of an experimental mesocosm and bioinformatics approaches to examine how salinity and dispersal of aquatic communities impact bacterioplankton community structure and function. Findings indicate that salinity, but not dispersal, reduced bacterial taxonomic (i.e., based on species identify and abundance) and phylogenetic (based on relatedness of species) diversity and community composition changed along the experimental salinity gradient. The compositional shifts were accompanied by changes in carbon mineralization rates. A unique group of bacterial taxa, many of which were unable to be classified, represented each salinity environment. Together, changes in bacterial community composition were associated with changes in carbon cycling functions. Possible impacts to carbon cycling and storage, and biogeochemical cycles in general, include reduced storage potential and slowed cycling rates. By considering microbial community responses, it may be possible to manage freshwater wetland communities to promote carbon storage for climate change mitigation.

SALINIZATION IMPACTS ON HISTORICALLY FRESHWATER BACTERIAL COMMUNITIES

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CHAPTER 1

INTRODUCTION

Microorganisms are recognized for their critical role in the health of ecosystems as they function to regulate climate and enhance water quality (Barberán et al. 2014, Ducklow 2008). Microorganisms carry out essential global biogeochemical processes (i.e., nitrogen and carbon metabolism) and are responsible for controlling large flows of carbon (Schimel and Schaeffer 2012). Microorganisms regulate carbon and nutrient transformations through redox reactions (Herbert et al. 2015; Ducklow 2008; Azam and Malfatti 2007). Decreasing rates of microbial respiration of carbon dioxide (CO_2) and methane (CH_4) can increase carbon storage potential and climate regulation. However, natural and human-induced environmental stressors can impact microbial communities and alter associated ecosystem functions that we value as services (i.e., benefits) and dis-services in coastal wetland ecosystems.

Microbiomes, which are the entirety of microorganisms and their genetic material within an environment, are composed of organisms that vary in response and in tolerance to environmental changes. Microbial community composition is known to have some effect on ecosystem functions, such as biogeochemical processes. The strength of the relationship between microbial community structure and ecosystem function varies because communities that are composed of unique groups of taxa are capable of carrying out both redundant and unique functions (Strickland et al. 2009, Allison and Martiny 2008, Rocca et al. 2015). Both natural and human-induced environmental change is known to impact microbial community structure and ecosystem functions. The stress from environmental change, such as sea level rise and salinization, is predicted to intensify in the coming decades with continued human development and climate change (IPCC SRES 2000). It is critical to understand how microorganisms will respond to short-

and long-term environmental disturbances in order to predict how microbial community structure and ecosystem functions might be altered. This knowledge can inform strategies to manage microbiomes in a way that can mitigate climate change through controlling rates of organic matter decomposition to enhance carbon storage activities. Wetlands are responsible for large flows of carbon through ecosystems by processes such as sequestration, where organic carbon is not accessible for microbial respiration, and decomposition, where rates of organic matter breakdown are slowed in the anoxic conditions of aquatic environments. The rates of these processes can change due to natural or anthropogenic activities, resulting in carbon storage (i.e., carbon fixation rates outpace respiration) or carbon release (i.e., respiration rates outpace carbon fixation rates).

The effects of human and naturally induced environmental change are particularly evident and impactful in coastal regions where land development, natural resource extraction, and climate change intersect. For example, the increase in global ocean temperature is causing thermal expansion of ocean water, glacier melt, and rising sea levels on the coasts. This is causing salt water to move upriver, inundating existing coastal salt marshes and invading formerly isolated freshwater wetlands (Weston et al. 2006). According to the Intergovernmental Panel on Climate Change, sea level is predicted to increase by 30-100 cm by the year 2100 (IPCC SRES 2000; Craft et al. 2009) and salinization of freshwater wetlands will dramatically increase over time. Though both fresh and salt water microbes have been studied, it is unclear how the composition and functions of wetland microbial communities at the fresh/salt water interface will change with increasing salinization. Sea level rise (SLR) and saltwater intrusion in previously freshwater wetlands can reduce wetland ecosystem functions such as increasing amounts of toxic sulfides, decreasing inorganic nitrogen removal, and decreasing carbon storage; all of these reduced functions have negative implications for water quality, climate regulation, wetland accretion, and

the health of wetland biota (Herbert et al. 2015, Weston et al. 2006, Craft et al. 2009). Salinization in wetlands can occur from other sources as well. Storm surge occurs when increased wave action from coastal storms washes over land and into freshwater environments. With increasing storm frequency (e.g., hurricanes) due to climate change, this salinization process could increase. Another process related to climate change is the potential for drought, which would reduce freshwater run-off into surface waters and thereby increase salinity levels. Other processes of salinization include the use of road salts in upper latitudes and geomorphology changes of coastal habitat due to dredging of navigation channels (Herbert et al. 2015). Since microbial community composition underpins biogeochemical process rates, environmental stressors, such as increased salinity, impact microbial composition and function. Specifically, as salinity increases in these historically freshwater ecosystems, aerobic methane oxidation is expected to decrease due to the increasing availability of sulfate in saltwater (Herbert et al. 2015).

Salt water intrusion into previously freshwater wetlands is also expected to alter microbial mediated nutrient cycling. Soils exchange gases with the atmosphere through microbial respiration. Specifically, tracking the flux of CO_2 is important in examining salinity impacts on carbon emissions and identifying unintended ecosystem dis-services due to ongoing salinization. It is less clear how carbon cycling rates shift when freshwater and saltwater microbial communities interact in a contemporary context. As wetlands shift from fresh to salt water, the concentration of terminal electron acceptors (i.e., Fe(III), Mn(IV)) is increased, which prompts CO_2 production (Herbert et al. 2015). Microorganisms are particularly important to carbon cycling in terrestrial and aquatic ecosystems. For example, in wetlands, carbon can be stored as organic matter, leading to carbon sequestration, which is considered an ecosystem service capable of mitigating climate change effects. In contrast, carbon can also be released as the greenhouse gases methane and

carbon dioxide which is considered a dis-service. (Chambers et al. 2011, Schimel and Schaeffer 2012) Environmental stressors are altering the microbial communities governing these carbon cycling rates.

The salinity tolerance trait is considered a complex trait, meaning that it is coded by many genes. This trait is assumed to not easily be gained or lost by microorganisms. Two mechanisms are responsible for osmotic or salinity stress tolerance in microorganisms. First, microorganisms can adapt by excluding harmful solutes (e.g., sodium, chloride) while gathering beneficial solutes necessary for metabolism instead. Second, some microorganisms can create organic compounds to reduce the concentration gradient between the external environment and the cell (Wichern et al. 2006). More specifically, microbes can either 1) accumulate ions that can be later used in times of osmotic stress to maintain balance inside the cells, or 2) acquire compatible solutes by means of synthesis or uptake from the environment that will assist the organism in counteracting effects of osmotic stress (Kempf and Bremer 1998, Weston et al. 2011, Galinski and Truper 1994). Some studies suggest that long-term salinity changes, like those due to sea level rise, may occur slowly enough that the organisms can adapt. Specifically, they suggest that "pulses" of salinity influx, such as localized flooding due to storm surge in a climatic event, will more negatively affect freshwater aquatic communities compared to constant exposure to salinization. Unexpected pulses of salinity are believed to cause greater damage than long-term salinity changes due to the inability of organisms to adapt or change quickly following one of these events (Chambers et al. 2011).

One comparison study of marine or freshwater bacterial communities revealed that many other studies do not compare the two community types; in part because the distinction between marine and freshwater bacteria have never been well defined (Methe et al. 1998). In a synthetic literature review, researchers described the make-up of assemblages of bacterial communities in

freshwater and marine environments. Alphaproteobacteria were most abundant in marine environments, and Betaproteobacteria were most abundant in freshwater environments (Methe et al. 1998). The Alphaproteobacteria are found in almost all habitats and are important players in the global nitrogen cycle due to phylum members facilitating atmospheric nitrogen fixation (Newton et al. 2011). One clade of Alphaproteobacteria, SAR11, accounts for a third of the cells that are present in surface seawater (Morris et al. 2002). There are also freshwater Alphaproteobacteria; however, SAR11 is a very well-known and well-studied marine clade. Coastal watersheds provide ideal locations to examine microbial communities along a salinity gradient. For example, the most abundant fraction of Proteobacteria detected in the Tar River in eastern North Carolina was Betaproteobacteria, which increased after Hurricane Irene (a disturbance), potentially due to the influx of freshwater (Balmonte et al. 2016). The Betaproteobacteria are most common in freshwater and have a relatively low abundance in marine environments (Newton et al. 2011). Microbes in freshwater and marine environments respond to changes in environmental conditions; in particular, they respond to irregular variations, which can prompt alterations in community composition (Balmonte et al. 2016). The emergence, loss, and persistence of microbial taxa in a community can result in community function shifts. Resilient communities can be sensitive to environmental change and are capable of returning to their pre-disturbance state if that community consists of stress-tolerant taxa (Balmonte et al. 2016).

The microbial species that will survive pulses of environmental salinity change will have specialized traits. These traits, called effect traits, can be directly linked to ecosystem functioning (Martiny et al. 2015), and can determine an organism's effect on ecosystem services. Salinity preference is identified as one of the most complex trait measurements, classifying it based on how many genes are directly involved in coding the trait and how the trait is integrated with other

mechanisms (Martiny et al. 2015). Because specialized traits are needed for organisms to tolerate increasingly saline environments, major shifts in microbial composition and diversity are expected (Rath et al. 2016; Trivedi et al. 2016). Salinization of freshwater wetlands can shift dominant biogeochemical processes due to the alteration of water chemistry, which changes availability, concentration, and equilibrium of chemical substrates available for microbial metabolism (Herbert et al. 2015). Salinization effects on wetland carbon emissions (CH_4 and CO_2) and the balance of carbon must be further investigated. Carbon accumulation in freshwater wetland sediments has the potential to play an important role in reducing greenhouse gas (GHG) concentrations and lead to climate change mitigation (Herbert et al. 2015).

These essential biogeochemical cycles, such as carbon cycling, occur in relation to the structure of the community. There are connections between the community structure and the community function. When environmental conditions change, the community changes and the functions are altered. One process that is known to affect community composition is environmental filtering, where abiotic factors are causing traits to be constrained to certain limits (Pavoine et al. 2011). The interactions of microorganisms within an ecosystem are assumed to be difficult to quantify. It is common for microbial functions to be studied alongside microbial community composition as they are assumed to collectively respond to environmental change (Allison and Martiny 2008). However, researchers argue the importance of microbial communities to changes in the rate of ecosystem processes. As the community shifts, organisms that are already better adapted to new conditions may become more active and abundant, thereby shifting the dominant ecosystem processes (Allison et al. 2013).

The goal of this study is to examine how salinization impacts microbial diversity and function of freshwater microbial communities as estuarine aquatic communities and saltier

conditions move in and persist. I hypothesize that rising salinity will decrease microbial diversity and alter composition of microbial communities when compared to historically freshwater microbial communities since specialized microbial traits are necessary to tolerate or thrive in saline environments. I also hypothesize that salinity-induced shifts in microbial community composition will directly influence carbon cycling rates (i.e. decomposition, CO_2 respiration). I also expect that microorganisms that exist in more saline conditions will have a more similar phylogenetic signal compared to freshwater microorganisms. Since salinity tolerance is considered a complex trait, I expect that phylogenetic diversity will be lowest in the most saline tank and increase as water gets fresher. To test this hypothesis, I 1) characterized microbial communities along a salinity gradient and determined associated microbial traits; 2) related community structure and function differences to possible alterations in microbial communities as saltier conditions move in and persist; 3) quantified relative carbon cycling rates in response to salinity-induced aquatic community shifts; and 4) related changes in taxonomic and phylogenetic microbial diversity to carbon cycling rates. I used a combination of experimental mesocosm and bioinformatics approaches to examine how salinity and estuarine aquatic communities impact freshwater microbial community structure and function. Understanding how salinization impacts freshwater wetland microbial community structure and ecosystem function relationships can inform the management of carbon storage capacity in coastal wetlands experiencing increased salinization.

CHAPTER 2

METHODS

Experimental Design. Microbial community composition and function were characterized based on aquatic samples that were collected from a replicated mesocosm experiment conducted from June-July 2015 (Werba 2016) (**Figure 1**). Graduate Student Jo Werba (2016) generated the experimental design and set-up involved in addressing how salinization and introduction of brackish aquatic communities affects zooplankton community composition and ecosystem function. Werba and undergraduate Spencer Wilkinson implemented microbial field sampling as detailed in the "Microbial Sample Collection and Processing" section. To replicate a freshwater-saltwater aquatic system, peat moss (to serve as a nutrient pulse), sand (to serve as a benthic substrate), and water were added to 150-gallon stock watering tanks (filled to 100 gallons). Each mesocosm pond was adjusted to one of 4 different salinities (measured in practical salinity units) using Instant Ocean® Sea Salt (4 levels: 0, 5, 9, 13 psu). Each tank was then seeded with an inoculation consisting of water, zooplankton and microbes collected from natural ponds in the outer banks of North Carolina (see **Supplemental Table S1** for coastal pond location and information). These inoculates, twenty 1 L samples of water, were collected and filtered through 62.5 μm mesh across a one-hundred meter transect at each of the 5 coastal ponds and subsequently dispersed into each mesocosm tank. It is important to note that zooplankton were the focus of the original experimental design by Werba (2016) and bacterial communities represented in the source communities were likely particle- and zooplankton-associated. Each tank was covered with a shade cloth to reduce the opportunity for other organisms from colonizing the mesocosms. Populations introduced via the initial inoculation were allowed to stabilize for 6 weeks before sampling began. After this acclimation period, mesocosms received additional colonist of zooplankton and

microbes via simulated dispersal events from separate mesocosm ponds established as source tanks for salt (13 psu) and freshwater (0 psu) zooplankton and microbial communities. The two source tanks served as dispersal treatments, and consisted of either a freshwater (0 psu; fresh dispersal treatment) community or a mix of communities from freshwater (0 psu) and saltwater (13 psu; fresh+salt dispersal treatment). Dispersal treatments consisted of 2 L of water from dispersal (source) tanks into the experiment (treatment) tanks. Sample collection occurred every 9 days (due to average time for completion of one generation cycle) over the 6-week mesocosm experiment for a total of six time points. The salinity × dispersal setup was replicated 4 times to generate 16 tanks (4 at each salinity level) with the fresh dispersal treatment, and 4 times to generate 16 tanks (4 at each salinity level) with the fresh+salt dispersal treatment (Werba 2016). Tanks were well-mixed before each sampling event. About halfway through the experiment, all tanks at 13 psu were reseeded with zooplankton due to low abundance. During each sampling event, 1 L of water was collected from each tank for downstream microbial and biogeochemical analyses. For each tank, the following were measured using a YSI Pro: dissolved oxygen, ammonium (NH_4^+), temperature, and pH. Community analysis was focused on three of the time points (days 0, 18, and 45; 11 June 2015, 29 June 2015, and 25 July 2015, respectively), representing the initial, middle, and end of the experiment. Besides salinity levels, measured nitrogen and phosphorus concentrations were similar among treatment tanks (**Supplemental Figure S1**).

Decomposition Assay. Litter bag decomposition rates were used to measure relative carbon cycling rates associated with microbial decomposition across salinity and dispersal treatments. Three bags each containing dried leaf litter from one of three common wetland plants; *Spartina spp., Acer rubrum*, and *Phragmites australis* were placed in each tank. Each species originates from different salinity environments, enabling the comparison of decomposition rates of various

plants at changing salinity levels. *Spartina spp.* is a saltwater wetland grass, *Acer rubrum,* or red maple, is commonly found in freshwater wetlands, and *Phragmites australis* is a widely established non-native, invasive grass found in both salt and freshwater wetlands. Leaves from each of the plants were collected and air-dried, and then weighed to record initial weight. *P.australis* and *A. rubrum* litter were placed in 24-inch mesh mariculture bags and subsequently transferred into each tank. *S. alterniflora* has smaller leaves and was placed into mesh bags with smaller holes to prevent the loss of plant matter through the openings. Each of the species' leaves were harvested and air dried in late May, and the leaf litter bags remained in the tanks for the entire duration of the experiment. After 6 weeks (on day 45), we removed the litter bags, air-dried, then oven-dried the samples for 48-hours and weighed. The loss of plant matter, determined by subtracting the final weight of each dried litter bag from the initial weight of each dried litter bag, over six weeks represented the litter decomposition rate (Werba 2016).

Carbon Mineralization Assay. On the final mesocosm sampling date, 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 an LI-820 Infrared Gas Analyzer (IRGA). On the first day (Day 0), 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 one, three, and seven in order to determine CO_2 respiration rates over time. To determine the CO_2 production of each aquatic sample, the initial (day 0) reading was subtracted from the analyzed day's reading. Based on the day's known standard CO_2 calibration curve

readings, we calculated the unknown CO_2 concentration of each sample. To calculate the CO_2 respiration rate, the concentration of CO_2 calculated from the calibration curve is converted to volume units (ppm) using the following equation:

$$Cm\ (\mu g\ CO_2\ ^{-C}\ L^{-1}\ headspace) = (Cv \times M \times P) / (R \times T),$$

where Cv is the volume (ppm) of CO_2, M is the molecular weight of carbon, B 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 $soil^{-1}$ day^{-1}.

