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How Do Drying and Rewetting Events Affect Nutrient Fluxes and Bacteria Dynamics in Subtropical Estuarine Sediments?

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How Do Drying and Rewetting Events Affect Nutrient Fluxes and Bacteria Dynamics in Subtropical Estuarine Sediments?

by

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Dedication

I would like to dedicate this to my family, friends and professors who supported, guided, and motivated me during this journey. I would especially like to thank my wife, Naomi, for having the patience and determination to help see me through.

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Abstract

How Do Drying and Rewetting Events Affect Nutrient Fluxes and Bacteria Dynamics in Subtropical Estuarine Sediments?

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Drying and rewetting occur frequently in coastal marsh sediments due to oscillations of rising and falling tides, and episodic droughts and floods. Similarly, drying events also occur within freshwater systems due to changing precipitation patterns. However, little is known about how these events affect biogeochemical processes in sediments. In this study we examined the effects of drying on the release of nutrients from sediments to overlying waters, together with associated bacterial dynamics. We incubated dried and rewetted salt marsh sediments collected from the Nueces River mouth at the Nueces Salt Marsh (NSM) and from a freshwater section of the Mission River (MR) in South Texas. During the incubations, we quantified the nutrients released and changes of bacterial abundance and community structure in slurries of wet and dry sediments under anoxic conditions. Our results showed that ammonium concentrations increased steadily for both NSM and MR dry treatment incubations, reaching a maximum of 203 and 51 μM respectively, as compared to only 124 and 2 μM in the wet treatments.

Phosphate concentrations steadily increased throughout the incubation in the NSM dry treatment, but not in the wet treatment where concentrations remained below 5 μM. In contrast, we observed an opposite trend in the MR sediment with phosphate concentrations in the dry treatment remaining below those in the wet treatment throughout the incubation. The atomic C/N ratios for NSM and MR sediments ranged from 10 to 14 for both MR and NSM treatments, however they were significantly lower in the supernatants of the NSM dry treatment (<5) than those in supernatants of the NSM wet treatment and in both MR wet and dry treatments (>12). Although both NSM and MR had higher ammonium releases in the dry treatments than the wet ones, patterns in phosphate release and C/N ratios of dissolved organic matter differed in these two sediments, likely resulting from the differences in salinity and grain size distribution. Bacteria that developed in the slurry of NSM dried sediment included *Bacillus*, *Anaerobacillus, Haloplasma,* and *Vibrio*; these species were perhaps involved in decomposing sedimentary organic matter, including lysates from biota killed by the drying. The MR sediment slurry developed a different microbial community, where *Gemmobacter*, *Rhodobacter*, and *Mycoplasma* were most notable in the dried treatment. Overall, this study demonstrates that drying and rewetting events can increase nutrient fluxes out of marsh sediments and affect bacterial communities, important in estuarine biogeochemical processes. Information on this topic is important in the context of the increasing frequency of extreme droughts and floods and rising sea levels associated with global change.

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INTRODUCTION

Balance of nutrients in estuaries is critical to maintaining healthy and productive estuarine ecosystems (Bricker et al., 2008; Justić et al., 1995). This balance rests delicately on the various levels of nutrients and organic matter entering the estuary, whether from normal river flow regimes or strong episodic storms that rinse surrounding watersheds (Crain, 2007; Livingston et al., 1997). These processes introduce high levels of organic matter and nutrients into the ecosystem, which can promote higher levels of productivity, and may lead to harmful algae blooms (HABs) and hypoxia (Costanza et al., 2008; Rabalais et al., 2002). High turbidity and lower light levels can also decrease primary productivity (Cloern, 1987). Sediments in coastal estuaries, such as those from tidal flats, shore banks and river systems, help maintain these balances.

Coastal marsh sediments are responsible for multiple important ecological functions. They provide support and protection against coastal erosion and storm surge (Costanza et al., 2008; Gedan et al., 2011), trap and encapsulate pollutants and excess nutrients (Etheridge et al., 2015; Nixon et al., 1996; Valiela et al., 1973), and host microorganisms with high diversity (Nedwell et al., 1999). The water content in estuarine sediments is vital in providing these functions effectively (de Leeuw et al., 1990). It is known that soil desiccation along with drying and rewetting events disrupts microbial enzyme efficiencies, alter microbial diversity and community composition, and increases phosphate concentrations impacting trophic states in terrestrial and freshwater sediments (Alster et al., 2013; Fazi et al., 2008; Gilbert et al., 2014). However, little research has focused on estuarine sediments from salt marshes and riverbanks, which undergo frequent drying/rewetting events from tidal oscillations or in tidal areas that are

also subject to prolonged droughts. Drying and rewetting events drastically change important properties of salt marsh sediments, such as sorption and water retention capacities and lability of sedimentary organic matter (Gilbert et al., 2014; Liu et al., 2008). When tides rise or rains come, the sediment becomes oversaturated, diminishing its ability to capture nutrients and water. The retained nutrients and pollutants are "rinsed" with flowing water. Ultimately this excess nutrient runoff is transported to coastal watersheds and estuaries, and may lead to eutrophication (Pinckney et al., 2001). Estuarine river systems often experience large influxes of water from strong episodic storms that increase nutrient concentrations and fluxes into coastal areas, impacting organic matter and nutrient dynamics (Mooney and McClelland, 2012). These rivers supply most of the freshwater added to estuaries and coastal bays, which helps maintain the health and productivity of coastal systems (Attrill, 2000; Palmer et al., 2011). Freshwater inflow is vital for mitigating salinity levels, providing nutrients and supporting biodiversity (Dettmann, 2001; Livingston et al., 1997; Montagna et al., 2002). However, these inflow-caused nutrient fluxes can also be problematic, as eutrophication can alter benthic microbial community structure, affect microbial processes, and impact critical ecosystem functions (Meyer-Reil and Koster, 2000). Coastal marsh sediments can experience frequent drying/rewetting events, especially in arid climates, but can also experience prolonged periods of saturation causing the sediments to be waterlogged. Decomposition of organic matter in waterlogged sediments can cause anoxic conditions, production of sulfides, and mineralization of nitrogen (N) and phosphorous (P) (Adam, 1990; Baldwin and Mitchell, 2000; Ponnamperuma, 1972).

Sedimentary microorganisms regulate the biogeochemical cycling of nutrients in sediments, and participate in water column nutrient cycling when suspended due to fluctuating tides, currents, and precipitation (Denef et al., 2001). Different classes of

bacteria dominate different stages of decomposition of organic matter sourced from phytoplankton and marsh plants. They also affect the cycling of nutrients such as N and P (Kirchman, 1994; Nedwell et al., 1999). Marine bacteria are intrinsic to nutrient cycling and estuarine eutrophication mitigation. Genera such as *Candidatus Pelagibacter* are important in the global carbon budget by cycling dissolved organic carbon (DOC), and consuming oxygen during organic matter respiration (Carini et al., 2012). Other genera such as *Bacillus, Sulfurimonas, and Arcobacter* affect the cycling of nutrients such as S, N, P, and DOC (Han and Perner, 2015; Priest, 1977; Wirsen et al., 2002). Bacteria that thrive at lower salinities also affect the health and productivity of estuarine ecosystems. Freshwater and euryhaline bacteria play key roles in the transformation and conversion of nutrients, especially at the oligohaline zones (Holmes et al., 2000). Common genera in low salinity waters include *Gemmobacter, Mycoplasma,* and *Rhodobacter*. Bacterial community structure is affected by environmental conditions, including salinity, temperature, and the water content of the sediment (Potts, 1994; Priest, 1977), thereby affecting the biogeochemical cycling of nutrients. Microenvironments in sediment particles shift between iron/manganese, nitrate, sulfate and CO_2 reduction depending on the presence of oxygen and availability of organic matter. Different types of microorganisms are involved in these processes, and drastic events such as a drying and rewetting, alter the community and shift metabolic pathways.