Microbial Sample Collection and Processing. Sample collection occurred every 9 days over the time of the 6-week mesocosm experiment for a total of six time points. During each sampling event, we collected 1 L of water from each tank. Each 1 L bottle of mesocosm water was homogenized and 200 mL of the water sample were concentrated onto 0.22 μm filters within 24 hours of field sampling (Supor-200; Pall Gelman, East Hills, NY) to collect microbial community samples**.** Filters were transferred into 2 mL sterile tubes and stored at -80^{o} C until molecular analyses. We extracted DNA from filters collected at three (of the six) time points representing the initial, middle, final sampling dates (Days 0, 18, 45) using the PowerWater DNA Isolation Kit (MO BIO Laboratories).

Microbial Community Sequencing. To examine shifts in microbial community composition and diversity, aquatic microbial communities in each mesocosm were characterized using paired-end targeted Illumina sequencing of the 16S rRNA gene (bacteria, archaea) (Caporaso et al. 2011). We extracted and purified the DNA found in the water of each mesocosm using the PowerWater DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA) using the sample collected a 0.22 μm

supor filter post-filtration. 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). PCR products were combined in equimolar concentrations and sequenced using paired-end (2×250 bp) approach using the Illumina MiSeq platform at Indiana University's Center for Genomics and Bioinformatics.

Raw bacterial sequences were processed using the Mothur pipeline (version 1.39.5; Kozich et al. 2013). As a brief description of this process, 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 UCHIME algorithm (Edgar et al. 2011). Next, I created operational taxonomic units (OTUs) by splitting sequences based on taxonomic class and then binning these OTUs by 97% sequence similarity.

Statistical Analyses. I examined how experimental treatment parameters of salinity, dispersal, and date influenced bacterial diversity metrics (Shannon H′ diversity, Pielou's J evenness, and richness) using analysis of variance (ANOVA). These bacterial diversity metrics were analyzed for the treatment tanks and the source water tanks. Using a principle coordinates analysis (PCoA) based on Bray-Curtis dissimilarity, I visualized bacterial community response to experimental treatments of salinity and sampling date. I used a permutational analysis of variance (PERMANOVA) to measure variation explained by salinity and dispersal on bacterial community composition over sampling date. Using ANOVA, I examined how salinity and dispersal and the interaction between salinity and dispersal influenced carbon mineralization rates. I also quantified the relationship between carbon mineralization rates and measured salinity in each experimental tank.

To further analyze bacterial community structure and carbon cycling function relationships, we used a series of Mantel tests and multivariate models to examine the relationships between bacterial community composition and both decomposition and carbon mineralization. I used a distance-based redundancy analysis (dbRDA) based on Bray-Curtis dissimilarity to determine the potential relationships between bacterial community composition and carbon cycling functions (i.e., decomposition of three plant litter types and carbon mineralization rate). I included decomposition of the three plant species used, *Acer rubrum, Spartina alterniflora,* and *Phragmites australis,* and carbon mineralization to analyze if these parameters explained patterns in bacterial community composition. Next, I used Mantel matrix comparisons to compare bacterial community composition (based on Bray-Curtis dissimilarity matrix) and carbon cycling functions (carbon mineralization, leaf litter decomposition). I then used an indicator species analysis to reveal which bacterial species were most representative of each salinity treatment. For this analysis, I included only bacterial taxa with a relative abundance greater than 0.05 in each of the tank salinities. Finally, I used Venn diagrams to examine how bacterial taxa overlap among source salt and source freshwater dispersal tanks along the experimental salinity gradient.

I completed all statistical calculations in the R environment (R v3.2.3, R Core Development Team 2013) using the vegan, ade4, and VennDiagram packages (Dray et al., 2017; Oksanen et al., 2017; Hanbo 2017) and custom functions. We performed PERMANOVA using the *adonis* and used the *dbrda* function in the vegan package, we used the *mantel.rtest* function in the ade4 package, we ran the *draw.pairwise.venn* and *draw.triple.venn* in the VennDiagram package, and we calculated UniFrac distances using the mothur software package (version 1.39.5).

CHAPTER 3

RESULTS

Bacterial Diversity Responds to Salinity More than Dispersal

Taxonomic Diversity. Salinity treatment influenced bacterial community diversity metrics more than the dispersal treatment. Using Shannon H′to measure the species diversity in the communities of the experimental tanks, salinity significantly influenced bacterial diversity over time (ANOVA, salinity×time: $F_{2,89}$=4.215, p=0.018). Bacterial diversity increased from freshwater to saline conditions at Days 0, but diversity appeared to decline from fresh to saltwater conditions for the fresh and fresh+saltwater dispersal treatment at the end of the experiment on Day 45 (**Figure 2**). Bacterial evenness (Pielou's J′) was used to measure and compare all species abundances within the community. Similar to bacterial diversity patterns for the experimental tanks, salinity altered bacterial species evenness over time (ANOVA, salinity×time: $F_{2,89}$=3.701, p=0.029), showing increased evenness as salinity increased for Days 0, but evenness declined at salinities 5 and 9 resulting in similar values at along the salinity gradient measured at Day 45 (**Figure 3**). Species richness represented the number of different OTUs in a community according to dispersal and salinity treatments. For the experimental tanks, bacterial species richness was highest in the most saline treatment on Days 0 and 18, and tended to decrease across the salinity gradient on Day 45 (ANOVA, salinity×time: $F_{2,89}$=5.290; p=0.291; **Figure 4**). The salinity and dispersal treatment interaction did not affect diversity (ANOVA, salinity×dispersal: $F_{1,79}$=1.49, p=0.226), richness (ANOVA, salinity×dispersal: $F_{1,79}$=0.994, p=0.322), and evenness (ANOVA, salinity×dispersal: $F_{1,79}$=1.29, p=0.260). In addition, there was no linear relationship between measured salinity and bacterial species diversity (R^2=-0.002, p=0.3739), evenness (R^2=-0.0002, p=0.326), and richness (R^2=-0.008, p=0.570). Based on the analysis of the dispersal source tanks only, species diversity,

evenness, and richness were similar between freshwater and saltwater sources over time (ANOVA, diversity: $F_{1,21}$=0.732, p=0.402, **Figure 5**; evenness: $F_{1,21}$=0.473, p=0.499, **Figure 6**; richness: $F_{1,21}$=1.584, p=0.222; **Figure 7**). Salinity, but not dispersal, treatment accounted for variation in bacterial diversity, richness, and evenness over time in our 6-week experiment.

Phylogenetic Diversity. Salinity, dispersal, and date influenced phylogenetic diversity (PD) to varying degrees. Phylogenetic diversity is explained as the sum of the distances between branches of a phylogenetic tree, with shorter distances equating to lower phylogenetic diversity. The main effects of salinity and date had a significant effect on PD. For Day 0, PD tended to increase with increasing salinity. However, by Day 18 and Day 45, PD decreased with increasing salinity. The interaction between date and salinity also had a significant relationship with PD, where PD increased along the fresh to saltwater gradient at Day 0 but decreased along the salinity gradient at Days 18 and 45 (ANOVA, date: $F_{2,79}$=7.25, p=0.001; salinity×date: $F_{2,79}$=6.38, p=0.003). Notably, the PD significantly decreased with increasing salinity for both the fresh and the fresh+salt dispersal treatments by Day 45 of the experiment (**Figure 8**). However, there was no significant effect on PD due to dispersal, or the interaction of salinity and dispersal (ANOVA, dispersal: $F_{1,79}$=0.081, p=0.776; salinity×dispersal, $F_{1,79}$=0.321, p=0.573), and salinity as a main effect influenced PD to a small degree (ANOVA, salinity: $F_{1,79}$=3.16, p=0.079).

Bacterial Community Composition Shifts According to Salinity and Dispersal

Salinity and sampling date changed bacterial community structure over time (PERMANOVA, salinity×date: R^2=0.027, p=0.002). For only the source dispersal tanks made of source fresh and source salt water from coastal ponds, dispersal (PERMANOVA, dispersal: R^2=0.287, p=0.001) accounted for the most variation in the source tank bacterial community composition; date (PERMANOVA, date: R^2=0.133, p=0.003) also had a significant effect on

community composition (**Table 1)**. The primary axis, which explained 29.1% of variation, showed a clear distinction between the source fresh and source salt tank communities. The secondary axis, which explained 9.8% of the variation, showed separation of the communities by date; the freshwater source community showed day 0 and 18 the furthest apart, with the day 45 community more similar to day 0. For the saltwater source community, day 18 and 45 communities are furthest apart, with day 0 located in the middle (**Figure 9**). When experimental tanks were considered, main effects of salinity and time accounted for the most variation in bacterial community composition (PERMANOVA, salinity: R^2=0.115, p=0.001, date: R^2=0.052, p=0.001), while dispersal did not affect bacterial community structure (PERMANOVA, dispersal: R^2=0.007, p=0.795) (**Table 2**). The bacterial communities in the treatment tanks separated into salt vs. freshwater environments along the primary axis, which explained 17.2% of the variation among communities, while distinct bacterial communities grouped according to increasing salinity (5, 9, 13) and separated along the secondary axis, which explained 7.4% of the variation in bacterial community composition (**Figure 10**). I also examined how dispersal type (shape) and salinity (color) to affected community composition (**Figure 11**). The main effects of dispersal, salinity, and date explained the most variation in bacterial community composition (PERMANOVA, dispersal: R^2=0.112, p=0.001; salinity: R^2=0.080, p=0.001; date: R^2=0.072, p=0.001; **Table 3**). In addition, the interaction of date and dispersal (R^2=0.060, p=0.024) and date and salinity (R^2=0.032, p=0.001) also explained variation in bacterial community composition, but to a lesser degree (**Table 3)**. The primary axis, which explained 20.8% of the bacterial community variation, showed a distinct separation between the saltwater and freshwater communities. Along this axis, the salinity 0 community is distinctly different from the other saline communities (5, 9, 13; **Figure 11**). The secondary axis, which explained 6.4% of the variation, showed some separation of

communities by date and salinity (**Figure 11**). The bacterial communities associated with source fresh and source saltwater continued to be grouped with salinity 0 and salinity 13 communities, respectively. For the dispersal treatments, fresh and fresh+salt, were similar in community composition at certain salinities. For salinity 0, we see that community composition at day 0 for the fresh+salt dispersal reflected a lot of variation due to the mixing of the fresh and saltwater source communities; there is a large change in community composition from day 0 to day 18. At days 18 and 45, both fresh and fresh+salt dispersal treatments and the source fresh communities were all relatively similar. The control source (no dispersal) remained similar to the salinity 0 communities for days 0, 18, and 45. For salinities 5 and 9, the bacterial communities remain relatively constant from day 0 through day 45. For the day 0, fresh+salt dispersal treatment, a large amount of variation again reflected the mixing of the fresh and salt communities. The composition of the fresh+salt bacterial communities in salinity 13 had distinctly changed composition from day 0 to day 18. From day 18 to day 45, the communities shifted to be more similar to the original day 0 communities. The source fresh treatment at salinity 13 showed day 18 and 45 to be furthest apart, with the day 0 community composition somewhere in between (**Figure 11**).

Venn diagrams were used to visualize the shared and unique taxa between source dispersal communities (source 0 or 13) and among bacterial communities of source compared to each of the salinity treatments (0, 5, 9, 13). The source salt and source fresh communities shared only 7.5% of the total taxa observed (**Figure 12**). Between source fresh, salinity 0, and salinity 5 communities, only 10.7% of total taxa were shared between salinity 5 and source fresh and 38.2% of taxa in the salinity 5 treatment had the highest amount of unique taxa (**Figure 13**). A Venn diagram comparing source fresh, salinity 5, and salinity 9 shared 8.6% of total taxa, which was the lowest percentage of shared taxa between source fresh and salinity 9; only 7.4% of total taxa observed were shared

among source fresh, salinity 5, and salinity 9 (**Figure 14**). Between source fresh, salinity 9, and salinity 13, source fresh and salinity 9 shared 7.4% of all taxa observed and only 6.2% of total taxa observed were shared between source fresh, salinity 9, and salinity 13 (**Figure 15**). A Venn diagram of source salt, salinity 0, and salinity 5 had the lowest percentage of shared taxa (7.8%) between source salt and salinity 0 (**Figure 16**). A comparison of source salt, salinity 5, and salinity 9 showed the highest percentage of shared taxa between salinity 5 and 9 at 33.7% of the total taxa observed (**Figure 17**). In addition, source salt and salinity 13 communities shared 100% of taxa, while source salt and salinity 13 shared 39% of all taxa observed (**Figure 18**).

Indicator species analysis revealed bacterial taxa (OTUs) that were unique to each salinity treatment. Associating these organisms with a salinity level can help to determine key taxa contributing to shifts in bacterial community structure (abbreviated **Table 4**; see https://github.com/PeraltaLab/CSI_Dispersal/tree/master/data for full table). The indicator value (indval) statistic shows a species (OTU) that exists in abundance at a certain salinity; the indval also shows that a particular salinity treatment is characterized by a community comprised of a large number of that same species. The salinity of each site type is shown as a cluster in this analysis. In cluster 1 (salinity 0), there were a high number of OTUs with large indicator values. Due to the great diversity of microbial communities, many bacterial sequences were unresolved to the 'species' level but instead are 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 (indval=0.990). Polynucleobacter (genus) was the next highest indicator (indval=0.983) of salinity 0 treatment. In cluster 2 (salinity 5), Betaproteobacteria (class; indval=0.937) represented salinity 5 environments, followed by *Flavobacterium* (genus; indval=0.889) and Alcaligenaceae (family; indval=0.852). Cluster 3

(salinity 9) and cluster 4 (salinity 13) were less clear; of the top 5 OTU indval ratings, 4 of 5 OTUs in cluster 3 and 3 of 5 OTUs in cluster 4 were unclassified and were unresolved beyond the Bacterial domain (**Table 4**). For cluster 4 (salinity 9) Planctomycetes had the second highest indicator value, and was only 1 of 4 classified OTUs indicative of salinity 9 (phylum; indval=0.891). In cluster 5 (salinity 13), only 2 OTUs of the top 5 indicator values reported were classified; the second highest indicator was *Haliea* (genus; indval=0.869) and the fourth was Alphaproteobacteria (class; indval=0.847).

Bacterial Community Composition and Carbon Cycling Functions Correlated Along Salinity Gradient

Salinity had a strong effect on carbon mineralization rates of bacterial communities at the end of the experiment. Salinity but not dispersal significantly impacted carbon mineralization rate (ANOVA, salinity: $F_{1,39}$=15.9, p=0.0002, dispersal: $F_{1,39}$=2.51, p=0.121; **Figure 19**). In addition, measured salinity accounted for 13% of the variation in carbon mineralization rates (R^2=0.130, p=0.026; **Figure 20).** I further examined the relationships between bacterial community composition and rates of carbon mineralization and decomposition of plant species litter (*Acer rubrum*, *Spartina alterniflora*, *Phragmites australis*). Decomposition of *A. rubrum* and *S. alterniflora* litter accounted for 20% of the variation in bacterial community composition, with *A. rubrum* having a very strong relationship (dbRDA model R^2=0.206; *A. rubrum:* $F_{1,26}$=2.40, p=0.007; *S. alterniflora:* $F_{1,26}$=1.81, p=0.025). Decomposition rates of *P. australis* did not relate to bacterial community (dbRDA, $F_{1,26}$=0.919, p=0.569). In addition, carbon mineralization rates significantly explained variation in bacterial community composition (dbRDA, $F_{1,26}$=1.629, p=0.036). In another measurement of bacterial structure-carbon cycling function relationships,

bacterial community composition and the decomposition rates of all three litter species were positively correlated (Mantel r=0.231, p=0.006; **Figure 21**).

CHAPTER 4

DISCUSSION

Salinity Impacted Taxonomic and Phylogenetic Diversity Over Time

The general effects of sea level rise have been and continue to be documented (IPCC SRES (2000); however, the effects of salinization on freshwater microbial communities that have never been exposed to saline conditions are not completely understood. This study specifically focused on determining how historically freshwater wetland aquatic communities and carbon cycling function would respond to a short-term salinization event. My results revealed that salinity significantly affected bacterial taxonomic and phylogenetic diversity, while dispersal treatments did not influence bacterial species diversity, richness, or evenness. Both bacterial diversity (Shannon H′), and bacterial evenness, Pielou's J′, significantly increased over time along the fresh to saltwater gradient, which is in contrast to the original hypothesis that diversity would decrease with increasing salinity. Bacterial communities tended to respond to salinity, rather than dispersal, because it is such a strong environmental filter. There are many factors that play a part in determining how bacterial community composition will change in response to an influx of saline-dominant communities following a salinization event. Both stochastic processes (such as drift) and deterministic processes (such as the interaction of biotic and abiotic factors) within an ecosystem can determine patterns of community assembly via four processes: selection, dispersal, diversification, and drift (Nemergut et al. 2013, Vellend 2010). Abiotic factors in an environment often act as environmental filters, selecting for organisms that are adapted to establish and persist. In fact, community assembly is more related to a species' traits than their taxonomy (Nemergut et al. 2013). Salinity tolerance is considered a complex trait among microbes, so adapting to saline conditions upon arrival is no easy feat (Martiny et al. 2015). Environmental selection often

determines microbial community assembly, and past research has shown that strong environmental selection pressures produce phylogenetically clustered communities (i.e., environmental filters select for organisms with certain traits; Nemergut et al. 2013).

Distribution of a microbial species is sometimes used as a proxy for dispersal. Because dispersal of microbes is often a passive process, we recognize that the longest transport of microbial species is via wind, water, and attachment to mobile organisms (Nemergut et al. 2013). In the case of our study, we added a mix of freshwater community and mesohaline (13 psu) aquatic communities in equal proportion as dispersal treatments along an experimental salinity gradient. The dispersal treatments represented natural dispersal methods of aquatic communities via water; a fresh community being mixed with a saline community can occur in storm events with coastal flooding, as well as gradual mixing of these environments via sea level rise. These methods of dispersal are on different time-scales, but each is potentially damaging to microbial communities.