Droughts and drying events can alter microbial communities, metabolic pathways and organic matter decomposition in soils, and cause an increase in nutrient release rates (Fierer and Schimel, 2002; Kaiser et al., 2015; Schimel et al., 2007). Drying alters bacterial community structure and diversity (Fierer et al., 2003), water retention ability, and soil stability (Liu and Lee, 2006). Decomposition experiments on various terrestrial soils indicate that drying/rewetting events affect C and N dynamics, respiration rates and

microbial communities (Fierer and Schimel, 2002). Drying/rewetting events impacts estuarine sediments in a similar fashion, however these impacts are often magnified because of the dynamic and delicately balanced ecosystem functions occurring in these environments. Chemical processes such as ion exchange and sorption/desorption impact levels of available nutrients such as ammonium concentrations in the water column, processes which are exaggerated by increasing salinity (Rysgaard et al., 1999; Seitzinger et al., 1991). Therefore, it is important to improve our understanding of the effects of drying/rewetting events on ecosystem processes and functions in both marine and fresh estuarine waters.

The objective of this study was to evaluate the release of nutrients and bacterial community changes during decomposition of dried/rewetted salt marsh and freshwater river sediments under anoxic conditions. We chose to conduct the decomposition under anoxic conditions to mimic what is most commonly found in coastal marsh sediments that are subjected to drying events and prolonged saturation. We hypothesized that more nutrients are released from dried sediments than fresh wet sediments, for both salt marsh and freshwater sediments, and that the bacterial communities differ between the dried and fresh wet treatments. Understanding the dynamics of nutrients and the bacterial community in sediments from both salt marsh and freshwater sediments under drying-rewetting conditions will improve our understanding of the effects of droughts and tidal events on estuarine systems. The knowledge should help inform future management decisions and legislation regarding inflows to estuaries by providing critical information about how drying/rewetting events affect the biogeochemistry of these important environments.

MATERIALS & METHODS

Sampling Locations

Nueces Salt Marsh (NSM)

The sampling site, Station 451, was studied previously regarding plant cover (Dunton et al., 2001). It is located at the Nueces River Delta (NRD) in south Texas (Fig.1), which is characterized by a strong negative salinity gradient due to a negative freshwater balance, limited tidal exchange, and high rates of evapotranspiration (Forbes and Dunton, 2006). The sediment at this site is dominated by silts and clays (~60%), but also contains abundant sands (Liu et al., 2013; Wang et al., 2016).

Mission River (MR)

The Mission River sampling site (Fig. 1), located at 28°11'02.29"N, 097°12'47.84"W near the FM 2678 Mission River overpass, experiences widely variable flow rates depending on the magnitude and frequency of local precipitation events that in turn affect the levels of nutrients entering the river (Güneralp et al., 2013; Mooney and McClelland, 2012). The river is formed by the convergence of the Blanco and Medio creeks located in central Refugio county of South Texas and flows to the Gulf coast where it empties into Mission and Copano bays. The sampling site is not influenced tidally so the salinity remains fresh. Data on the grain size distribution at this site are unavailable, but clay and silts are dominant (74%) at an adjacent site to NSM (within 5 miles) (Liu et al., 2013).

Sediment collection and pretreatment

Sediments of NMR and MR were collected from the bank edge using a constructed corer with a diameter of ~ 8 cm. The salinity was 32 at the NSM site and 0.7 at the MR site during sampling in the month of June 2013. Sediment cores measuring 19

cm in depth were taken from sediment ~3 cm below the water surface. Undisturbed overlying water samples were taken from both sites. Core and water samples were placed on ice immediately after collection and transported to lab until sample preparation. Hydrographic data was recorded from the site at the time of sample collection using a YSI data sonde.

Once at the lab, seawater was vacuum filtered (0.45 μm polycarbonate) to remove particles. The sediment was mixed homogenously and filtered through a 500-μm sieve to remove large particles such as shell pieces and plant debris. Two hundred grams of the sediment were dried at 40°C until the weight became constant, typically within 72 h. Another two hundred grams of sediment were refrigerated. Water percentage of the sediment was calculated by weighing a small fraction of wet sediment before and after drying. The calculated water lost by the drying was replaced by distilled water. The rewetted dried sediment will be referred to as "dry" sediment hereafter. Sediment collection and pretreatment were the same for both the MR and NSM sites.

Experimental setup and sample processing

Incubation bottles (30 ml) were prepared by weighing 3 g of dried sediment from each site and adding 3 mL and 2.5 mL of distilled water to the NSM and MR samples respectively to rewet, based on the water loss calculation. The wet samples were prepared by weighing 6 g of wet sediment into 30 mL incubation bottles. The NSM and MR filtered water was deoxygenated by bubbling N_2 gas to make anoxic, as verified with a DO probe (Appendix Fig. 1). This deoxygenated water was then carefully added to each 30 mL bottle to bring the total volume of each vial to 30 mL, including sediment and pore water. N_2 was again bubbled over the mouth of each bottle, which were secured with Teflon coated rubber septa to ensure anoxic conditions in each bottle. All bottles were

incubated in the dark at room temperature until sampled over the course of the two-month incubation.

On each sampling day, two duplicate bottles were sacrificed for both wet and "dry" sediments, respectively. DO was measured immediately in each bottle. The sediment and water from each bottle were poured into a 50 mL centrifuge tube, the pH measured, and the slurry was centrifuged at 3000 rpm for 20 min. This relatively gentle centrifugation force was selected to allow bacteria suspended in the water to remain in the supernatant (Peterson et al., 2012). Ten mL of the supernatant was filtered through a 0.2 μm syringe filter (cellulose acetate) for analyses of nutrients and dissolved organic matter. The syringe filters were saved and frozen at -20°C for bacterial pyrosequencing. An additional 1 mL of the supernatant was mixed with a 4% formaldehyde solution in a 1.5 mL Eppendorf tube and stored in a refrigerator for bacterial abundance analysis. One gram of the centrifuged solid was placed in an Eppendorf tube and frozen for elemental analysis.

Nutrient and organic analyses

Ammonium in supernatant was analyzed using an assay adapted from established protocols (Bolleter et al., 1961; Strickland and Parsons, 1970). Soluble active phosphate (SRP) was determined using the ascorbic acid method adapted from previous protocols (Murphy and Riley, 1962; Strickland and Parsons, 1970). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) concentrations were determined by a Shimadzu TOC-V analyzer. Duplicate analyses agreed within 10%. Organic carbon and nitrogen content in sediments were determined using a CHN analyzer; after the sediment was freeze-dried and acidified under acid fumes to remove inorganic carbon (Hedges and Stern, 1984). Dissolved organic nitrogen (DON) was calculated based on the difference

between TDN and ammonium. The incubation was conducted under anoxic conditions, so we assume that the only form of inorganic nitrogen was ammonium.

Bacterial abundance and community analysis

Bacterial cells were enumerated using an Accuri ® C6 flow cytometer under 488 nm excitation after staining with SYBR Green II (Molecular Probes, 1:100 v/v) (Liu et al., 2013). Cell counts were done in duplicate samples.

DNA from the syringe filters were extracted and analyzed through the bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) by the Research and Testing Laboratory (RTL), Lubbock, TX, following established protocols (Smith et al., 2010; Liu and Liu, 2013). The Eubacterial primers 28F 5′TTTGATCNTGGCTCAG-3′ and 519R 5′- GTNTTACNGCGGCKGCTG-3′ were used to amplify about ~500 bp region of 16S rRNA gene according to the RTL protocols (www.researchandtesting.com) for bacterial diversity (Dowd et al., 2008; Smith et al., 2010).