Researchers argue that dormancy in dispersal mechanisms can affect results gathered from experiments, as in my study, that examine the effects of dispersal on community composition (Aanderud et al. 2016). Microbes that exhibit the dormant life history strategy may enter dormancy upon arrival in an unsuitable habitat, and organisms with traits that confer an advantage in the new environment may establish and dominate the community. These actions can cause misinterpretations due to potentially dormant organisms being detected and associated with community functions, even though those dormant organisms may not be actively metabolizing; therefore, dormant organisms not active in the community may be mismatched with actively occurring functions. Dormancy was not the focus of this experiment, therefore more research is required to determine the role that dormancy plays on bacterial community assembly during a salinization event. A previous study showed that dormancy was more prevalent in freshwater than

in hypersaline environments; highly adapted microbial communities in more extreme environments (hypersaline lakes) entered dormancy less often than freshwater microbes in order to survive (Aanderud et al. 2016). Activity in both fresh and hypersaline lakes increased with increasing salinity, suggesting salinity as an environmental filter determines activity (Aanderud et al. 2016). Bacterial taxa in dormancy did not rise in the extreme (hypersaline) environments; rather, microbial activity increased, perhaps due to the specialized adaptations for optimal metabolism and growth at high salinity (Aanderud et al. 2016). If an organism adapted for high salinity life goes dormant in an ideal environment, the organism risks the chance to establish and persist; if the organism goes dormant when it could actually be thriving, no metabolizing, growth, or reproduction can occur.

It is also important to consider the intensity and length of the disturbance affecting a microbial community. The disturbance in this experiment was the addition of salinity and saline-adapted microbial species to historically freshwater communities. This disturbance was intended to represent salinization due to sea level rise; however, the results are also relevant to short-term salinization events such as storm surge. At the end of the experiment, phylogenetic diversity (PD) was low for both dispersal treatments at the highest salinity, indicating that the bacteria persisting in those tanks were more related to each other (lower PD). This observation supported the hypothesis that PD would be lowest in the most saline tank, due to salinity tolerance being a complex trait. With increased dispersal, it can be assumed that the introduced, newly added species may also be forced to diversify through methods such as horizontal gene transfer (HGT) and increased mutation rates. Recent studies show that selection may act on traits that are subject to HGT, which can alter historic processes by transferring genetic material (i.e,. a trait) that would allow a historically non-existent organism to establish and persist in a previously inhospitable

environment (Papke & Gogarten 2012). When an organism gains a trait that would allow them to establish and persist in an historically inhospitable environment, they are able to explore new fitness landscapes and diversify (Nemergut et al. 2013); however, gaining a trait is not always a simple process, as some traits are complex and cannot be easily inherited (Martiny et al. 2015). Ecological drift (constant changes in relative abundance of organisms) may also potentially affect community assembly. In particular, low abundance organisms (i.e., majority of microbial species) are more vulnerable to drift (Nemergut et al. 2013). In addition, dormancy may protect these organisms from local extinction, but more research is required to determine how dormancy and rates of extinction in microbial communities impact community assembly (Nemergut et al. 2013). Overall, my study showed that the bacterial communities tended to respond to salinity, rather than dispersal, because it is such a strong environmental filter and because salinity tolerance is a deeply conserved trait.

Salinity Caused Shifts in Bacterial Community Composition

Changes in salinity resulted in distinct bacterial community patterns. Similar to bacterial diversity analyses, dispersal treatment did not impact community composition. Bacterial community structure is a determining factor for community function. An organism's traits determine its physiology and interactions with biotic and abiotic factors; the collective traits of a bacterial community interact with the environment to control ecosystem functioning, including the transformation of nutrients and energy for use by community members and the entire ecosystem at large (Martiny et al. 2015).

I identified key bacterial taxa associated with distinct bacterial communities along the salinity gradient. Different microbial taxa were representative of each of the four different salinity levels, in support of the hypothesis that 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 of a 0 salinity environment were OTUs that were matched to known taxa at the level of Proteobacteria (phylum), Rhodospirillales (order), and *Polynucelobacter* (genus). 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; most often found in fresh water habitats, many species within this order contain photosynthetic pigments and function as photoheterotrophs. Genus *Polynucelobacter* is a cosmopolitan bacterial genus; sequences and strains of this genus are globally found in a wide variety of freshwater ecosystems. Each of these three bacterial taxa are typical of freshwater environments; in this experiment, these three taxa were the highest indicators of salinity 0 (fresh) environments. The top three taxa determined to be representative of a 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, 4 of the top 5 OTUs remained unclassified up to the phylum level, while *Planctomycetes* (phylum) was identified as an indicator taxa. This phylum is typically found in estuarine, marine, and also fresh water (Newton et al. 2011). No species of

Planctomycetes has been cultured, so little more is known about the particular characteristics of the members of this phylum. 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 4 indicators 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 3 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) It is important to continue to incorporate gene sequencing to make inferences about traits (structure) and related functions.

Though these unclassified OTUs in salinity 9 and 13 tanks limit our conclusions, we do observe trends in dominant taxa that shift from fresh to saltwater treatment tanks. None of the top three indicator OTUs were classified as the same organism across the salinity gradient. Therefore, these results provide some support that each tank salinity selected for taxa unique to that salinity level. Global climate change may play a large role in facilitating increased dispersal of microbes; the predicted increases in extreme weather events may potentially promote increased spread of microbes to new, adaptable environments (Litchman 2010). As previously discussed, microbes have several mechanisms for adapting to environmental change; this experiment did not address which mechanisms were utilized. Future studies focused on bacterial community structure and function under increasing salinity would benefit from examining mechanisms of adaptation that taxa may use when placed under environmental stress

Salinity But Not Dispersal Influenced Community Structure-Function Relationship

In this experiment, we used the carbon cycling rates (carbon mineralization and decomposition) to make inferences about the functional changes as they relate to structural changes of a community along the salinity gradient. Again, we observed that salinity, but not dispersal, significantly impacting carbon mineralization rate; in turn, carbon mineralization rate explained variation in bacterial community composition. Coastal wetlands and their aquatic microbial communities play an important role in linking terrestrial, fresh, and marine carbon cycles through transport, mineralization, and storage of carbon (Ardón et al. 2016). Results of another experiment examining shifts in carbon export due to salinity stress in microcosms also showed that salinity was an important driver of dissolved organic carbon concentrations (Ardón et al. 2016). In our study, the litter bag approach revealed that litter type resulted in different rates of litter decomposition. Specifically, decomposition of *Acer rubrum* and *Spartina alterniflora* accounted for variation in bacterial community composition. Decomposition of *Phragmites australis* did not relate to bacterial community composition. It is important to note that *P. australis* is a non-native, widely successful species (Amsberry et al. 2000), whereas the other two species, *A. rubrum* and *S. alterniflora*, are native species to fresh and salt water wetlands communities, respectively. Salinity has altered decomposition to varying degrees. A recent review of salinization impacts on freshwater wetlands theorized that increased salinity should accelerate the mineralization of organic matter. However, multiple studies are referenced that include reports of salinity causing an increase, decrease, and no effect on organic matter decomposition in wetlands (Herbert et al. 2015). In measuring bacterial structure and carbon cycling function relationships in our study, there was a positive relationship between decomposition rates and all three abovementioned plant species.

CHAPTER 5

CONCLUSIONS

Global climate change is a widespread threat to all ecosystems. Due to changes in climate, the pycnocline, which is the stratification of water due to salinity and temperature differences, has the potential to strengthen and decrease amounts of dissolved oxygen in bottom waters; decreased oxygen concentration will negatively affect biota and disrupt biogeochemical cycles (Rabalais et al. 2009). Particularly concerning to historically freshwater wetland microbial communities is the threat of salinization due to sea level rise. Salinity as an environmental filter remained a strong influence on bacterial community composition throughout the experiment. Because of this, we can see the potential changes in freshwater wetland bacterial community structure and function that may occur due to short-term salinization events. However, sea level rise is a long-term process, and this experiment represented more of a pulse disturbance to a freshwater community. Though this short-term study allowed some inferences to be made about the fate of historically freshwater communities with salinization, it may be of interest to repeat the study to compare short-term changes observed here to long-term salinization effects. The changes associated with bacterial community structure and carbon cycling function are important to determine as rates of carbon release and storage can change in unexpected ways. Because microbes are responsible for major biogeochemical cycles and regulate large flows of carbon, it may be possible to mitigate the associated effects to freshwater wetland communities.

REFERENCES

Aanderud, Z.T., Vert, J.C., Lennon, J.T., Magnusson, T.W., Breakwell, D.P., and Harker, A.R. (2016). Bacterial dormancy is more prevalent in freshwater than hypersaline lakes. Frontiers in Microbiology 7, Article 853.

Allison, S.D., Lu, Y., Weihe, C., Goulden, M.L., Martiny, A.C., Treseder, K.K., Martiny, J.B.H. (2013). Microbial abundance and composition influence litter decomposition response to environmental change. Ecology 94(3), 714-725.

Allison, S.D., and Martiny, J.B.H. (2008). Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences 105, 11512-11519.

Amsberry, L., Baker, M.A., Ewanchuk, P.J., and Bertness, M.D. (2000). Clonal integration and the expansion of *Phragmites australis*. Ecological Applications 10(4), 1110-1118.

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, 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.

Azam, F., and Malfatti, F. (2007). Microbial structuring of marine ecosystems. Nature Reviews 5, 782-791. doi: 10.1038/nrmicro1747

Balmonte, J.P., Arnosti, C., Underwood, S., McKee, B.A., and Teske, A. (2016). Riverine bacterial communities reveal environmental disturbance signatures within the *Betaproteobacteria* and *Verrucomicrobia*. Frontiers in Microbiology 7 (1441).

Barberan, A., Casamayor, E.O., and Fierer, N. (2014). The microbial contribution to macroecology. Frontiers in Microbiology, 5(203).

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., Gormley, N., Gilbert, J.A., Smith, G., and Knight, R. (2012). Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. The ISME Journal 6, 1621–1624.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., … 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 of the United States of America, 108 (Supplement_1), 4516–22. https://doi.org/10.1073/pnas.1000080107

Chambers, L.G., Reddy, K.R., and Osborne, T.Z. (2011). Short-term response of carbon cycling to salinity pulses in a freshwater wetland. SSAJ 75 (5), 2000-2007.

Christian, R. R. (1984). A life-table approach to decomposition studies. Ecology 65(5), 1693-1697.

Craft, C., Clough, J., Ehman, J., Joye, S., Park, R., Pennings, S., Guo, H., Machmuller, M. (2009). Frontiers in Ecology and the Environment 7(2), 73-78. doi: 10.1890/070219

Dray, S., Blanchet, G., Borcard, D., Guenard, G., Jombart, T., Legendre, P., & Wagner, H. H. (2017). adespatial: Multivariate multiscale spatial analysis. R package version 0.0-8. Retrieved from https://CRAN. R-project. org/package= adespatial.

Ducklow, H. (2008). Microbial services: challenges for microbial ecologists in a changing world. Aquatic Microbial Ecology 53, 13-19. doi: 10.3354/ame01220

Edgar, R.C., B.J. Haas, J.C. Clemente, C. Quince, and R. Knight. (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27(16), 2194-2200.

Galinski, E.A., Truper, H.G. (1994). Microbial behavior in salt-stressed ecosystems. FEMS Microbiology Reviews 15, 95-108.

Hanbo, C. (2017). VennDiagram: generate high-resolution Venn and Euler plots. R package version 1.6.18. URL https://cran.r-project.org/web/packages/VennDiagram/VennDiagram.pdf

Herbert, E.R., Boon, P., Burgin, A.J., Neubauer, S.C., and Franklin, R.B. (2015). A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. Papers in Natural Resources 6(10).

Intergovernmental Panel on Climate Change. (2000). IPCC Special Report: Emissions Scenarios. ISBN: 92-9169-113-5.

Kempf, B., Bremer, E. (1998). Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. Archives of Microbiology 170, 319-330.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology 79(17), 5112-5120.

Kraft, N.J.B., P.B. Adler, O. Godoy, E.C. James, S. Fuller, and J.M. Levine. (2015). Community assembly, coexistence and the environmental filtering metaphor. Functional Ecology 29, 592-599.

Litchman, E. (2010). Invisible invaders: non-pathogenic invasive microbes in aquatic and terrestrial ecosystems. Ecology Letters 13, 1560-1572.

Loreau, M. (2010). Linking biodiversity and ecosystems: towards a unifying ecological theory. Philosophical Transaction of the Royal Society B 365, 49-60. doi: 10.1098/rstb.2009.0155

Martiny, J. B., Jones, S. E., Lennon, J. T., Martiny, A. C. (2015). Microbiomes in light of traits: a phylogenetic perspective. Science, 350(6261), aac9323.

Methe, B.B., Hiorns, W.D., and Zehr, J.P. (1998). Contrasts between marine and freshwater bacterial community composition: Analyses of communities in Lake George and six other Adirondack lakes. Limnology and Oceanography 43 (2), 368-374.

Morris, R.M., Rappe, M.S., Connon, S.A., Vergin, K.L., Siebold, W.A., Carlson, S.A., and Giovannoni, S.J. (2002). SAR11 clade dominates ocean surface bacterioplankton communities. Nature 420, 806-810.

Nemergut, D.R., Schmidt, S.K., Fukami, T., O'Neill, S.P., Bilinski, T.M., Stanish, L.F., Knelman J.E., Darcy, J.L., Lynch, R.C., Wickey, P., and Ferrenberg, S. (2013). Patterns and processes of microbial community assembly. Microbiology and Molecular Biology Reviews 77(3), 342-356.

Newton, R.J., Jones, S.E., Eiler, A., Mahon, K.D., and Bertilsson, S. (2011). A guide to the natural history of freshwater lake bacteria. Microbiology and Molecular Biology Reviews, 75 (1), 14-49.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H. (2017). Vegan: community ecology package. R package version 2.4-3. URL https://cran.r-project.org/web/packages/vegan/vegan.pdf

Papke, R.T. and Gogarten, J.P. (2012). How bacterial lineages emerge. Science 336(45), 45-46.

Parada, A. E., Needham, D.M., and Fuhrman, J.A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18, 1403–1414.

Pavoine, S., Vela, E., Gachet, S., Bélair, G. De, Michael, B., Pavoine, S., … Bélair, G. De. (2017). Linking patterns in phylogeny, traits, abiotic variables and space: a novel approach to linking environmental filtering and plant community assembly. Journal of Ecology 99(1), 165–175.

Peralta, A.L., Matthews, J.W., and Kent, K.D. (2010). Microbial community structure and denitrification in a wetland mitigation bank. Applied and Environmental Microbiology, 4207-4215.

R Core Development Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Rabalais, N.N., Turner, R.E., Diaz, R.J., and Justic, D. (2009). Global change and eutrophication of coastal waters. ICES Journal of Marine Science 66, 1528-1537.

Rath, K.M., Meheshwari, A., Bengtson, P., Rousk, J. (2016). Comparative toxicities of salts on microbial processes in soil. Applied and Environmental Microbiology 82(7).

Rocca, J.D., Hall, E.K., Lennon, J.T., Evans, S.E., Waldrop, M.P., Cotner, J.B., Nemergut, D.R., Graham, E.B., Wallenstein, M.D. (2015). Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. The ISME Journal 9, 1693- 1699. doi: 10.1038/ismej.2014.252

Schimel, J.P., and Schaeffer, S.M. (2012). Microbial control over carbon cycling in soil. Frontiers in Microbiology, 3.

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M. A. (2009). Testing the functional significance of microbial community composition. Ecology, 90(2), 441-451.

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I.C., Jeffries, T.C., Zhou, J., and Singh, B.K. (2016). Microbial regulation of the soil carbon cycle: evidence from gene-enzyme relationships. The ISME Journal, 1-12. doi: 10.1038/ismej.2016.65

Vellend, M. (2010). Conceptual synthesis in community ecology. The Quarterly review of biology, 85(2), 183-206.

Werba, J. (2016). Zooplankton community structure and ecosystem function across a salinity gradient. MS thesis. East Carolina University.

Weston, N.B., Dixon, R.E., and Joye, S.B. (2006). Ramifications of increased salinity in tidal freshwater sediments: geochemistry and microbial pathways of organic matter mineralization. Journal of Geophysical Research 111, G01009. doi: 10.1029/2005JG000071

Weston, N.B., Vile, M.A., Neubauer, S.C., and Velinsky, D.J. (2011). Accelerated microbial organic matter mineralization following salt-water intrusion into tidal freshwater marsh soils. Biogeochemistry 102, 135-151.

Wichern, J., Wichern, F., and Joergensen, R.G. (2006). Impact on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100-108.

Wilkinson, J.S., Werba, J., McCoy, M.W., and Peralta, A.L. (2016). Salinity Impacts on freshwater aquatic communities. East Carolina University Research and Creative Achievement Week, April 4-8, 2016. Greenville, NC. Poster Presentation.