The sequencing raw data were converted into FASTA files, and analyzed using a custom-scripted bioinformatics pipeline (Handl et al., 2011; Ishak et al., 2011), including quality trimming, clustering, chimera checking, and denoising. After the short reads below 250 bp were deleted, denoising and chimera checking was accomplished using the UCLUST and UCHIIME algorithms (Edgar, 2010; Edgar et al., 2011). The sequences were clustered into operational taxonomic unit (OTU) clusters. For each cluster, the seed sequence was put into a FASTA formatted sequence file, which was queried against a database originating from NCBI (http://nbci.nlm.nih.gov). The gene sequences were identified using Krakenblast (www.krakenblast.com). Each bacterium was identified to its closest relative and taxonomic level from fragments of gene sequences based upon BLASTn + sequence identity. The genus and higher-level taxonomic designations were

compiled using a secondary post-processing algorithm, and relative percentages of bacterial taxa were determined for each individual sample. The percentage of each organism was analyzed for each sample based on the relative number of reads. The sequences were classified at appropriate taxonomic levels.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) (Langille et al., 2013). This method assesses functional gene predictions and is a tool for reconstructing metagenomes by inferring gene content based on 16S ribosomal DNA sequences. 16S rRNA was analyzed using MOTHUR version 1.16.1, and PICRUSt metabolic predictions on closed OTUs were conducted at the 97% similarity level.

RESULTS

Changes in nutrients and pH during the incubation

Nueces Salt Marsh

The wet and dry sediments displayed differences in measured nutrients and pH in the slurries throughout the 3-week incubation. During the majority of the incubation, ammonium concentrations in the dried sediment remained about twice as high as those in the wet sediment (Fig. 2a). Ammonium levels in wet sediment showed minor fluctuations, increasing to a maximum of 124 μM on day 9 before leveling off at ~100 μM through day 18, before declining to a minimum of 43 μM by day 21. In contrast, concentrations of ammonium in dried sediment increased throughout the incubation, reaching a maximum of 250 μM at the end of the experiment.

Similar to ammonium, SRP levels differed between wet and dry sediments during the incubation, with the wet sediment maintaining concentrations ~4 μM, whereas concentrations in the dried sediment increased throughout the incubation, peaking at 19

μM on day 17 (Fig. 3a). SRP concentrations in both sediments showed an initial increase until day 5 before the decrease at day 7. After day 7, the wet sediment maintained relatively consistent low concentrations (<5 μM) for the remainder of the incubation. In contrast, SRP concentrations in the dried sediment kept increasing and peaked at levels three times higher than those of the wet sediment (Fig. 3a).

The dried sediment maintained a lower pH than the wet sediment throughout the incubation except the initial time point, and both treatments showed a sharp decrease in pH during the first 5 days before becoming relatively constant (Fig. 4a). The pH in the dried sediment remained at about 7.2 for the majority of the experiment whereas in the wet sediment remained at about 7.4. Both the dry and wet sediments reached a minimum on day 17 with pH levels of 7.09 and 7.36, respectively.

Mission River

During the six-week incubation for MR sediment, nutrients and pH in the slurries differed greatly between the wet and dry sediments. By day 7, ammonium levels for the dry sediment were twice as high as those in the wet sediment, and reached a maximum concentration of 51 μM on day 42 (Fig. 2b). In contrast, ammonium concentrations in wet sediments decreased with time, reaching a minimum concentration of 3.8 μM at the end of the experiment on day 42.

In contrast to the NSM sediment, SRP concentrations for the MR were higher in wet sediment than in the dry sediment throughout the incubation. The SRP concentrations for the wet sediments remained relatively constant throughout the incubation in the range of 2.4 to 3.3 μM. However, SRP concentrations in the dry treatment decreased until a minimum of 1.1 μM on day 22, and then increased to a maximum of 2.4 μM on day 33 (Fig. 3b).

Wet and dry treatments both showed a pattern of decreasing pH during the incubation (Fig. 4b). The wet sediment had an initial pH of 8.55 on day 1 and fluctuated between 8.30 and 8.10 before reaching a minimum of 7.90 on days 33-42. The dry sediment had an initial pH of 8.36 and showed two distinct drops over the incubation. The first drop, from 8.35 to 7.79, occurred between days 7 and 15, followed by a second drop, from 7.87-7.59, between days 26 and 33.

DOC and DON patterns during incubations

Nueces Salt Marsh

Concentrations of DOC for the wet sediment supernatant decreased to 532 μM on day 5 before gradually increasing to a maximum of 807 μM on day 14, and then gradually decreased for the remainder of the experiment (Table 1, Fig. 5a). DOC concentrations for the dry sediment were more than double those for the wet sediment throughout the incubation. There was a noticeable spike in DOC measured for the "dry" sediment between days 1 and 5, reaching concentrations over 1600 μmol L^{-1} before decreasing gradually for the remainder of the incubation, except a small spike at day 18.

DON (Table 1, Fig 6a) was calculated by subtracting ammonium concentration from the total dissolved nitrogen (TDN). Concentrations of DON in the dried sediment supernatant were 2-6 times as high as those in the wet sediment, and increased greatly from day 1 to day 15, reaching a plateau of 450 μM, then slightly decreased to 320 μM at the end of the incubation. DON concentrations in wet sediment supernatant were quite constant, maintaining concentrations <145 μM, but dropped slightly to 80 μM on day 21.

Element ratios of C/N in dissolved organic matter (DOC and DON) offer insight into the quality of dissolved organic matter released during the incubation. The C/N ratios for the NSM supernatant of the wet sediment treatments were significantly higher

than those in the dry treatment (t test, $p < 0.05$). The dry treatment showed an initial ratio of 12.0 before dropping sharply to 5.3 on day 1; the ratio continued to decline for the remainder of the incubation with ratios of 3.4 towards the end of the incubation (Table 1). The wet treatment showed no obvious trends with fluctuating C/N ratios throughout the experiment from 14 to 27.

Mission River

DOC concentrations for the dry sediment decreased sharply over the first 22 days of the incubation, declining from 1301 μM on day 1 to 695 μM by day 22 before maintaining concentrations around 700 μM for the remainder of the experiment (Table 1; Fig. 5b). In contrast, the wet treatment showed a much more gradual decreasing trend over the incubation, with lower initial concentrations of 835 μM, but varied only slightly thereafter, reaching a minimum concentration of 588 μM on day 26 and increasing to 675 μM by the end of the experiment.

DON in the wet and dry treatments showed a similar pattern during the incubation, except for a spike to 87 μM on day 33 in the dry treatment (Table 1, Fig 6b). The wet and dry sediments had initial DON concentrations of 38 and 49 μM, respectively, maintaining concentrations between 56 and 38 μM before reaching respective minimums of 35 and 27 μM on day 42 (Table 1). The dry treatment spiked sharply on day 33 at a concentration of 87 μM. Other than this outlier, DON concentrations for both wet and dry treatments remained close and displayed similar patterns throughout the incubation (Table 1).

C/N ratios of DOM for the MR wet and dry treatments differed throughout the experiment. The ratios in the wet treatment ranged from 12.4 to 21.5, peaking at 21.5 at day 1 (Table 1). C/N ratios in the dry treatment ranged from 23 and 28 for the first 11

days of the incubation before dropping to 16.2 at day 15 and staying constant thereafter. Note that the ratio dropped to 8 on day 33 for the dry treatment, which may be due to experimental error in the TDN observation as mentioned above.

Carbon and nitrogen content in sediments

Nueces Salt Marsh

Sediment carbon and nitrogen content showed similar patterns between wet and dry treatments (Table 2). The carbon and nitrogen content in the wet treatment declined slightly through day 7, from 0.84% and 0.093% to 0.51% and 0.067%, respectively. In contrast, the dried sediment treatment showed an opposite trend with increasing amounts of carbon and nitrogen from day 1 to day 5, from 0.462% and 0.063% to 0.669% and 0.076%, respectively.