Zimmerman, N., Izard, J., Klatt, C., Zhou, J., and Aronson, E. (2014). The unseen world: environmental microbial sequencing and identification methods for ecologists. Frontiers in Ecology and the Environment 12(4), 224-231. doi: 10.1890/130055

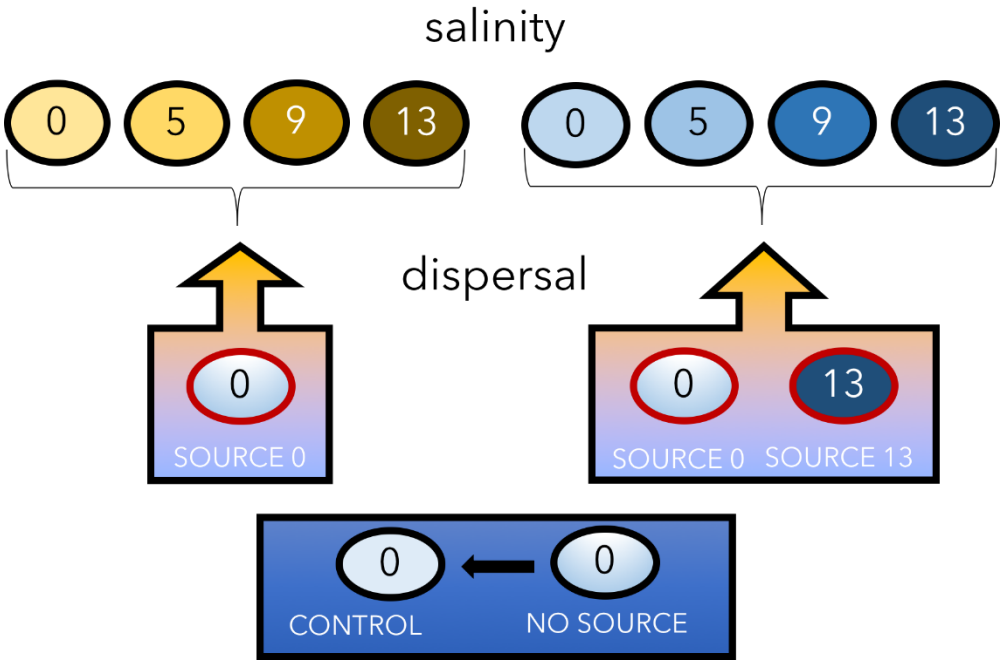


Figure 1. Experimental design of aquatic mesocosm experiment. Original experiment designed and carried out by Jo Werba (2016). The zooplankton/microbial dispersals and sampling were completed over a 6-week period. During each dispersal, aquatic communities were dispersed from source tanks. Half of the tanks were treated with dispersal source 0, while the other half were treated with both dispersal source fresh (0) and source 13. A control of salinity 0 received only source fresh (0). We analyzed samples from Days 0, 18, and 45 of the experiment (11 June 2015, 29 June 2015, 25 July 2015, respectively).

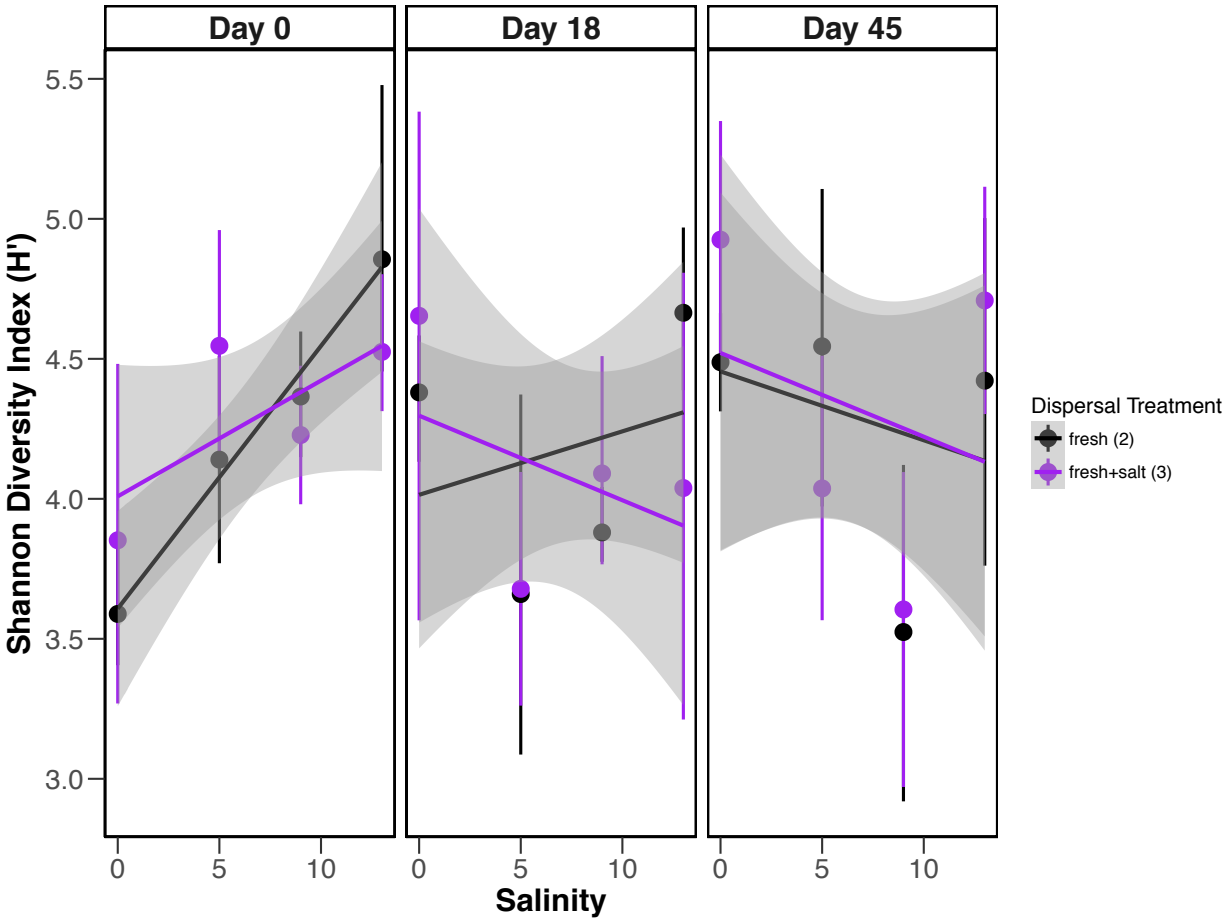


Figure 2. Shannon diversity (H′) of bacterial communities along a salinity gradient according to dispersal treatment. Black and purple circles represent the estimated mean of bacterial species diversity for the fresh and fresh+salt dispersal communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The black lines represent the trend of the diversity for the fresh dispersal treatment, and the purple lines represent the trend of the diversity for the fresh+salt dispersal treatment. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model.

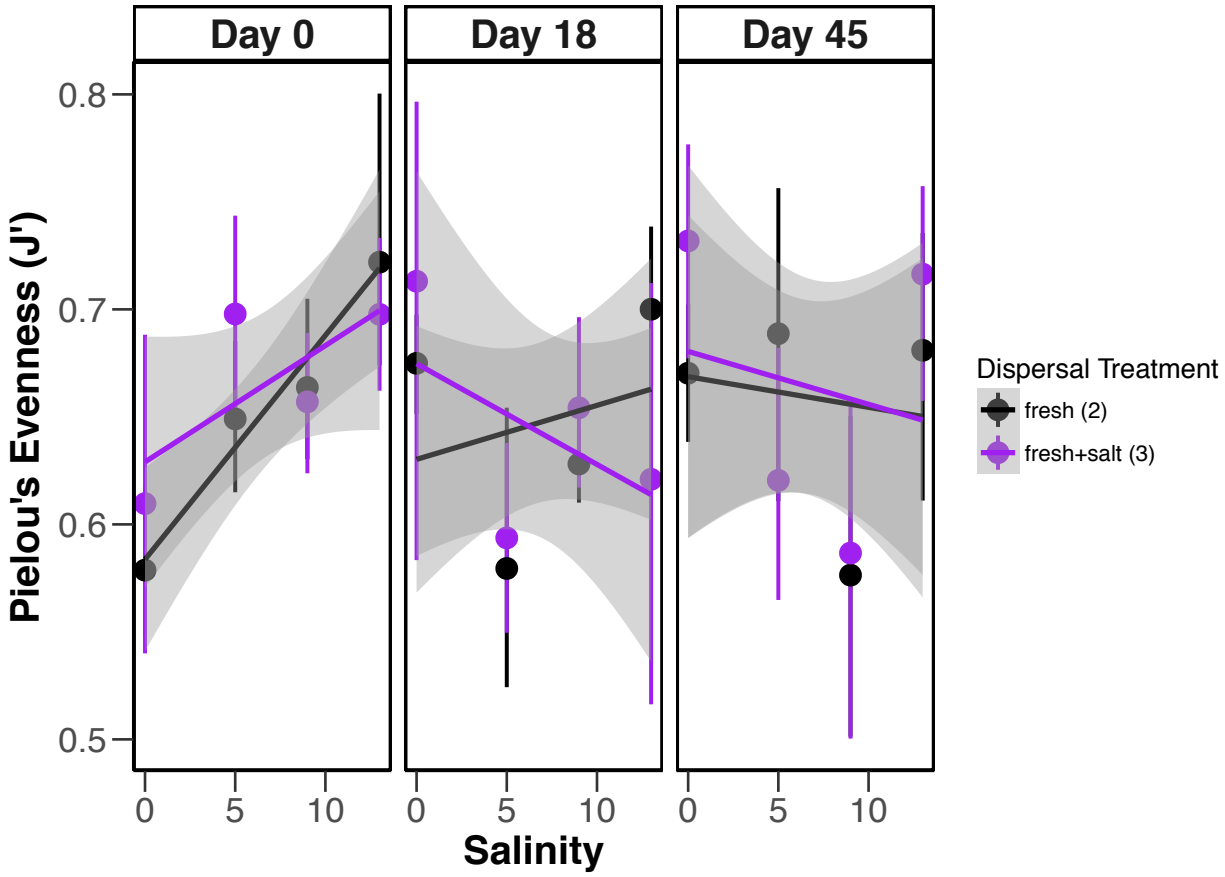


Figure 3. Species evenness (Pielou's evenness J′) of bacterial communities along a salinity gradient according to dispersal treatment. Black and purple circles represent the estimated mean of bacterial species evenness for the fresh and fresh+salt dispersal communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The black lines represent the trend of the evenness for the fresh dispersal treatment, and the purple lines represent the trend of the evenness for the fresh+salt dispersal treatment. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model.

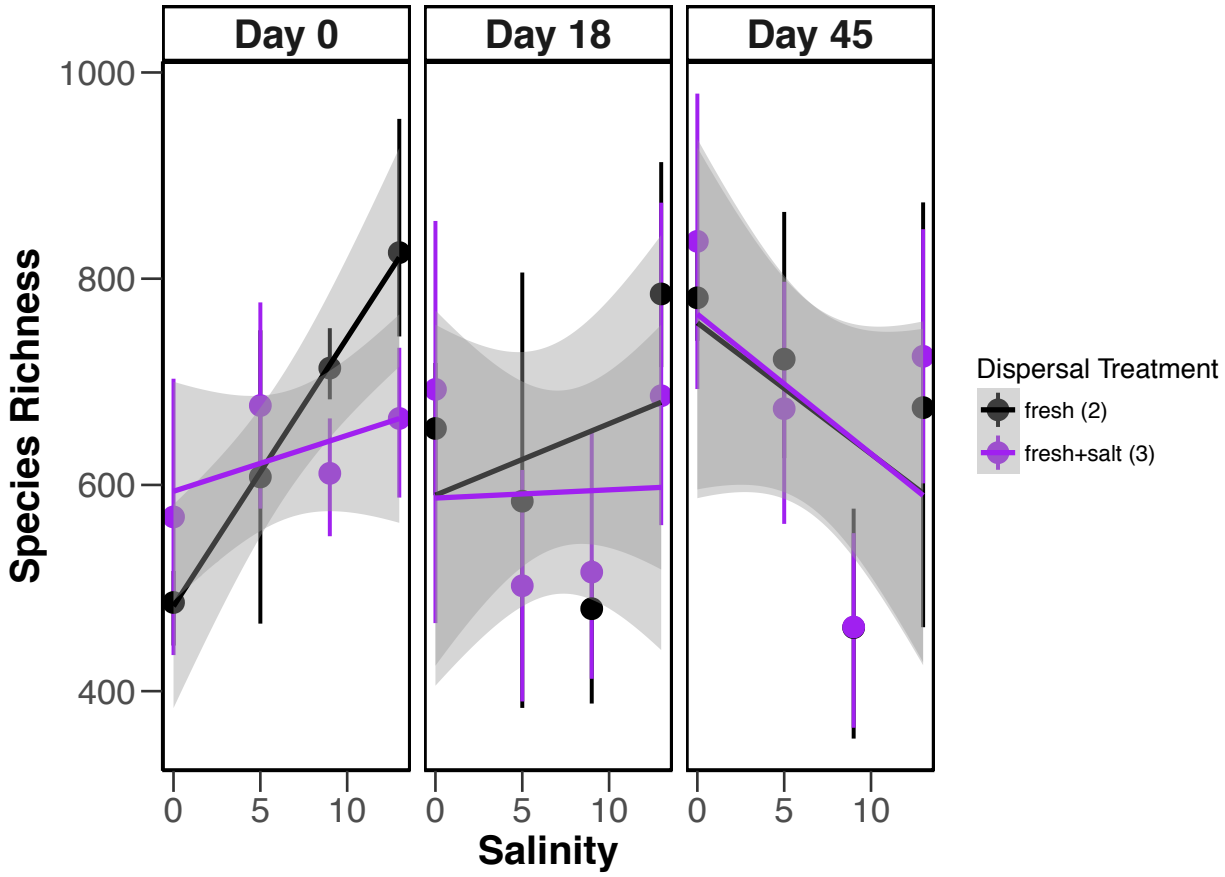


Figure 4. Species richness of bacterial communities along a salinity gradient according to dispersal treatment. Black and purple circles represent the estimated mean of bacterial species richness for the fresh and fresh+salt dispersal communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The black lines represent the trend of the richness for the fresh dispersal treatment, and the purple lines represent the trend of the richness for the fresh+salt dispersal treatment. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model.

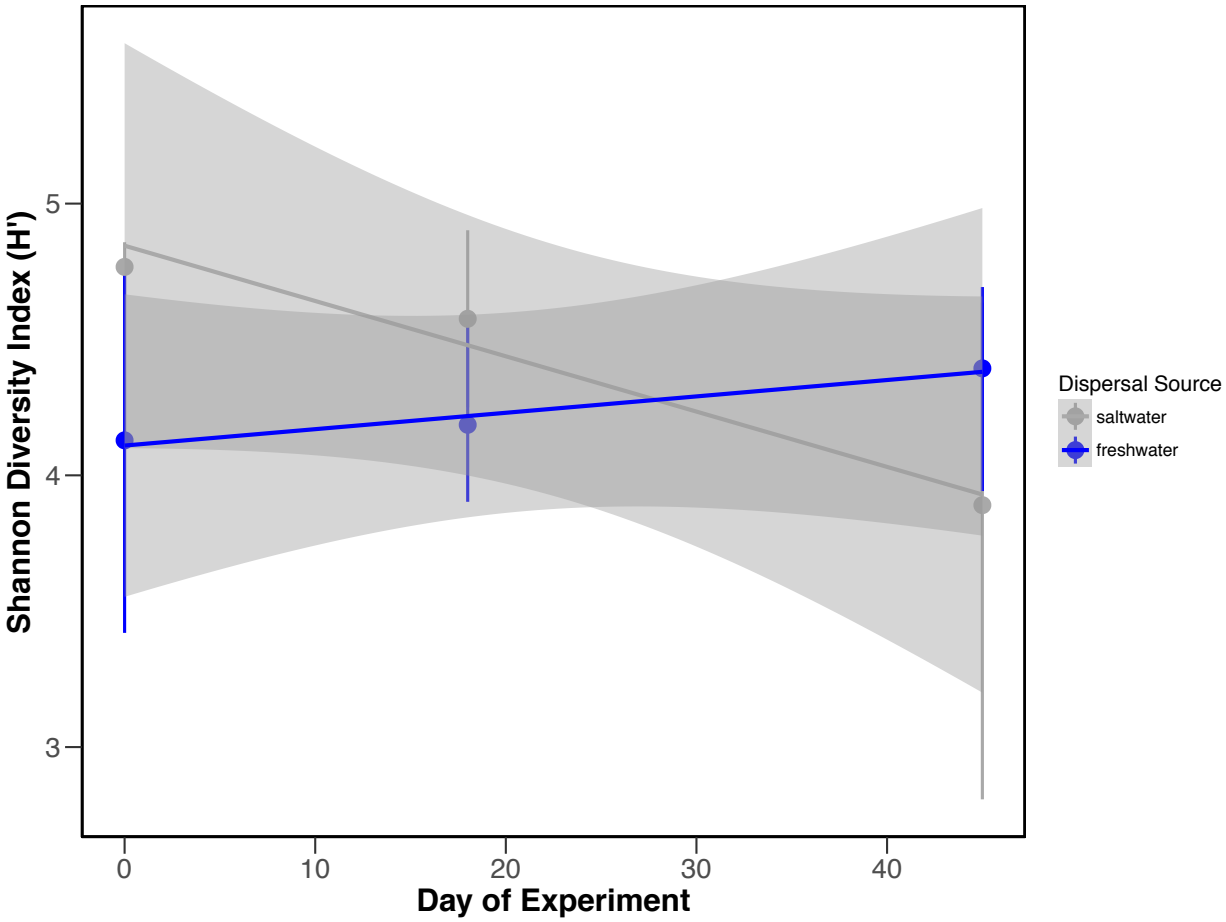


Figure 5. Shannon Diversity Index (H′) of bacterial communities over time according to dispersal source. Blue and gray circles represent the estimated mean of bacterial diversity for the freshwater and saltwater dispersal source communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The blue lines represent the trend of bacterial diversity for the freshwater dispersal source, and the gray lines represent the trend of the diversity for the saltwater dispersal source. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model.