The C/N ratios remained relatively constant for both wet and dry treatments during the experiment with no significant difference between the two treatments (t test, $p<0.05$), even though they were consistently higher in dried sediments than in wet sediments (Table 2). Dried treatment showed an initial increase in the C/N ratio from 9.8 at day 0 to 10.2 at day 5, after which the ratio remained at ~10 for the duration of the experiment. The C/N ratios of wet sediment fluctuated, with no obvious trend with incubation time. Data for the wet sediment on days 9 and 21 are not available.

Mission River

The MR carbon content for the dry and wet treatments maintained similar levels throughout the experiment ~0.4% (Table 2). The nitrogen content of the two sediment treatments also followed similar patterns over the course of the incubation, ranging between 0.035 – 0.040% for both treatments except a noticeable spike at day 26 with 0.053% nitrogen in the wet treatment (Table 2).

The C/N ratios of the MR sediment varied little in both the wet and dry treatments over the incubation, maintaining ratios around 12 – 13 (Table 2). The wet treatment had a maximum ratio of 13.7 at day 42 and a minimum ratio of 10.8 at day 26. The dry treatment had high ratios > 13.0 at days 4, 7, 26 and 42, and a minimum of 11.9 at day 19.

Bacterial abundance and diversity

Nueces Salt Marsh

Bacterial abundances in the supernatants of wet and dry sediments ranged from 1.1-8.6 x 10^6 cells mL^{-1}, and they were generally higher in wet sediment than in dry sediment (Fig. 7a). Bacterial abundances in both wet and dry treatments increased initially from day 1 to 5, peaking on days 5-7. The dry sediments peaked on day 5 at 5.02 x 10^6 cells mL^{-1}, whereas the wet sediment peaked at day 7 with 8.60 x 10^6 cells ml^{-1}. After day 5, bacterial abundance decreased for both wet and dry treatments until reaching a minimum at day 18 with abundances of 1.58 x 10^6 and 1.10 x 10^6 cells mL^{-1}, respectively, after which the abundance increased slightly.

The bacterial community was more diverse in the wet than in the dry treatment throughout the course of the experiment based on the Shannon-Weaver diversity index (SDI), although the dried sediment became more diverse during the duration of the incubation with an SDI approaching 4 (Table 3). The wet sediment community was dominated mainly by *C. Pelagibacter,* accounting for 22% of the population early in the experiment before shifting to domination by *Sulfurimonas* with 20% and *Arcobacter* with 24% of the population by day 16 (Fig. 8). In contrast, *Bacillus* and *Anaerobacillus* dominated the dried sediment during the first 5 days, accounting for 50% and 15% of the population, respectively (Fig. 9). After day 5, the community diversified and shifted to a

community including mainly *Vibrio* (~18%), *Haloplasma* (~20%) *and Terasakiella* (~15%). *Pseudoalteromonas* was also present in significant numbers ranging from 2.5% - 15% of the population throughout the dried sediment incubation.

Mission River

The bacterial abundances for the MR dry and wet treatments showed a similar pattern throughout the incubation (Fig. 7b). Both wet and dry supernatants had minimum bacterial abundance at day 1 with 1.83 x 10^5 cells mL^{-1}. The wet and dry treatments peaked at day 11 with abundances of 4.18 x 10^6 and 5.83 x 10^6 cells mL^{-1}, respectively, and the abundances stayed similar until day 26, when the abundance in the wet treatment increased from 4.61 x 10^5 cells mL^{-1} on day 26 to 2.41 x 10^6 cells mL^{-1} on day 42. The abundance in the dry treatment decreased slightly to 3.18 x 10^5 cells mL^{-1} on day 33 before increasing to 5.60 x 10^5 cells mL^{-1} on day 42.

The wet treatment had a more diverse community than the dry treatment, as shown from the higher SDIs, an average of 3.3, than those in the dry treatments with an average of 2.6 (Table 3). The wet treatment had an initial community dominated by *Staphylococcus*, *Turicella*, *Corynebacterium* and *C. Pelagibacter* at 25%, 14%, 15% and 7%, respectively (Fig 10). A drastic shift in the in the wet treatment community occurred on day 11, when *Rheinheimera* made up 75% of the population but dropped to below 3% by day 15. *Mycoplasma* appeared at day 15 with more than 3% and reached 35% on day 22. *Turicella*, *Staphylococcus*, *Streptomyces* and *C. Pelagibacter* accounted for less than 30% during the entire experiment.

The initial bacterial community in the dry treatment was dominated by *C. Pelagibacter,* which constituted ca. 50% of the population (Fig 11). *Turicella* and *Mycoplasma* accounted for ~10% and 2% of the population, respectively. At day 4,

Gemmobacter increased, and by day 15, dominated the population representing over 82% of the community before decreasing to < 1% at day 33. *Rhodobacter* and *Mycoplasma* were present throughout the incubation period, but their population levels never reached > 15% of the population. Similarly, *Staphylococcus* was present throughout the incubation with a minimum proportion of < 1.5% at days 1 and 26 and reaching a maximum proportion on day 42 accounting for > 15% of the population (Fig. 11).

Functional Gene Analysis

Nueces Salt Marsh

The 16S pyrosequencing analysis showed that various OTUs are associated with metabolic processes relevant to our anoxic incubation, including methanogenesis and methane oxidation, nitrogen reduction and sulfate reduction. In both wet and dry treatments, methane and nitrate processes are represented with higher OTU counts than sulfate reduction. In the wet treatment, methanogenesis and methane oxidation on average had 46% of the OTU counts; nitrate reduction accounted for about 40%; sulfate reduction only 14% (Fig. 12). Although the number of counts each day fluctuated, the percentages remained similar. In the dry treatment, much lower daily overall OTU counts were observed than the wet treatment (Fig. 13). Before day 9 OTU counts remained below 2000 for all processes before increasing more than two fold. Methanogenesis and methane oxidation along with nitrate reduction maintained on average 46% and 38% of OTU counts, respectively, while sulfate reduction had 16%.

DISCUSSION

Effects of drying on the physicochemical changes during the incubation

Many sediment properties, including nutrient sequestration, bacterial communities, and water retention capacity, are affected by drying (Baldwin and Mitchell,

2000; Evans and Wallenstein, 2012; Liu et al., 2008). These drying events also affect the decomposition of sedimentary organic matter and related nutrient cycling in sediments. Two contrasting systems were studied here, including respective sites in a tidally influenced salt marsh on the Nueces river delta, and within a freshwater system that feeds into an estuarine system. Using sediments from both fresh and marine sampling sites provided an opportunity to determine how these drying events alter and exaggerate nutrient fluxes into estuarine systems with differing salinities. The drying/rewetting events likely affect decomposition of sedimentary organic matter and further nutrient releases via at least two ways. The first is the physical degradation of the sediment structure itself. Once sediment is dried and loses interstitial water, structural bonds in the sediment break down, causing the sediment to lose sorption capacity for nutrients, particularly ammonium (Ford et al., 2007; Liu et al., 2008). Ammonium is adsorbed strongly onto clay mineral surfaces through electrostatic interactions (Müller, 1977). However, once the sediment is dried, the sorption capacity and ability to sequester nutrients is reduced, thus causing nutrient release (Liu et al., 2008). Ion exchange processes with cations such as sodium and potassium are stronger in saline water (Gardner et al., 1991; Rysgaard et al., 1999), thus perhaps explaining the initial higher ammonium concentrations in Nueces salt marsh sediments than in freshwater Mission River sediments. However, diminished ammonium sorption capacity does not explain the continuous release of ammonium throughout the incubation period (20-40 days) for dried NSM and MR sediments, because the ammonium sorption in sediments typically reaches equilibrium within hours (Wang and Lee, 1993).

The second pathway of nutrient release is sedimentary organic matter decomposition, including the original organic matter and biota killed by osmotic stress during drying, such as microorganisms and microfauna. Living cells that cannot adapt or

survive a traumatic drying event can lyse and release labile intracellular components to surrounding environments (Amalfitano et al., 2008; Gordon et al., 2008). However, the labile organic matter from biota is often remineralized rapidly (few days), and does not explain the continuous release of nutrients (ammonium and phosphate) throughout the incubation period. In addition, living biomass often accounts for only small fraction of the sedimentary organic matter based on mass balance calculation (Liu et al., 2008). Similarly, we estimated that bacterial biomass killed by the drying contribute 21% of the nitrogen, 41% of DOC, and 64% P released to the supernatant for the NSM, assuming that one bacterial cell contains approximately 40fg C, 10 fg of N and 2 fg of P (Fagerbakke et al., 1996; Vrede et al., 2002), a 50% bacterial mortality by the drying (Liu et al., 20018), and a typical bacterial abundance 3 x 10^9 cells g^{-1} in marsh sediments. The same calculations show that the killed biomass would account for 70% of the DOC released in the MR treatment. Note that the high C/N ratios of DOM in the MR slurry suggest that most of the DOM is not released from the killed biota. These calculations may overestimate the DOM released because bacteria mortality from the drying event may not be as high as estimated, and the cellular content of nutrients may be less. Therefore, the microbes killed by the drying event cannot solely explain the release of nutrients from dried sediments. A major fraction of the nutrient release may be caused by the degradation of non-living sedimentary organic matter. The dominant non-living sedimentary organic matter can become labile due to the structural changes caused by the drying, and its decomposition may lead to the nutrient release (Liu et al., 2008; Wang et al., 2016).

Our results suggest that these pathways had variable patterns during the incubation. Bacterial abundances were similar for both wet and dry sediments for NSM and MR over the course of the decomposition (Fig. 7a), so were the bacterial abundances

in the MR sediments (Fig. 7b). The abundances peaked between days 5 and 7 for both NSM sediments. When comparing the release of phosphate, an initial rise in phosphate levels peaked on day 5 before dropping by day 7 (Fig. 3a). This pattern suggests that an initial pulse in nutrients may result from the decomposition of labile organic matter sourced from the biota killed by the drying and rewetting. At day 7, phosphate levels dropped for both NSM wet and dry sediments and in duplicates, so it is unlikely an experimental outlier. It is possible that the decreases were caused by bacterial uptake, as fast growing bacteria consume a large quantity of phosphorus to build their biomass (Elser et al., 2000; Liu and Liu, 2017). After day 7, nutrients in the dry sediment continued to increase whereas those in the wet sediment remained relatively low for the remainder of the incubation. Phosphate level increases may have resulted from organic matter decomposition, with release of nutrients into the overlying water column. Interestingly, bacterial abundance decreased continually in both the NSM wet and dry sediments and showed similar patterns throughout the experiment.

Differences between NSM and MR sediment incubations

The NSM and MR sediment incubations showed similar trends in ammonium release, with increasing concentrations over time in dry treatments but decreasing concentrations in wet treatments, which would be expected as drying/rewetting enhances decomposition of sedimentary organic matter (Liu et al., 2008). However, a key difference in the incubation results is the higher concentrations of SRP for the MR wet treatment than the dry, whereas a steady release of SRP was observed in the dry NSM treatment (Fig 3). Previous research showed that phosphate (P) has a higher affinity to freshwater sediments (Hartzell, in press), which may explain the lower phosphate release from the MR sediment. Moreover, drying may change the three-dimensional structure of

sedimentary organic matter in such a way that exposes hydrophobic groups, while the hydrophilic groups, including the phosphate, may be buried into interiors through ionic interactions, leading to inaccessible phosphate (Liu et al., 2008). This burial of phosphate may be stronger in freshwater systems with depleted ions in dissolved phase. In contrast, saline water is enriched with negatively charged ions such carbonate and chlorine, which may help release phosphate. Further work is needed to test this speculation. Note that the decrease in SRP for the MR dry treatment between days 1 and 15 can be attributed to bacterial uptake, and the continuous release of SRP in the dry NSM treatment is likely from the release of trapped non-living organic matter that is released steadily during the incubation.

C/N ratios offer insight into the sources and lability of organic matter. For example, C/N ratios of 4-10 are indicative of marine sources, and the ratios can increase slightly for estuarine sediments as marine detritus falls to the sediments and becomes degraded (Müller, 1977). C/N ratios of the supernatants in the wet treatments for both sites stayed similar throughout the incubation, with a range of 9 – 13 (Table 2). However, the C/N ratios for the NSM dry treatment were significantly lower than those for the wet sediment supernatant, with ratios of 5 or less (Table 1). These significantly lower C/N ratios suggest that a significant amount of proteinaceous materials was released during the incubation for the NSM dry sediment supernatant (Fig 2a). As calculated above, the biota killed by the drying can only explains 21% of the DON. This result suggests that non-living organic matter buried inside the matrix may have become exposed during the drying and rewetting, and those protein-like materials were released preferentially to the solution, becoming accessible to microbes (Wang et al. 2016). Interestingly, C/N ratios of the dissolved organic matter in MR dried sediment were significantly higher than the NSM dry treatment, even though the C and N content (%)

and C/N ratios of sedimentary organic matter were similar at these two sites (Table 2). Salinity likely played a role in the observed differences. Again, the proteinaceous matter presumably contains more positively charged amine groups, so it is likely that cations in the saline water can preferentially release these types of organic matter at the NSM site when compared to the freshwater with low ionic strength at the MR site.

Bacterial communities in the supernatants

Bacterial abundances for both the MR wet and dry treatments followed similar patterns throughout the incubation. The abundances were generally higher in the wet treatments, but both wet and dry treatments fluctuated similarly over the decomposition (Fig 7). However, bacterial communities in wet and dry sediment slurries differed dramatically (Figs. 8 and 9, Table 3). At the initial stage of the incubation, dry sediment communities from the NSM were dominated by *bacillus*, a spore forming bacterium that is very resistant to harsh conditions (Priest, 1977). Thus, *bacillus* would be expected to survive the drying event and dominate initially after rewetting, consistent with what was observed (Fig. 9). *Pseudoalteromonas* were also present in higher abundance in dried sediment than in wet sediment. *Vibrio,* which can cause food poisoning in marine waters, also was more abundant in the dry sediment than the wet sediment. These bacterial often become dominated in the presence of labile substrates (Liu et al., 2013; Liu and Liu, 2013). In the dry sediment, however, the community was dominated mainly by nitrogen reducing bacteria such as *Terasakiella,* which are heterotrophic denitrifying bacteria that can inhibit sulfate reducing bacteria (Bødtker et al., 2009; Satomi et al., 2002). *Haloplasma* were also abundant; they are denitrifiers and only grow in anaerobic conditions (Antunes et al., 2008). These bacteria may be involved in the sulfate reduction

and methanogenesis, as these functional genes increased greatly late in the incubation (Fig. 13).

In the NSM wet sediment, a more diverse community structure was observed (Table 3). *Candidatus Pelagibacter* initially dominated the wet sediment before being taken over by *Sulfurimonas* and *Arcobacter* towards the end of the incubation. The abundance of *Sulfurimonas* began to increase from day 5 to 16, before *Arcobacter* began to increase through days 16 and 21 (Fig. 8). This result suggests that sulfate reduction was the major respiration process during the late incubation for these genera as *Sulfurimonas* and *Arcobacter* are sulfate reducers (Han and Perner, 2015; Miller et al., 2007). Compared with the dry treatments, functional genes of sulfate reduction and methanogenesis remained relatively constant, suggesting there was minimum disturbance to these already existing processes in wet sediments.