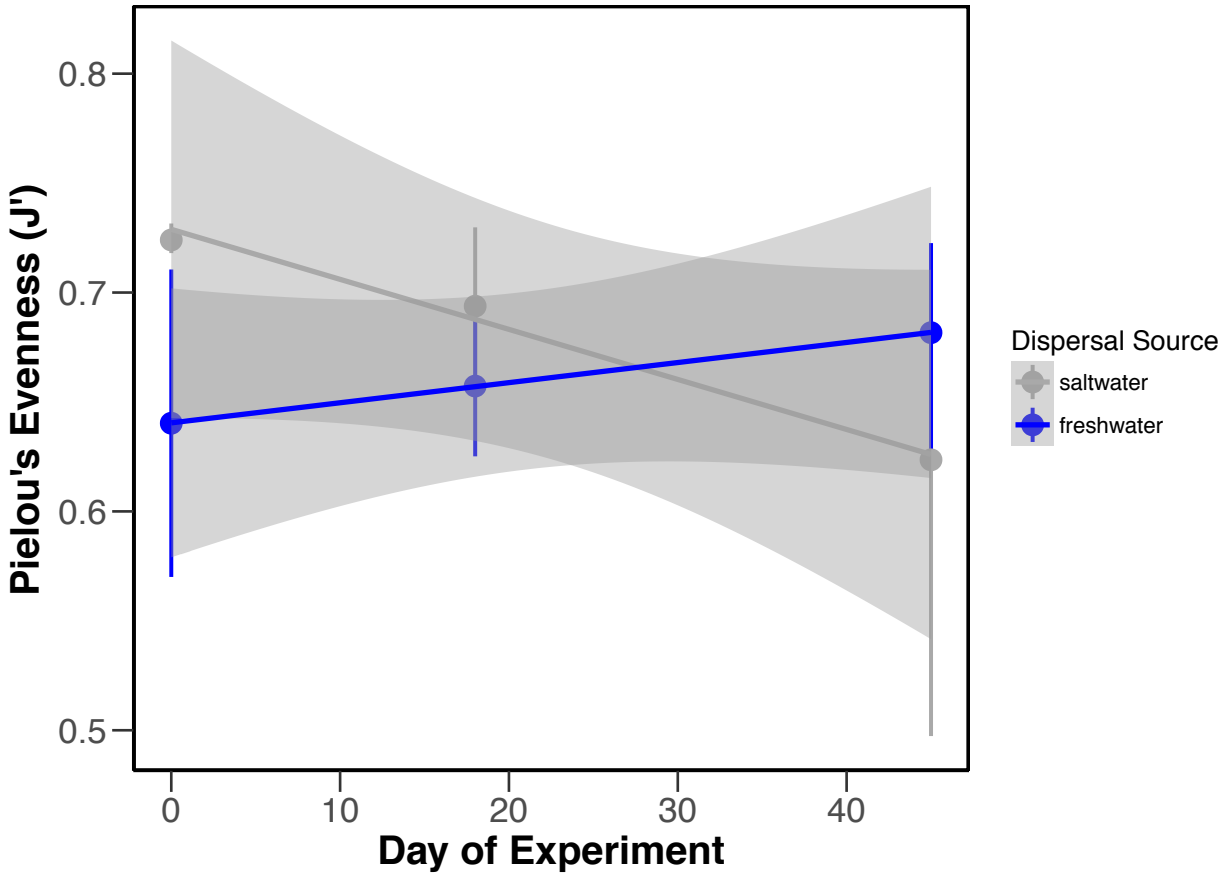


Figure 6. Species evenness (Pielou's evenness, J′) of bacterial communities over time according to dispersal source. Blue and gray circles represent the estimated mean of bacterial evenness for the freshwater and saltwater dispersal source communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The blue lines represent the trend of bacterial evenness for the freshwater dispersal source, and the gray lines represent the trend of the evenness for the saltwater dispersal source. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model.

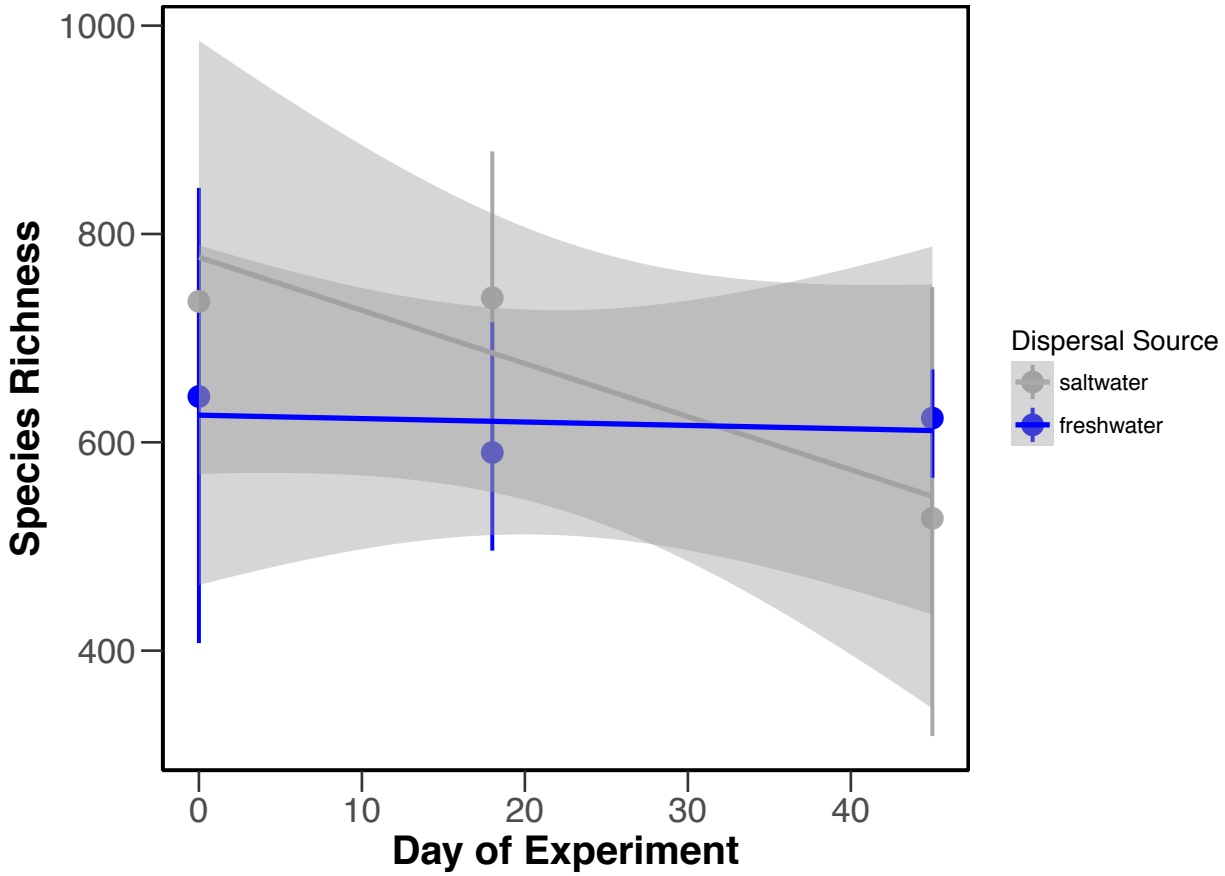


Figure 7. Species richness of bacterial communities over time according to dispersal source. Blue and gray circles represent the estimated mean of bacterial richness for the freshwater and saltwater dispersal source communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The blue lines represent the trend of bacterial richness for the freshwater dispersal source, and the gray lines represent the trend of the richness for the saltwater dispersal source. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model.

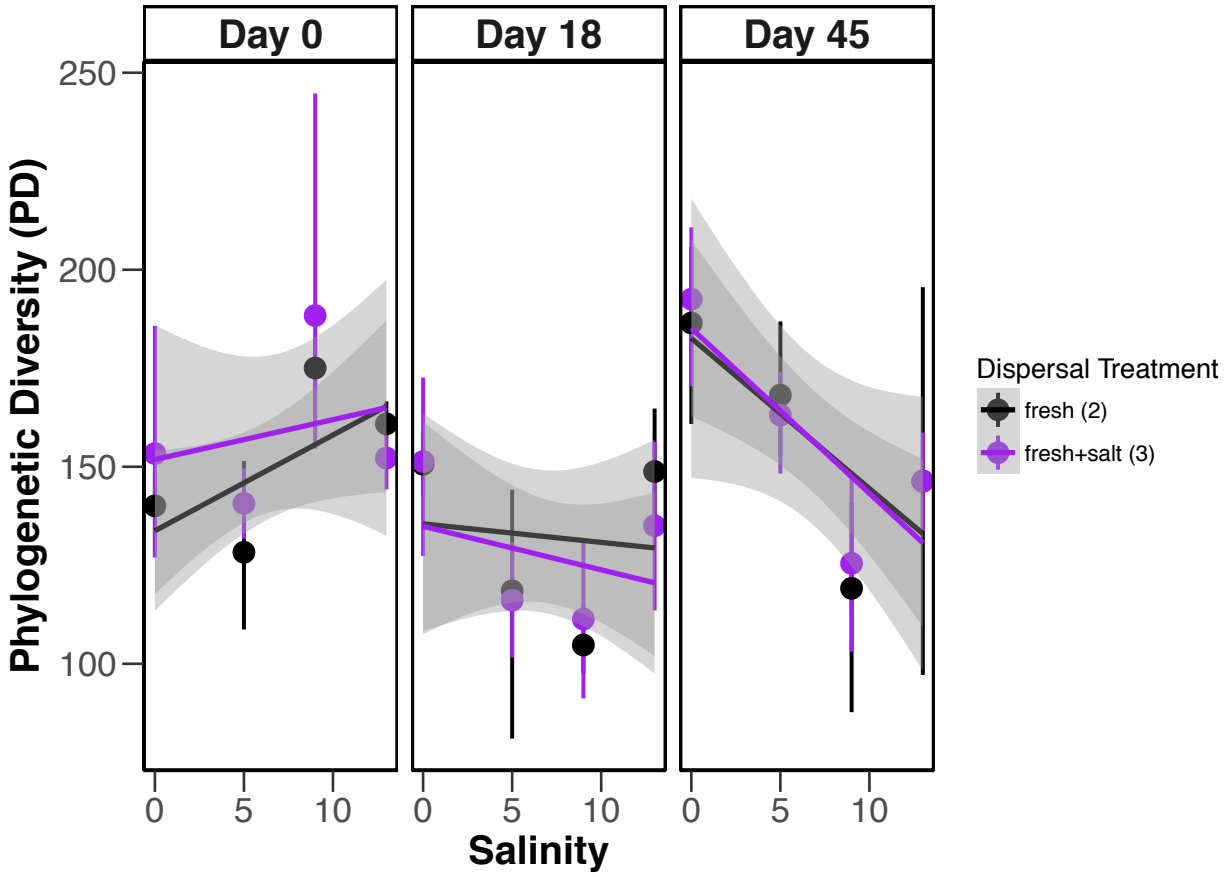


Figure 8. Phylogenetic diversity (PD) of bacterial communities along a salinity gradient according to dispersal treatment. Black and purple circles represent the estimated mean of bacterial PD for the fresh and fresh+salt dispersal communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The black lines represent the trend of the PD for the fresh dispersal treatment, and the purple lines represent the trend of the PD for the fresh+salt dispersal treatment. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model.

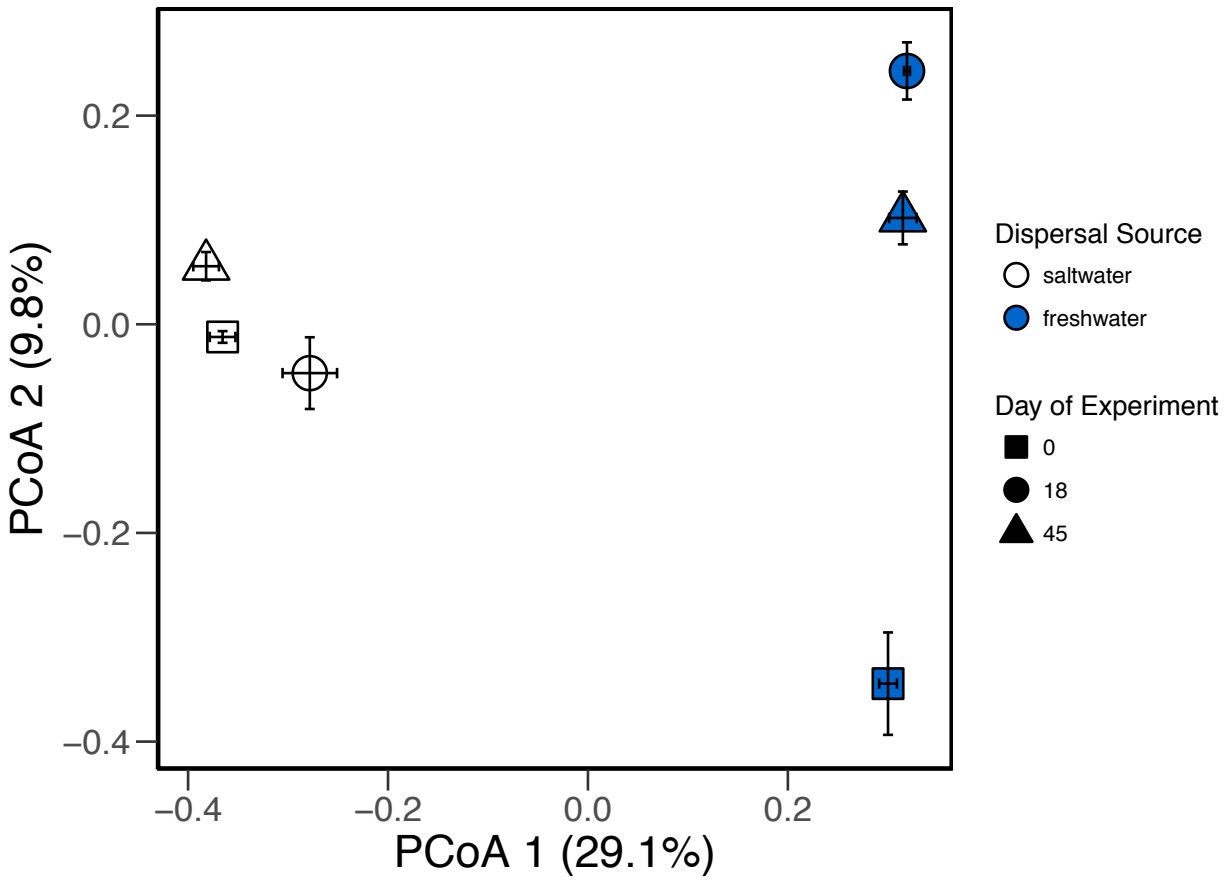


Figure 9. Principle coordinates analysis (PCoA) of only dispersal source bacterial communities. Each shape represents a date; square represents day 0, circle represents day 18, and triangle represents day 45. Each color represents a dispersal source bacterial community; white is the saltwater source tank and blue is the freshwater source tank. The error bars surrounding each point represent the bacterial communities from four experimental replicates. The axes of the PCoA show a percentage of variation that is explained by the analysis; PCoA 1 is the primary axis and PCoA 2 is the secondary axis.

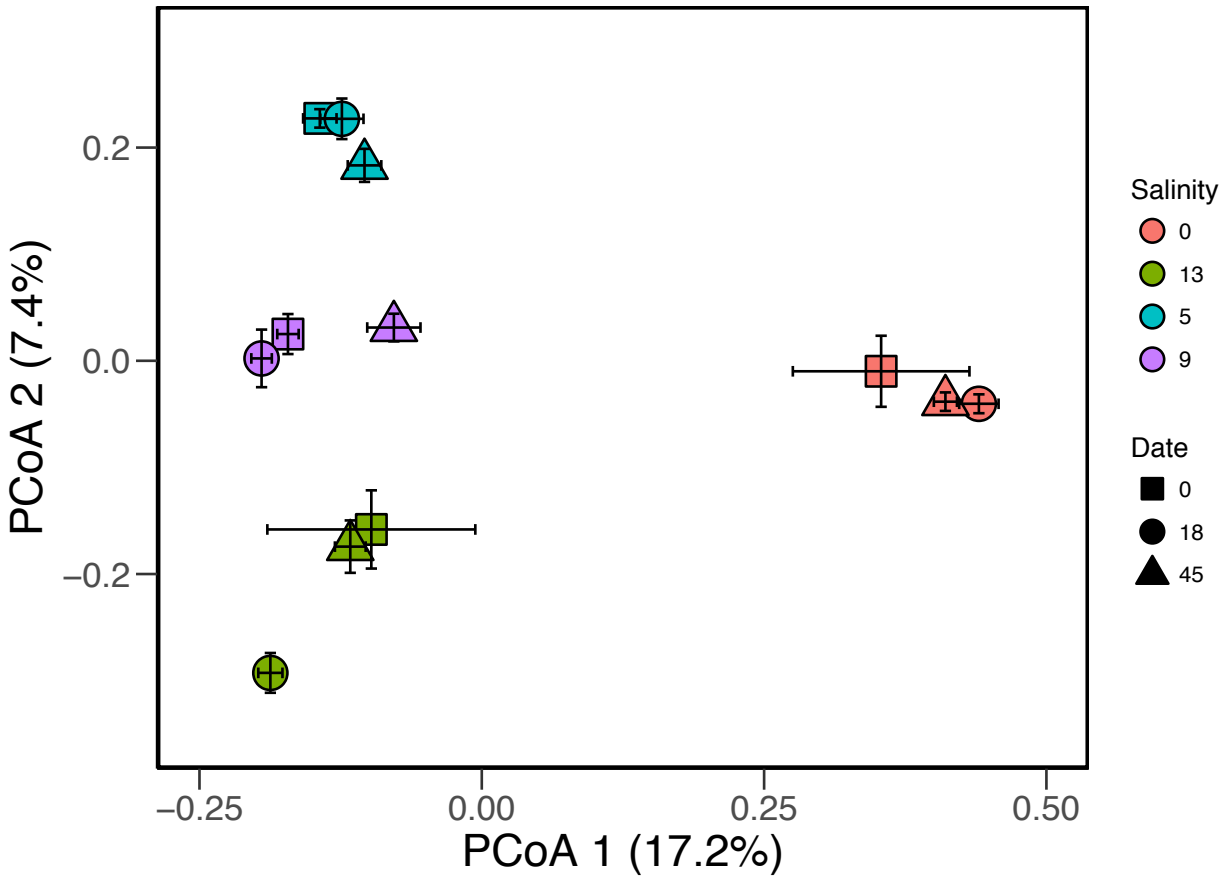


Figure 10. Principle coordinates analysis (PCoA) of bacterial communities exposed to dispersal treatments (no source communities) according to salinity. Each shape represents the day of experiment: square represents day 0, circle represents day 18, and triangle represents day 45. Each color represents a salinity level: pink is salinity 0, blue is salinity 5, purple is salinity 9, and green is salinity 13. The error bars surrounding each point represent the bacterial communities from four experimental replicates. The axes of the PCoA show a percentage of variation that is explained by the analysis; PCoA 1 is the primary axis and PCoA 2 is the secondary axis.