Similarly, the drying event affected the bacterial community structure and diversity of the MR treatments. *C. Pelagibacter* was present throughout the wet and dry incubations. *C. Pelagibacter* is common in both marine and fresh waters and can survive in extreme environments (Carini et al., 2012). The MR dry treatment showed a different community structure dominated by *Gemmobacter*, *Rhodobacter,* and *Mycoplasma*. *Rhodobacter* are capable of multiple metabolic pathways and anaerobic respiration, and can compete under unfavorable conditions such as low pH and anaerobic conditions (Tichi and Tabita, 2001; Wen et al., 2016). Drying events therefore not only affect the diversity and structure of the microbial community, but can also affect the cycling of organic matter and nutrients in the freshwater system.

CONCLUSIONS

Our results showed that drying enhanced the decomposition of sedimentary organic matter from a salt marsh and a freshwater river, leading to release of nutrients to overlying waters, particularly ammonium. We speculated that there are two main pathways of nutrient release from dried NSM and MR sediments. The initial nutrient flux may be caused by the decomposition of labile organic matter from biotas killed by the drying process. The prolonged release was mainly due to decomposition of non-living organic matter after the structural and physical changes during drying. Distinct differences were observed between salt marsh and freshwater river sediments; including much lower C/N ratios (<5) of dissolved organic matter from the NSM dry sediment and the binding of phosphate in the MR sediment. Even though the exact mechanisms remain uncertain, salinity may have affected the releasing or binding organic compounds and nutrients during the drying/rewetting process. We also observed changes in microbial community structure and functions that alter nutrient cycling and respiration pathways of organic matter. For example, the drying/rewetting event strongly altered the microbial community in both the NSM and MR sites, and lowered the counted OTUs for metabolic processes for the NSM site, and thus further affected nutrient release to the overlying water.

While it is difficult to compare these processes within marine and freshwater systems with many variables, our results showed that drying events affected both marine and freshwater sediments and enhanced the release of nutrients to the overlying water. Knowing the bacterial composition and how nutrients are released into the water column after drying events, can improve understanding of water management policies and estuarine conservation. Furthermore, the enhanced release of nutrients by drying and rewetting events should be considered when studying estuarine biogeochemical processes

in both marine and freshwater sediments that are subject to flood and drought oscillations.

TABLES AND FIGURES

Table 1: DOC and DON concentrations (μM) and organic carbon to organic nitrogen ratios (C/N) for supernatant of incubation slurries from Nueces Salt Marsh and Mission River sediments.

	Nueces Salt Marsh (NSM)						Mission River (MR)						
	Dry			Wet				Dry			Wet		
Time	DOC	DON	OC/ON	DOC	DON	OC/ON	Time	DOC	DON	OC/ON	DOC	DON	OC/ON
(days)	μM	μM		μM	μM		(days)	μM	μM		μM	μM	
0	1561	130	12.0	711	ND	ND	1	1301	49	26.4	835	38	21.5
1	1190	222	5.3	666	37	17.9	4	1074	43	24.8	768	44	17.2
5	1715	323	5.3	532	30	17.4	7	1101	39	28.0	748	42	17.7
7	1665	342	4.9	574	21	26.9	11	925	39	23.6	719	41	17.3
9	1698	376	4.5	699	35	19.8	15	784	48	16.2	707	56	12.4
14	1594	461	3.5	807	57	14.0	19	826	52	15.7	756	48	15.5
16	1273	421	3.0	607	30	20.1	22	695	39	17.8	677	46	14.5
18	1573	457	3.4	566	34	16.6	26	737	41	18.0	588	37	15.9
21	1178	344	3.4	591	37	15.6	33	699	87	8.0	710	48	14.7
							42	675	26	25.1	730	35	20.6

Table 2: Total organic carbon and total nitrogen (%) and C/N ratio for sediments of incubation slurries from Nueces Salt Marsh and Mission River sediments. ND – No Data.

Nueces Salt Marsh (NSM)							Mission River (MR)						
	Dry			Wet				Dry			Wet		
Time (days)	C %	N %	C/N	C %	N %	C/N	Time (days)	C %	N %	C/N	C %	N %	C/N
0	0.450	0.054	9.8	0.84	0.093	10.4	0	0.400	ND	ND	0.382	0.037	12.1
1	0.462	0.063	8.6	0.64	0.076	9.9	4	0.460	0.040	13.4	0.487	0.044	13.0
5	0.669	0.076	10.2	0.52	0.064	9.1	7	0.482	0.043	13.0	0.393	0.034	13.3
7	0.610	0.069	10.3	0.51	0.067	8.8	11	0.350	0.033	12.3	0.398	0.036	12.8
9	0.637	0.072	10.4	ND	ND	ND	15	0.431	0.039	12.8	0.360	0.033	12.6
14	0.701	0.077	10.6	0.58	0.065	10.3	19	0.373	0.037	11.9	0.423	0.038	13.1
16	0.749	0.085	10.3	0.54	0.069	9.2	22	0.381	0.035	12.7	0.414	0.038	12.9
18	0.571	0.066	10.1	0.63	0.076	9.7	26	0.444	0.039	13.2	0.490	0.053	10.8
21	0.674	0.076	10.3	ND	ND	ND	33	0.403	0.037	12.7	0.373	0.035	12.5
							42	0.433	0.038	13.2	0.421	0.036	13.7

Table 3: Shannon-Weaver Diversity Index of bacterial communities at genus level for supernatant slurries from Nueces Salt Marsh and Mission River sediments.

Nueces Salt Marsh			Mission River		
Day	Wet	Dry	Day	Wet	Dry
0	3.83	2.58	1	3.42	2.82
1	3.22	2.08	4	3.55	3.21
5	4.34	2.60	7	3.59	3.11
9	3.86	3.38	11	2.92	1.82
16	3.65	3.35	15	3.07	1.83
21	4.17	3.76	19	3.20	2.69
			22	2.69	1.72
			26	3.00	3.23
			33	3.61	2.59
			42	3.58	2.65

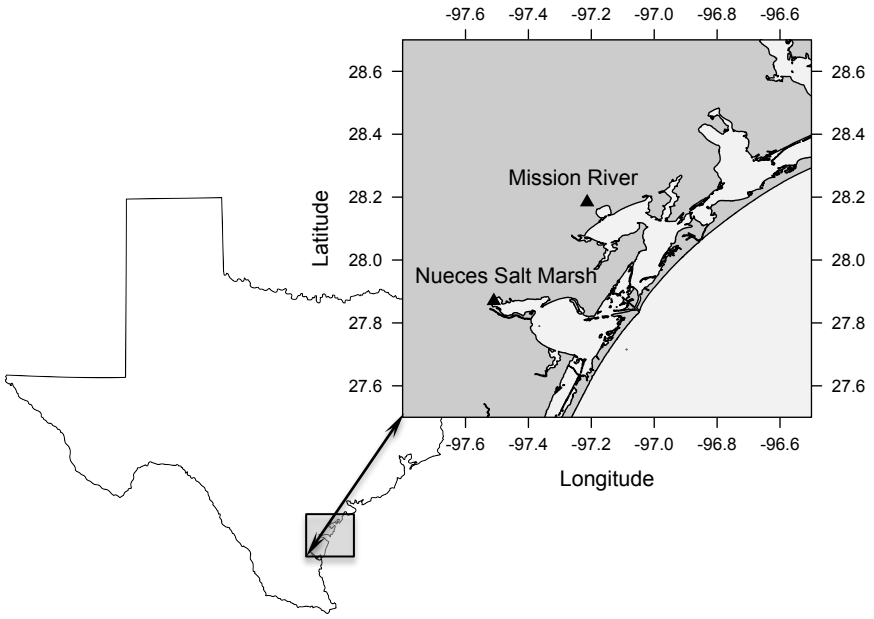

Figure 1: Sampling site locations for Nueces Salt Marsh (NSM) and Mission River (MR), Texas.