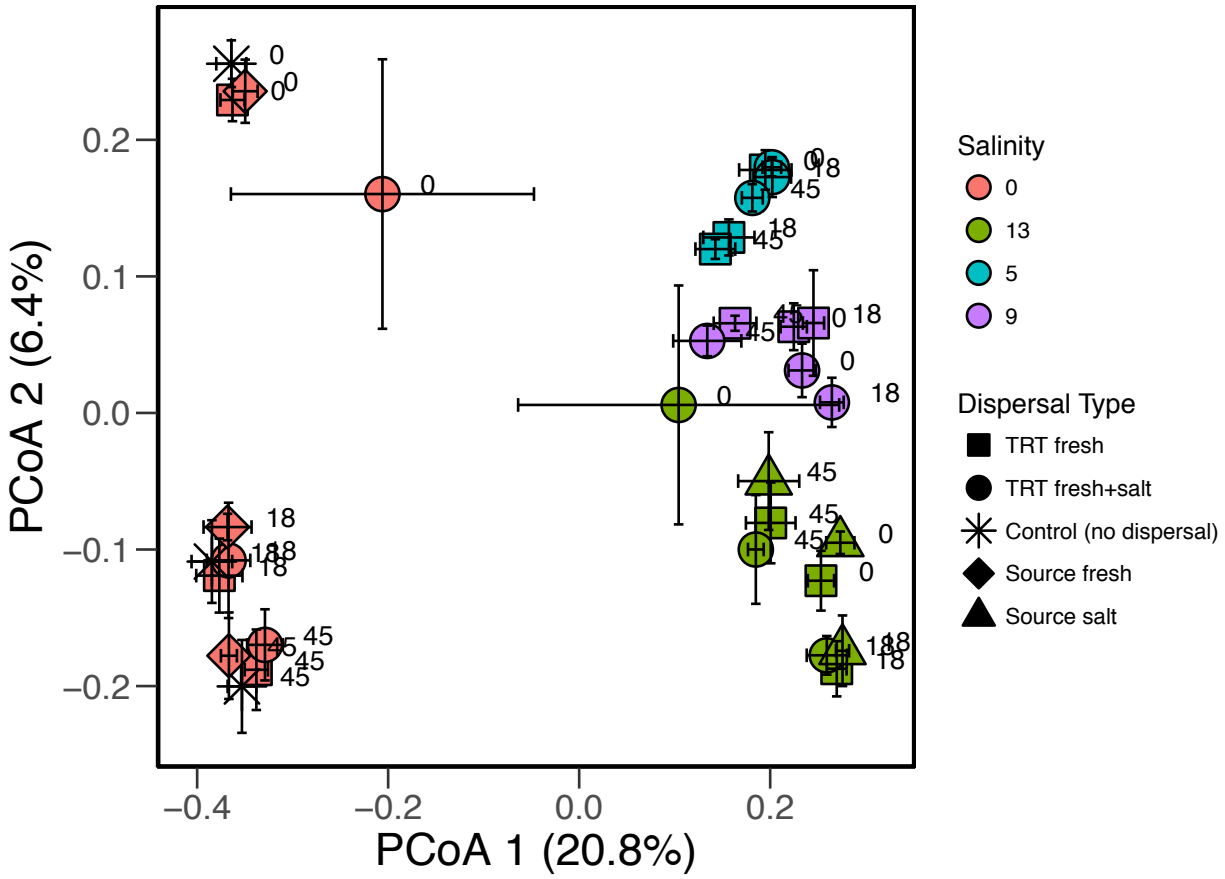


Figure 11. Principle coordinates analysis (PCoA) of all source and treatment bacterial communities according to salinity at each sampling date. Each shape represents a dispersal type: square represents fresh treatment, circle represents fresh+salt treatment, asterisk represents the control (no dispersal), diamond represents source freshwater, and triangle represents source saltwater. Each color represents a salinity level: pink is salinity 0, blue is salinity 5, purple is salinity 9, and green is salinity 13. The date of experiment is shown as a label to the right of each point on the figure (day 0, 18 or 45). The error bars surrounding each point represent the bacterial communities from four experimental replicates. The axes of the PCoA show a percentage of variation that is explained by the analysis; PCoA 1 is the primary axis and PCoA 2 is the secondary axis.

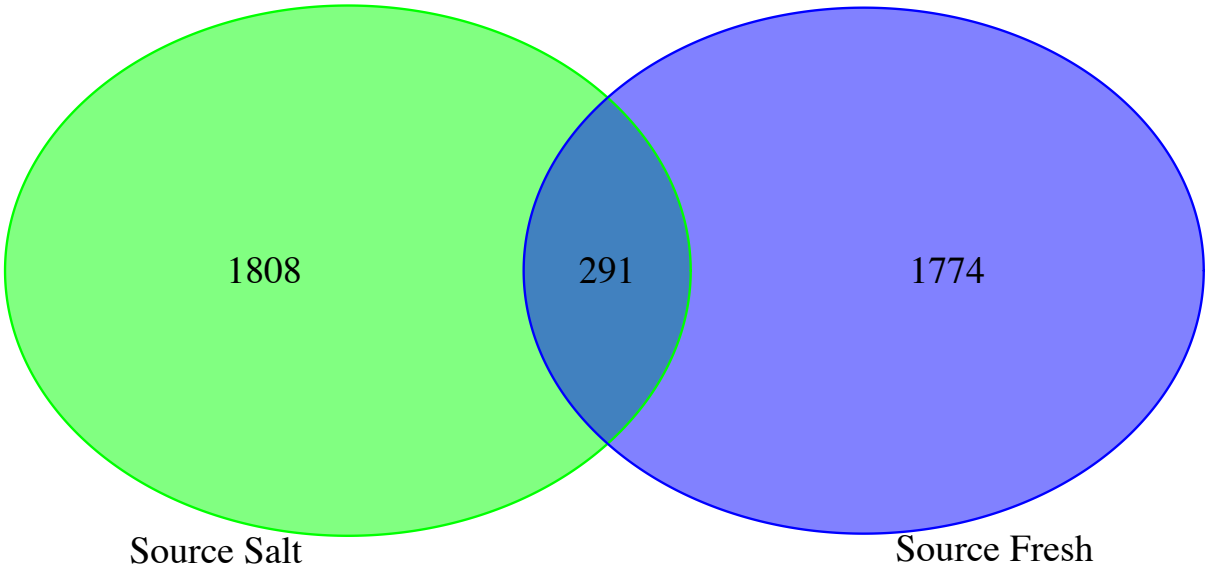


Figure 12. A Venn diagram comparing the number of unique and shared bacterial taxa (OTUs) of source freshwater and saltwater bacterial communities. The overlapping area represents the number of shared taxa, and the portions that do not overlap are unique taxa.

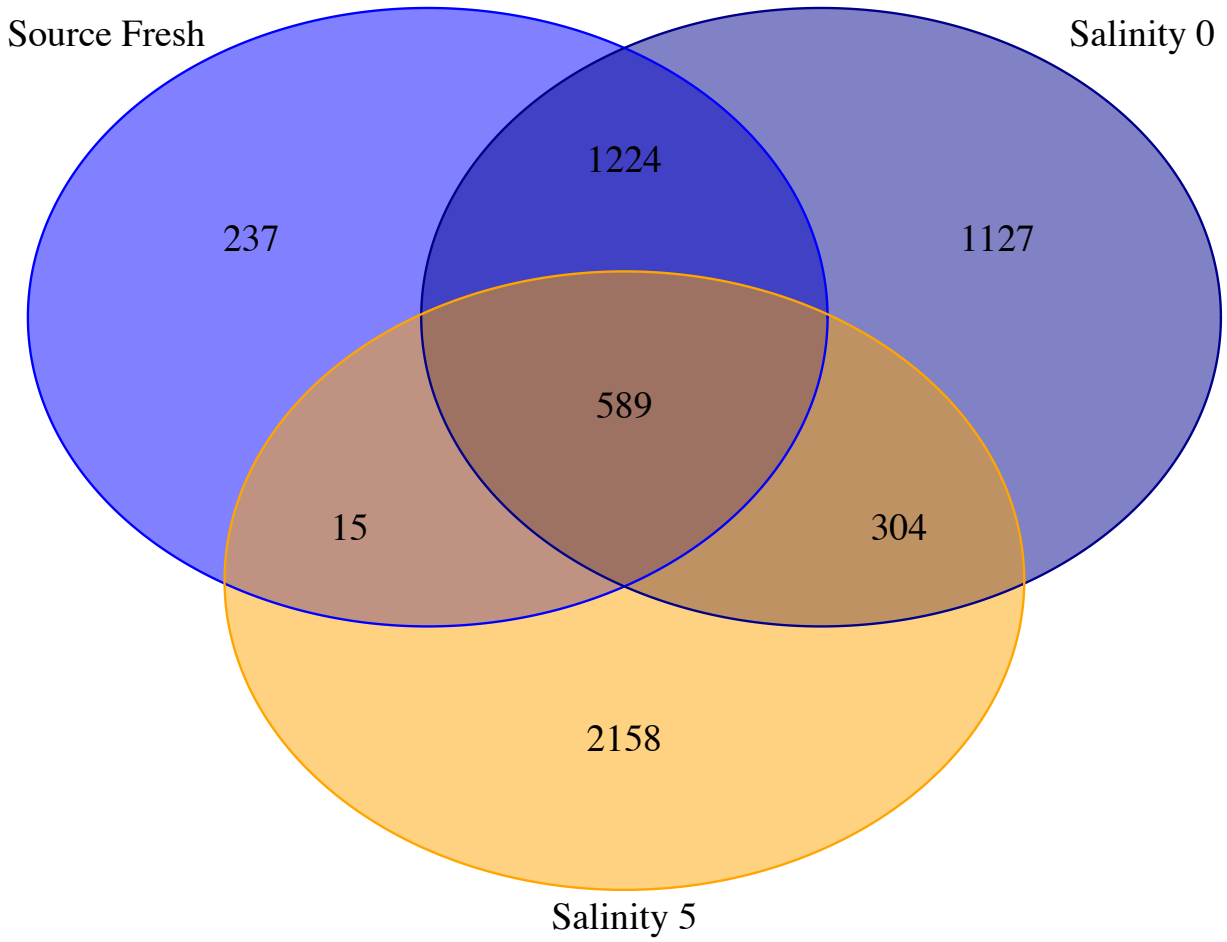


Figure 13. A Venn diagram comparing the number of unique and shared bacterial taxa (OTUs) of source freshwater, salinity 0, and salinity 5 bacterial communities. The overlapping area of two circles represents the number of shared taxa between those two tanks, the area where all three circles overlap is the number of shared taxa between all tanks, and the portions that do not overlap are unique taxa.

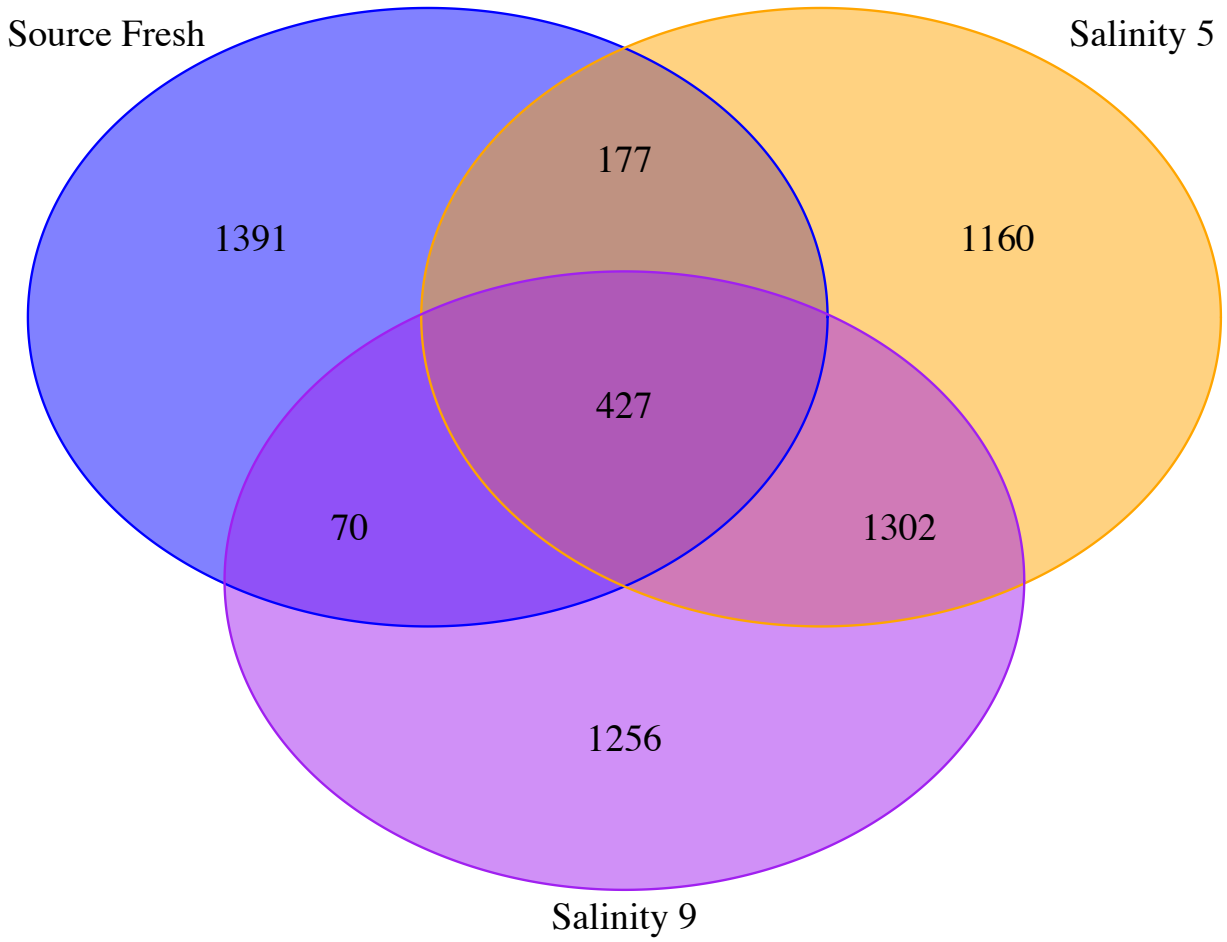


Figure 14. A Venn diagram comparing the number of unique and shared bacterial taxa (OTUs) of source freshwater, salinity 5, and salinity 9 bacterial communities. The overlapping area of two circles represents the number of shared taxa between those two tanks, the area where all three circles overlap is the number of shared taxa between all tanks, and the portions that do not overlap are unique taxa.