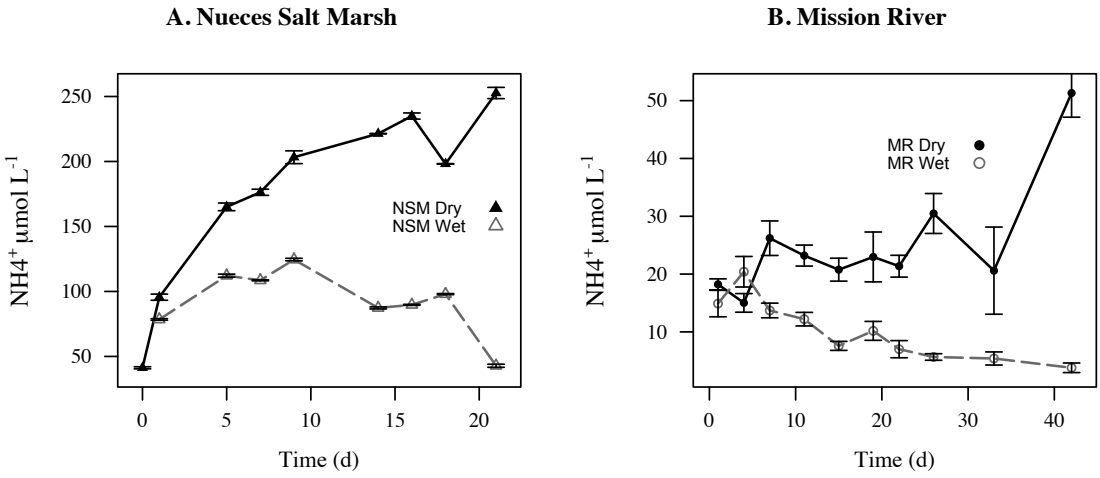

Figure 2: Ammonium concentrations with incubation time for (A) Nueces Salt Marsh (NSM) and (B) Mission River (MR) from sediment slurry supernatant. Error bars represent standard error for n=2.

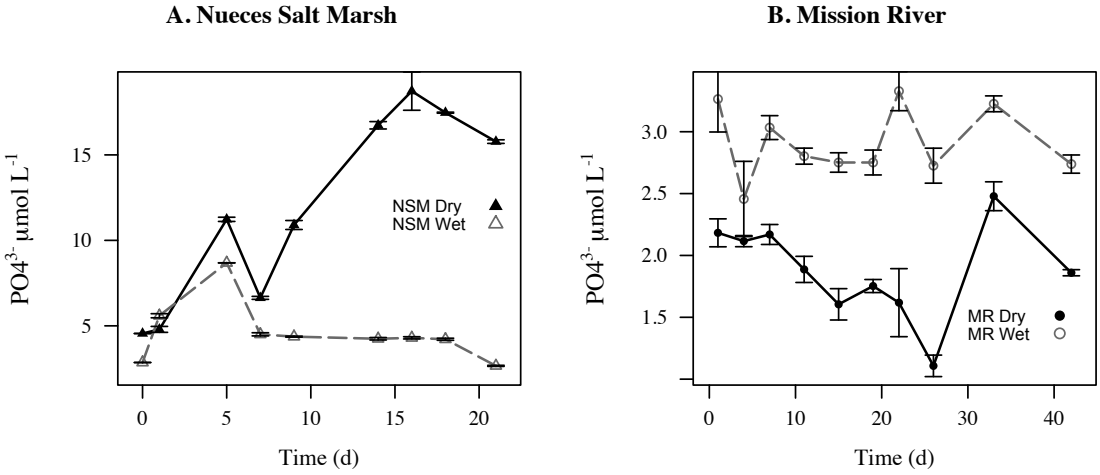

Figure 3: Concentrations of Soluble Reactive Phosphate (SRP) for (A) Nueces Salt Marsh and (B) Mission River from sediment slurry supernatant over time. Error bars represent standard error for n=2.

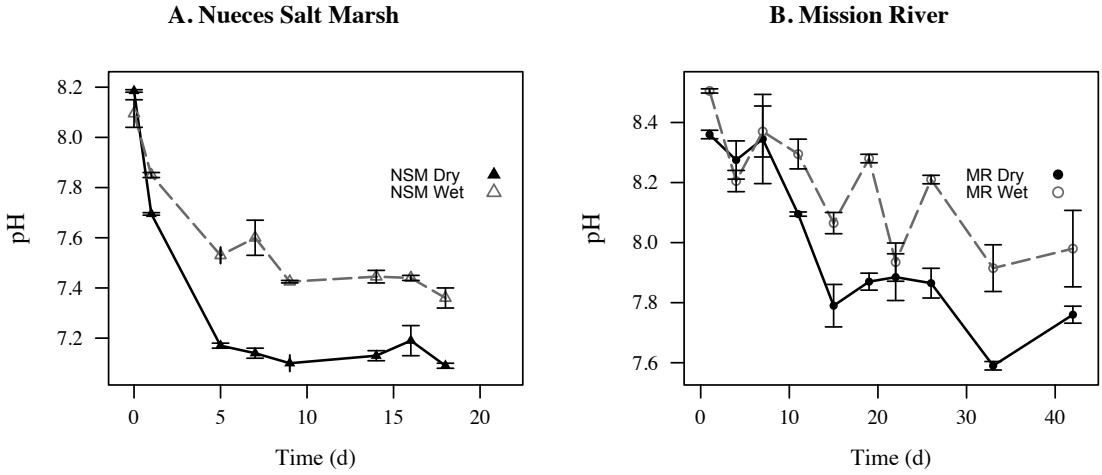

Figure 4: pH measurements for supernatant over duration of incubation experiment using sediment from (A) Nueces Salt Marsh and (B) Mission River. Error bars represent standard error for n=2. Note difference in y-scales.

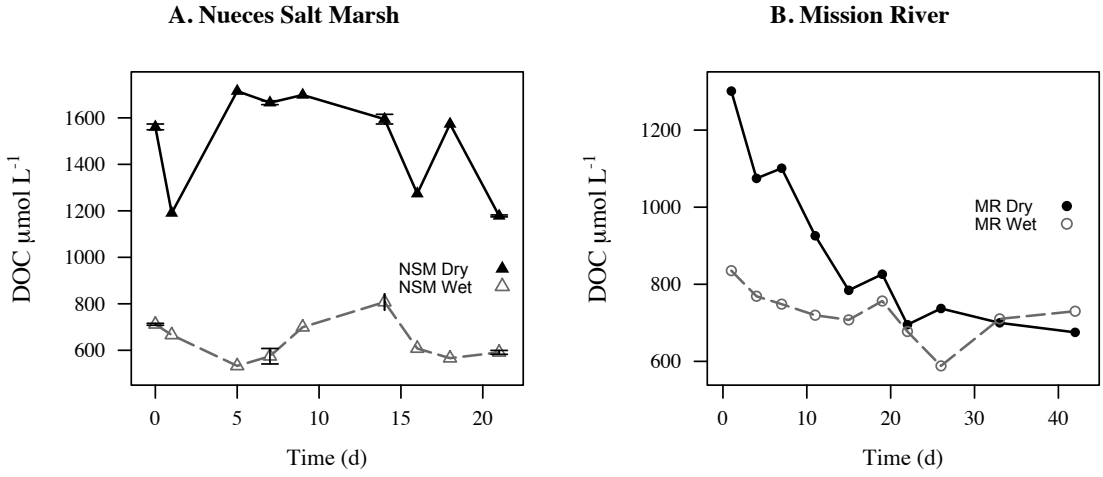

Figure 5: Dissolved Organic Carbon (DOC) concentration of sediment slurry supernatant for (A) Nueces Salt Marsh and (B) Mission River incubations.