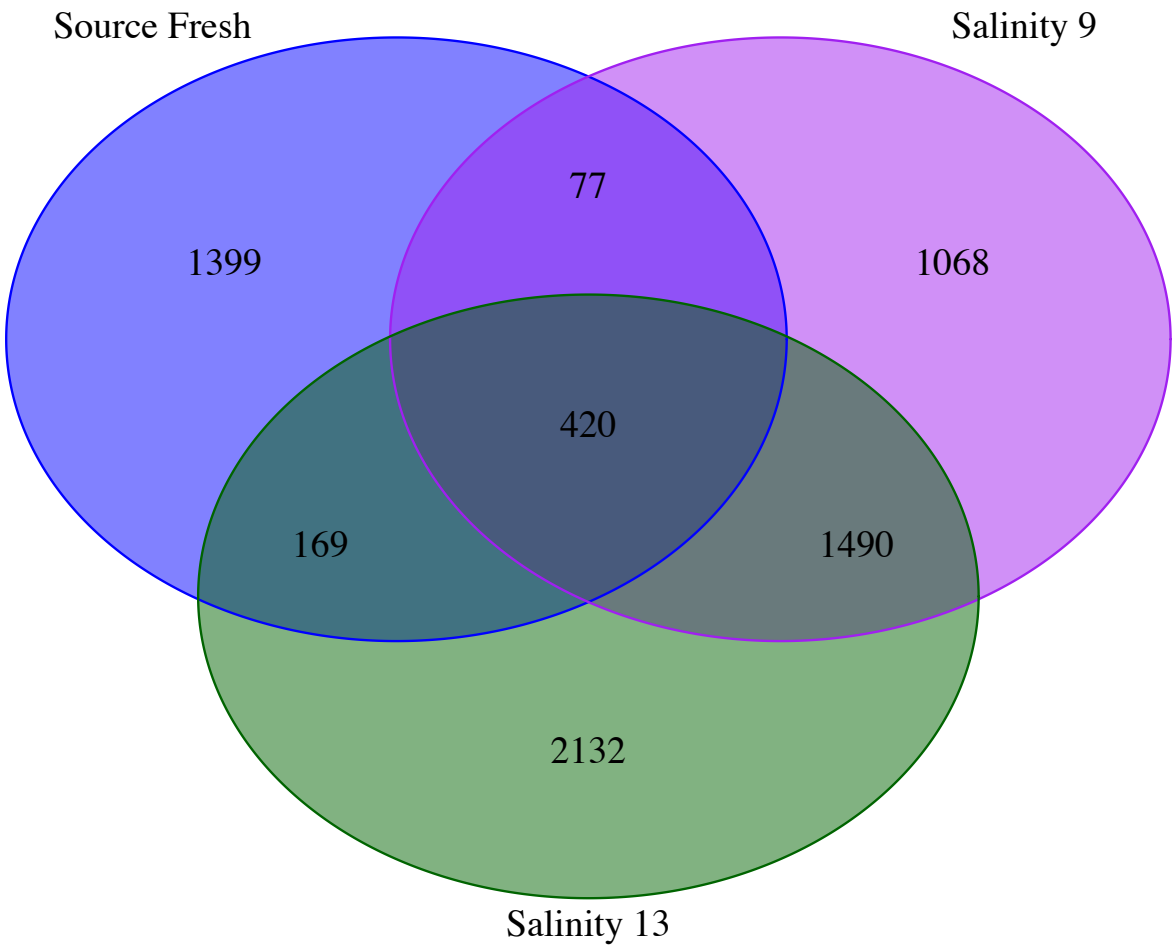


Figure 15. A Venn diagram comparing the number of unique and shared bacterial taxa (OTUs) of source freshwater, salinity 9, and salinity 13 communities. The overlapping area of two circles represents the number of shared taxa between those two tanks, the area where all three circles overlap is the number of shared taxa between all tanks, and the portions that do not overlap are unique taxa.

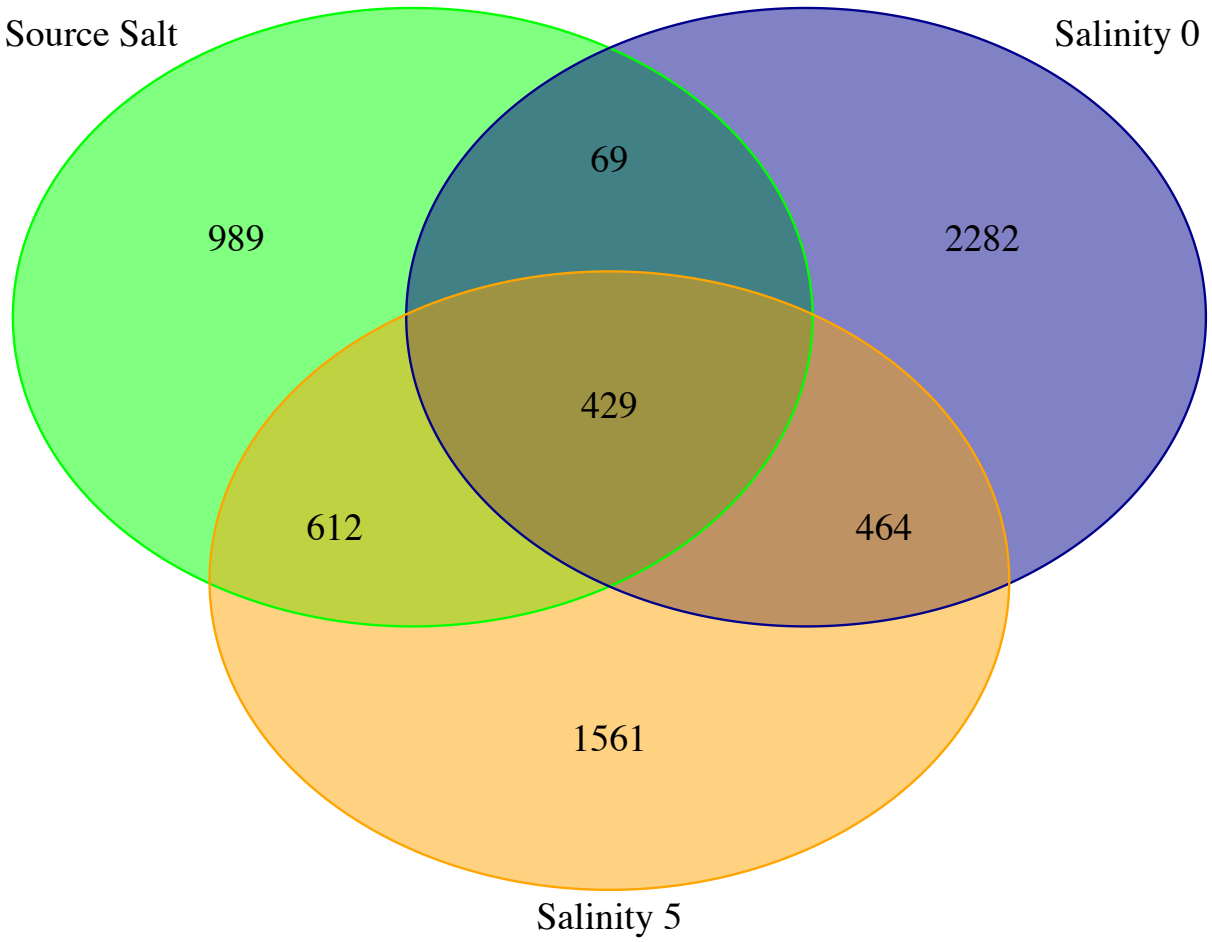


Figure 16. A Venn diagram comparing the number of unique and shared bacterial taxa (OTUs) in source saltwater, salinity 0, and salinity 5 bacterial communities. The overlapping area of two circles represents the number of shared taxa between those two tanks, the area where all three circles overlap is the number of shared taxa between all tanks, and the portions that do not overlap are unique taxa.

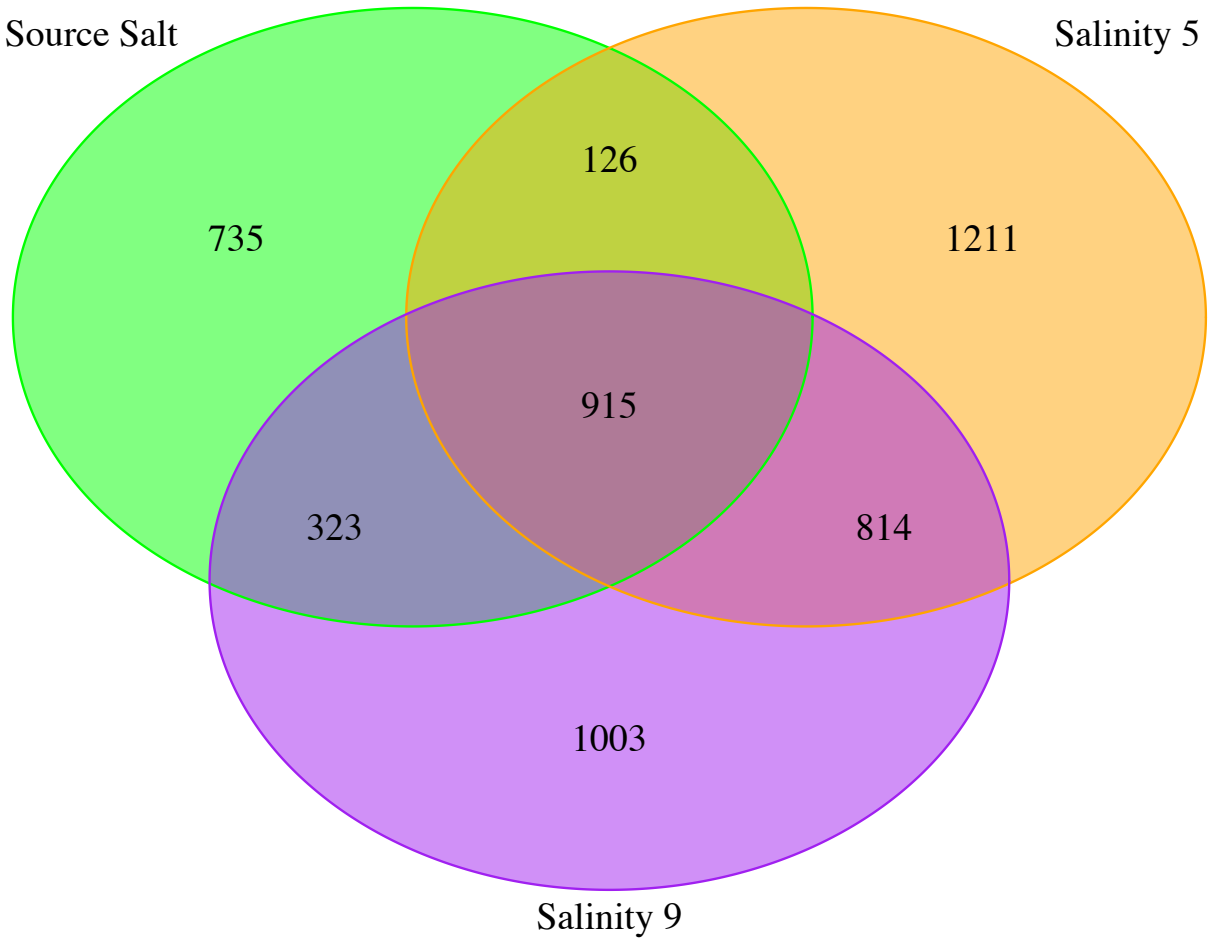


Figure 17. A Venn diagram comparing the number of unique and shared bacterial taxa (OTUs) in saltwater, salinity 5, and salinity 9 bacterial communities. The overlapping area of two circles represents the number of shared taxa between those two tanks, the area where all three circles overlap is the number of shared taxa between all tanks, and the portions that do not overlap are unique taxa.

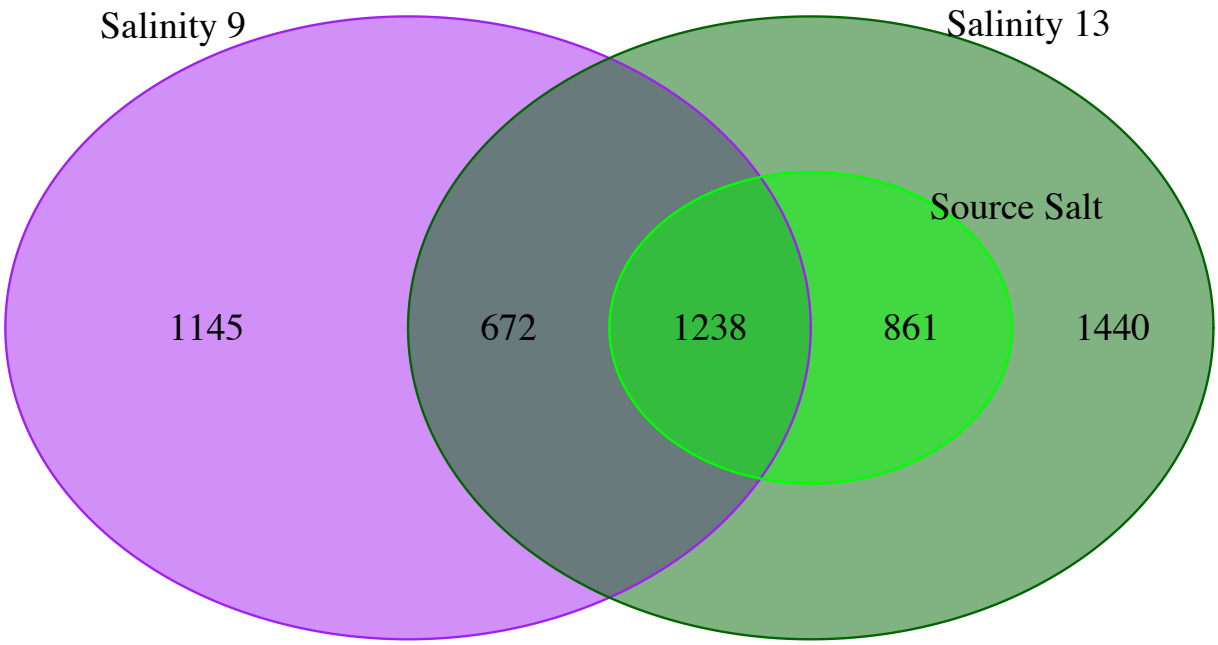


Figure 18. A Venn diagram comparing the number of unique and shared bacterial taxa (OTUs) in source saltwater, salinity 9, and salinity 13 bacterial communities. The overlapping area of two circles represents the number of shared taxa between those two tanks, the area where all three circles overlap is the number of shared taxa between all tanks, and the portions that do not overlap are unique taxa. The areas where one circle is enveloped by another represents that all taxa within the inner circle also exist in the outer circle.

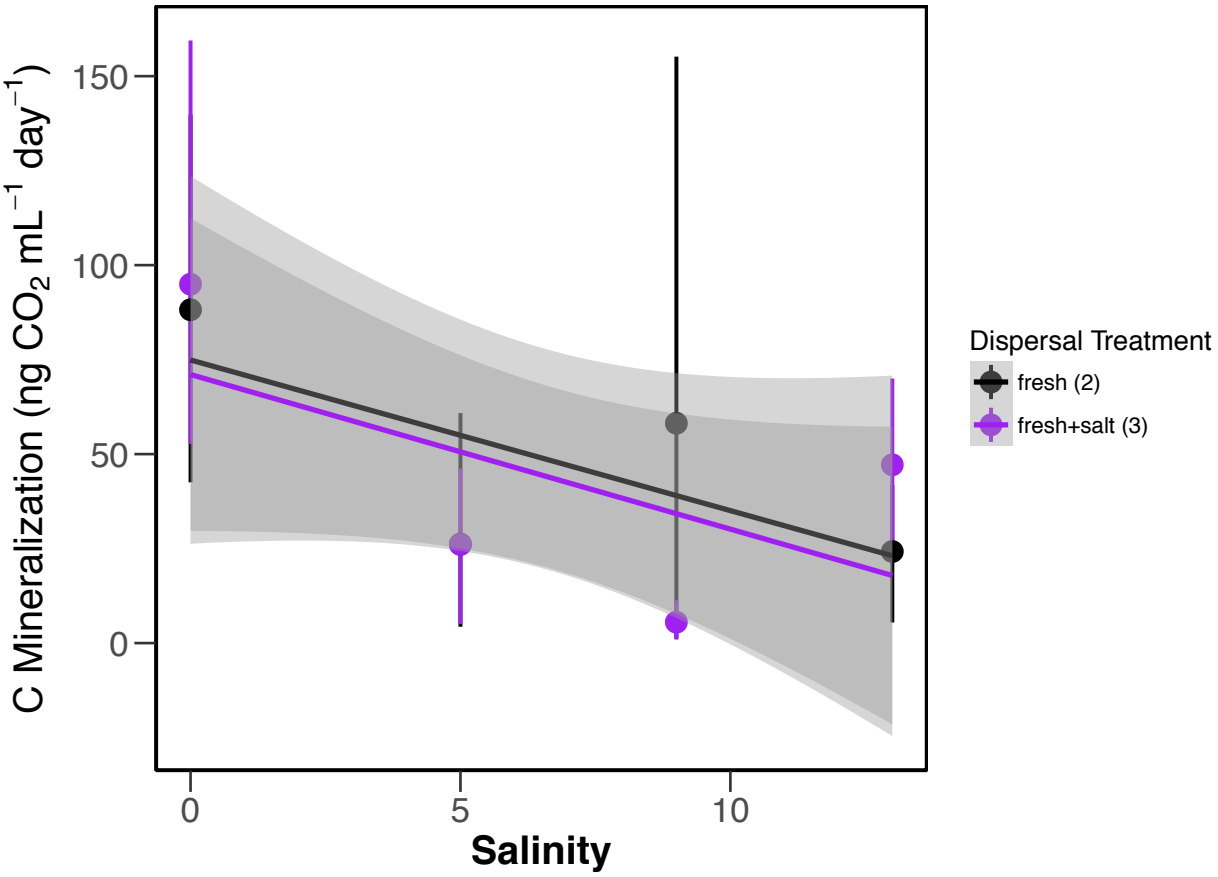


Figure 19. Carbon mineralization rate (nanograms of carbon dioxide per milliliter per day) of bacterial communities along a salinity gradient according to dispersal treatment. Black and purple circles represent the estimated mean of carbon mineralization rates for the fresh and fresh+salt dispersal communities, respectively, along the salinity gradient, with vertical lines representing estimated confidence interval (95%) around the mean. The black line represents the trend of the carbon mineralization rates for the fresh dispersal treatment, and the purple line represents the trend of the rates for the fresh+salt dispersal treatment. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model. Carbon mineralization rates measured at the end of the experiment (Day 45).

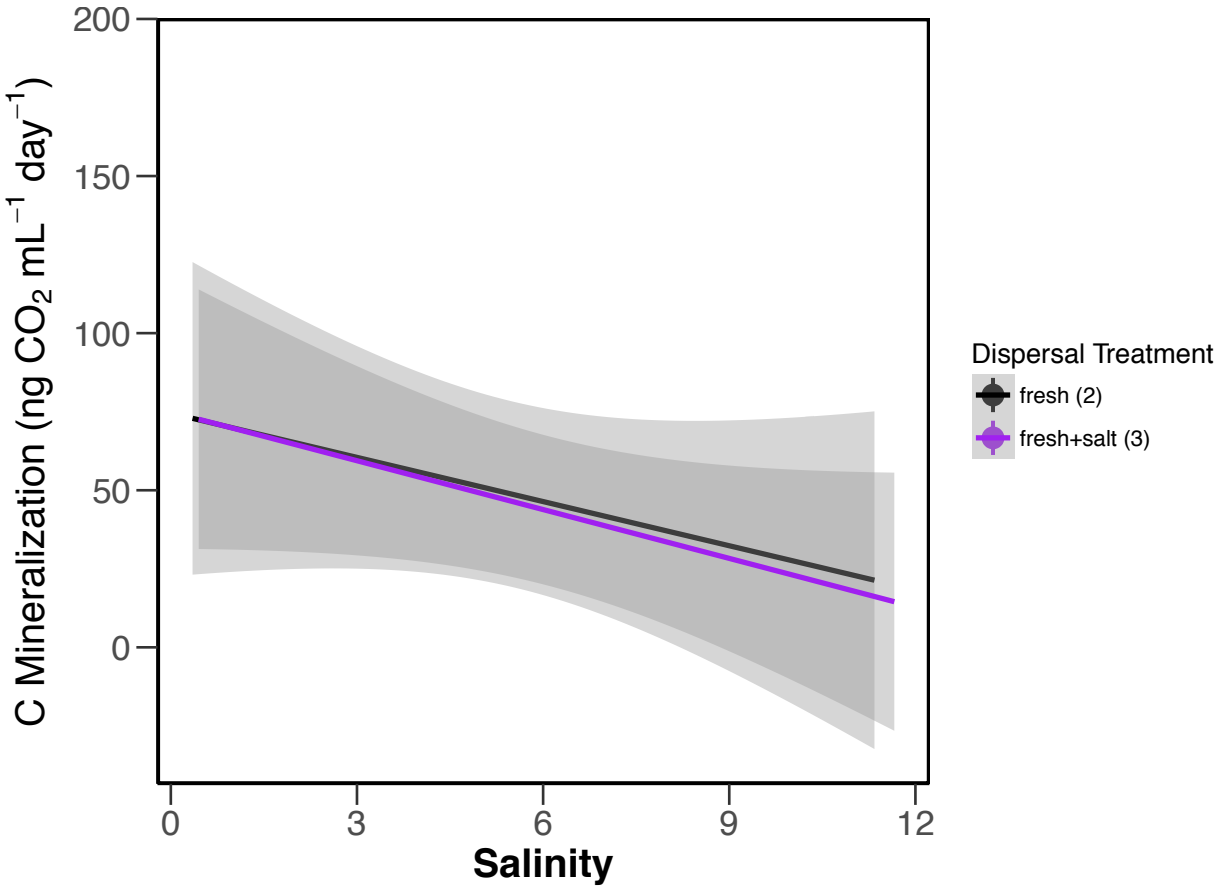


Figure 20. Carbon mineralization rate (nanograms of carbon dioxide per milliliter per day) of bacterial communities along measured salinity according to dispersal treatment. The black line represents the trend of the carbon mineralization rates for the fresh dispersal treatment, and the purple line represents the trend of the rates for the fresh+salt dispersal treatment. The gray confidence band around the points represents the 95% confidence level interval for predictions from a linear model. Carbon mineralization rates measured at the end of the experiment (Day 45).

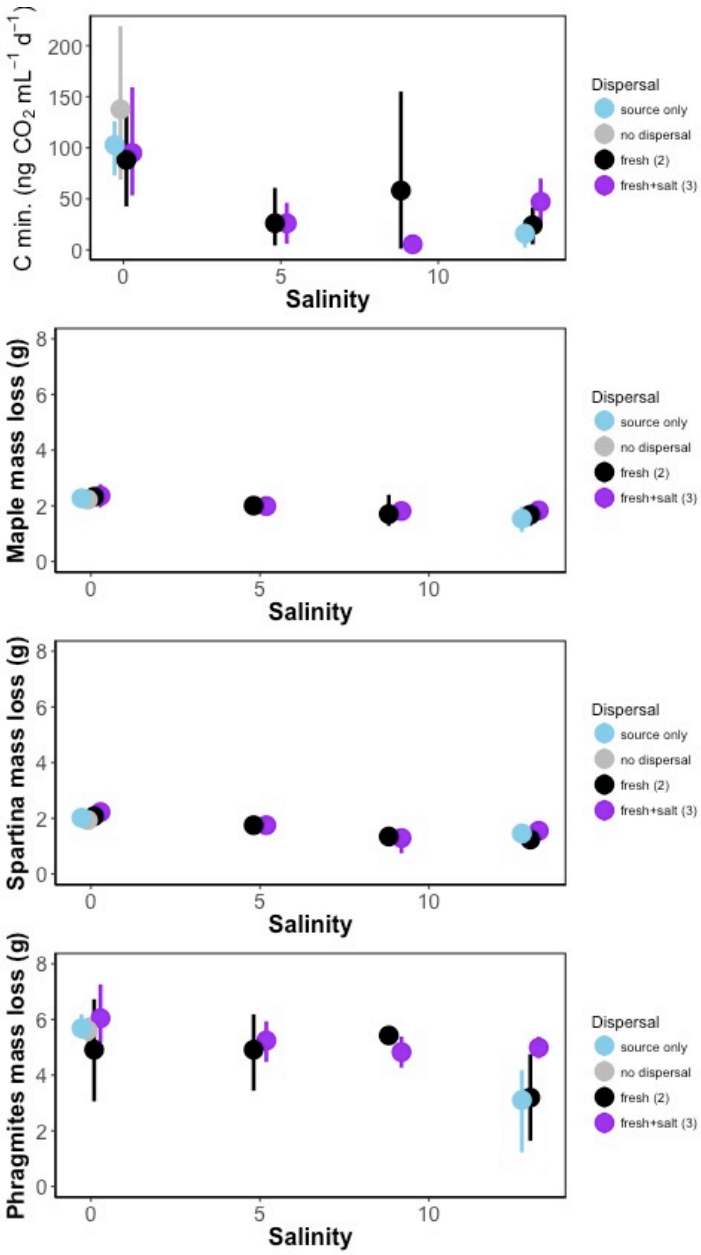


Figure 21. Ecosystem function measured as carbon mineralization and litter decomposition rates along salinity gradient according to dispersal type. The symbol colors represent dispersal type: light blue = source tanks only, gray = no dispersal type (control), black = fresh dispersal type, and purple = fresh+salt dispersal type. For the top plot, each point represents the mean carbon mineralization rate (nanograms of carbon dioxide per milliliter per day) as a function of salinity. For the second plot, each point represents the mean mass loss of *Acer rubrum* (red maple) in grams as a function of salinity. For the third plot, each point represents the mean mass loss of *Spartina alterniflora* (smooth cordgrass) in grams as a function of salinity. For the bottom plot, each point represents the mean mass loss of *Phragmites australis* (common reed) in grams as a function of salinity. The lines surrounding each symbol represent estimated confidence interval (95%) around the mean. Ecosystem function measured at the end of the experiment (Day 45).

Table 1. Permutational analysis of variance (PERMANOVA) table comparing dispersal source bacterial communities. Table displays degrees of freedom (df), sum of squares (sums of sqs), mean square (mean sqs), F statistic (F model), R^2 (measure of fit to regression line), and P-value.

Effect	df	Sums of Sqs	Mean Sqs	F model	R^2	P-value
Date	2	1.133	0.566	2.491	0.133	**0.003**
Dispersal	1	2.442	2.442	10.741	0.287	**0.001**
Date:Dispersal	2	1.069	0.535	2.351	0.126	**0.006**
Residuals	17	3.865	0.227	0.454		
Total	22	8.509	1.000			

Table 2. Permutational analysis of variance (PERMANOVA) table comparing bacterial communities according to date, dispersal type (no source communities), and salinity. Table displays degrees of freedom (df), sum of squares (sums of sqs), mean square (mean sqs), F statistic (F model), R^2 (measure of fit to regression line), and P-value.

Effect	df	Sums of Sqs	Mean Sqs	F model	R^2	P-value
Date	1	1.781	1.781	5.585	0.052	**0.001**
Dispersal	1	0.248	0.248	0.778	0.007	0.795
Salinity	1	3.905	3.905	12.242	0.115	**0.001**
Date:Dispersal	1	0.162	0.162	0.507	0.005	1.000
Date:Salinity	1	0.917	0.917	2.876	0.027	**0.002**
Dispersal:Salinity	1	0.297	0.297	0.930	0.009	0.519
Date:Dispersal:Salinity	1	0.263	0.263	0.825	0.008	0.703
Residuals	83	26.472	0.319	0.778		
Total	90	34.045	1.000			

Table 3. Permutational analysis of variance (PERMANOVA) table comparing bacterial communities according to date, dispersal type, and salinity. Table displays degrees of freedom (df), sum of squares (sums of sqs), mean square (mean sqs), F statistic (F model), R^2 (measure of fit to regression line), and P-value.

Effect	df	Sums of Sqs	Mean Sqs	F model	R^2	P-value
Date	2	3.474	1.737	5.993	0.072	**0.001**
Dispersal	4	5.441	1.360	4.692	0.112	**0.001**
Salinity	1	3.891	3.891	13.422	0.080	**0.001**
Date:Dispersal	8	2.905	0.363	1.253	0.060	**0.024**
Date:Salinity	2	1.545	0.772	2.664	0.032	**0.001**
Dispersal:Salinity	1	0.299	0.299	1.032	0.006	0.363
Date:Dispersal:Salinity	2	0.466	0.233	0.804	0.010	0.837
Residuals	105	30.438	0.290	0.628		
Total	125	48.459	1.000			

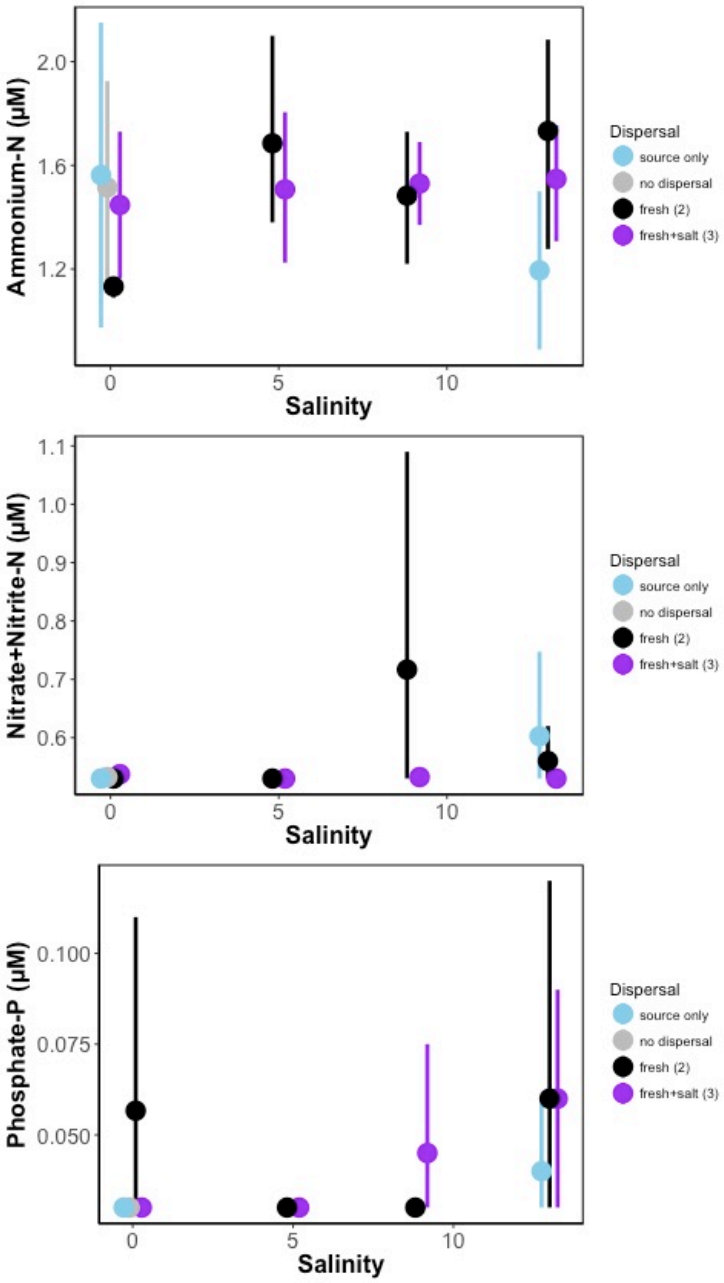
Table 4. Abbreviated species indicator analysis table (full table available at: https://github.com/PeraltaLab/CSI_Dispersal/tree/master/data; file: BacterialIndicators.txt). Table displays the OTUs with highest indicator values (indval) in each cluster, where clusters represent each salinity treatment (cluster 1 = salinity 0, 2 = salinity 5, 3 = salinity 9, 4 = salinity 13). The phylum, class, order, family, and genus are matched with each OTU.

OTU	Cluster	IndVal	Phylum	Class	Order	Family	Genus
Otu00152	1	0.991	Proteobacteria	Proteobacteria_unclassified	Proteobacteria_unclassified	Proteobacteria_unclassified	Proteobacteria_unclassified
Otu00049	1	0.990	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillales_unclassified	Rhodospirillales_unclassified
Otu00007	1	0.983	Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae	Polynucleobacter
Otu00105	1	0.980	Verrucomicrobia	Spartobacteria	Spartobacteria_order_incertae_sedis	Spartobacteria_family_incertae_sedis	Spartobacteria_genera_incertae_sedis
Otu00129	1	0.954	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified
Otu00204	1	0.952	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00201	1	0.947	Verrucomicrobia	Subdivision3	Subdivision3_order_incertae_sedis	Subdivision3_family_incertae_sedis	3_genus_incertae_sedis
Otu00017	1	0.940	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Chitinophagaceae	Sediminibacterium
Otu00033	1	0.929	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiales_unclassified	Verrucomicrobiales_unclassified
Otu00030	1	0.919	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonadaceae_unclassified
Otu00159	1	0.913	Bacteroidetes	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified	Bacteroidetes_unclassified
Otu00051	1	0.905	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	Roseomonas

Otu00144	1	0.905	Acidobacteria	Acidobacteria_Gp3	Acidobacteria_Gp3_unclassified	Acidobacteria_Gp3_unclassified	Acidobacteria_Gp3_unclassified
Otu00224	1	0.904	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonadaceae_unclassified
Otu00137	2	0.937	Proteobacteria	Betaproteobacteria	Betaproteobacteria_unclassified	Betaproteobacteria_unclassified	Betaproteobacteria_unclassified
Otu00041	2	0.889	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Flavobacterium
Otu00086	2	0.852	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Alcaligenaceae_unclassified
Otu00464	2	0.829	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00053	2	0.823	Bacteroidetes	Sphingobacteria	Sphingobacteriales	Cyclobacteriaceae	Cyclobacteriaceae_unclassified
Otu00347	3	0.944	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00216	3	0.891	Planctomycetes	Planctomycetes_unclassified	Planctomycetes_unclassified	Planctomycetes_unclassified	Planctomycetes_unclassified
Otu00067	3	0.825	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00016	3	0.772	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00230	3	0.722	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00110	4	0.870	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Otu00375	4	0.869	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Haliea
Otu00220	4	0.856	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified

Otu00221	4	0.847	Proteobacteria	Alphaproteobacteria	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified	Alphaproteobacteria_unclassified
Otu00241	4	0.823	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified

APPENDIX A: SUPPLEMENTAL INFORMATION



Supplemental Figure S1. Nutrient concentrations measured along salinity gradient according to dispersal type. The symbol colors represent dispersal type: light blue = source tanks only, gray = no dispersal type (control), black = fresh dispersal type, and purple = fresh+salt dispersal type. For the top plot, each point represents the mean ammonium concentration (µM) as a function of salinity according to dispersal type. For the second plot each point represents the mean nitrate plus nitrite concentration (µM) as a function of salinity according to dispersal type. For the third plot, each point represents the mean phosphate concentration (µM) as a function of salinity according to dispersal type. The lines surrounding each symbol represent estimated confidence interval (95%) around the mean. Nutrient concentrations measured at the end of the experiment (Day 45).

Supplemental Table S1. Site characteristics of the coastal ponds used as sources for freshwater and saltwater dispersal sources. Table displays the site name, date of collection, GPS location, temperature in degrees Celsius, salinity on date of collection, and pH on date of collection.

Site	Collection Date	GPS Location	Temperature (°C)	Salinity	pH
A2	19 June 2015	N 35°41.89' W 075°29.041'	28.4	15.12	7.94
A3	19 June 2015	N 35°36.632' W 075°28.074'	30.2	5.2	9.29
Bodie 1	3 May 2015	N 35°49.207' W 075°33.755'	31.9	9.46	8.59
CSI 1	19 June 2015	N 35°52.443', W 075°39.640	29.7	0.2	9.65