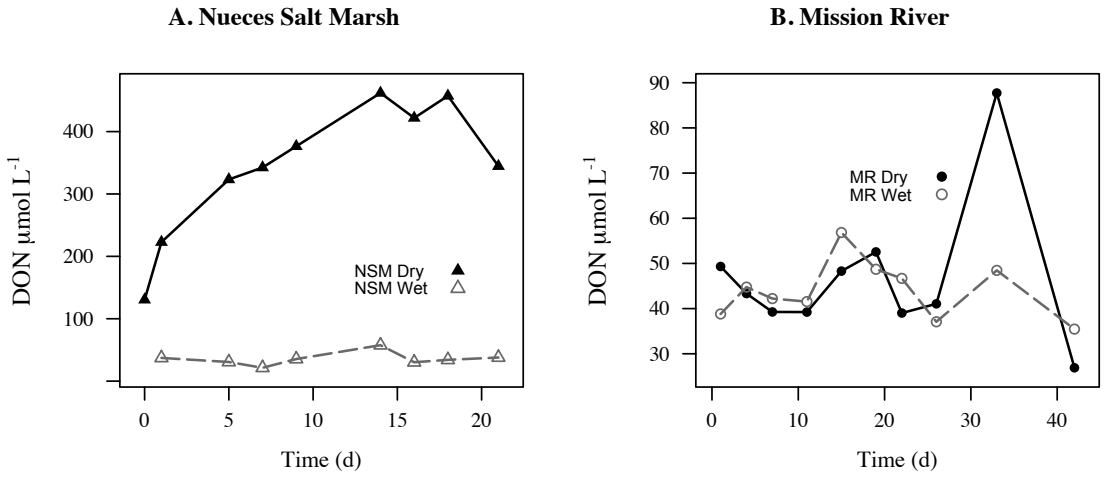

Figure 6: Dissolved Organic Nitrogen (DON) concentrations for supernatant of sediment slurries from (A) Nueces Salt Marsh and (B) Mission River incubations.

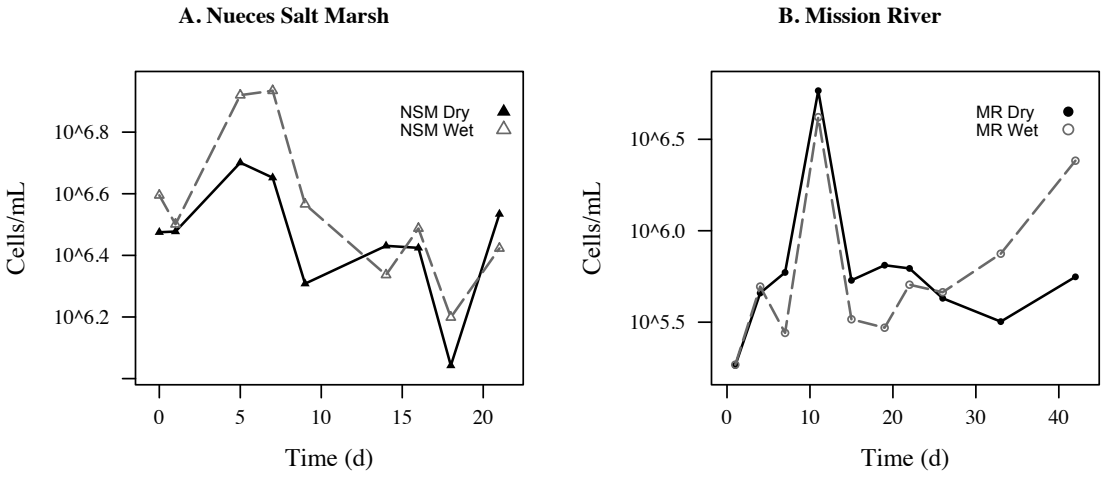

Figure 7: Bacterial abundance as enumerated by Accuri ® C6 flow cytometer under 488 nm excitation after being stained with SYBR Green II for NSM and MR. Note different y-scales.

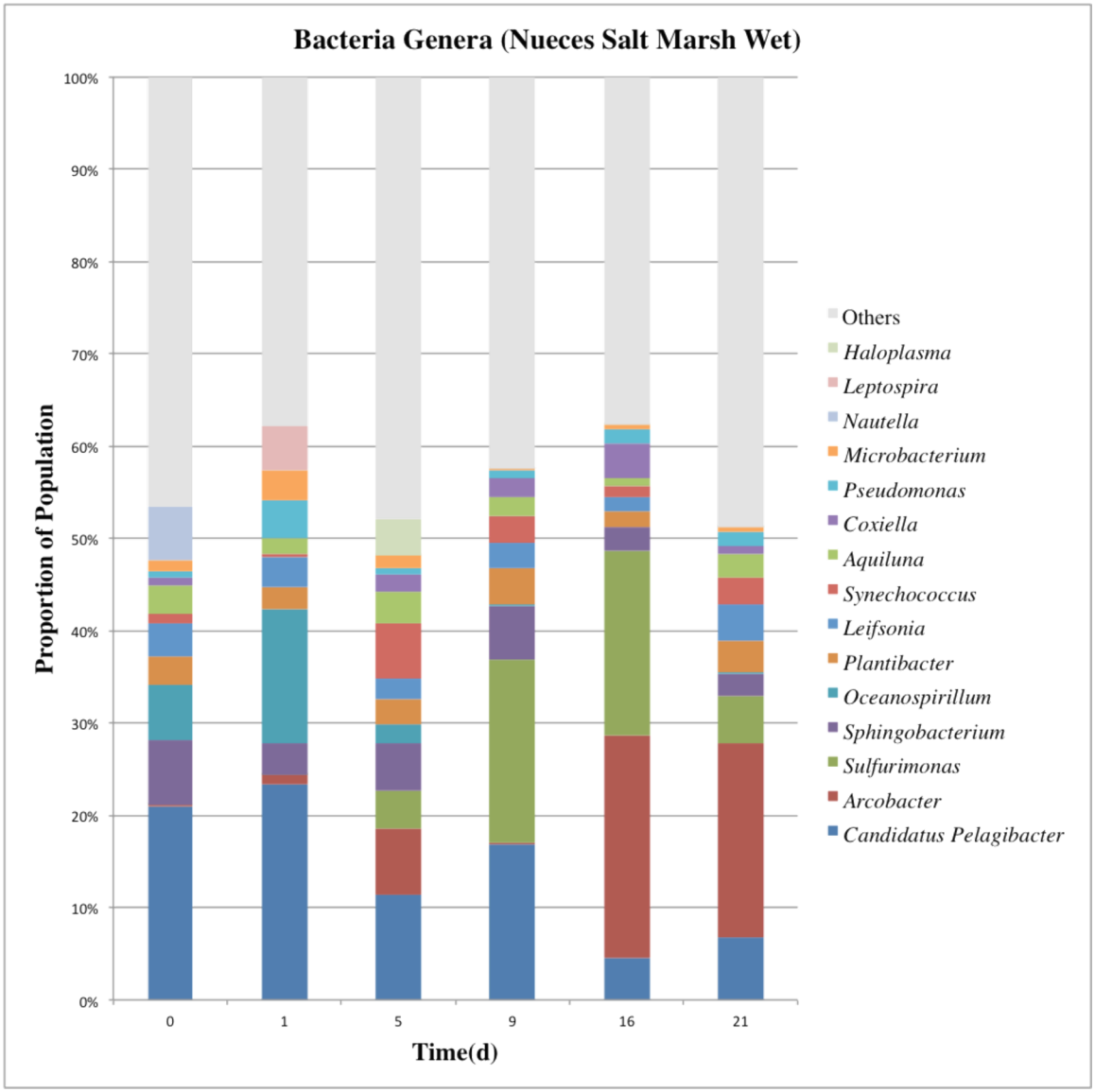

Figure 8: Bacterial community proportions at the genus level for Nueces Salt Marsh (wet treatment) sediment slurry supernatant. "Others" are those genera < 3% of the population.

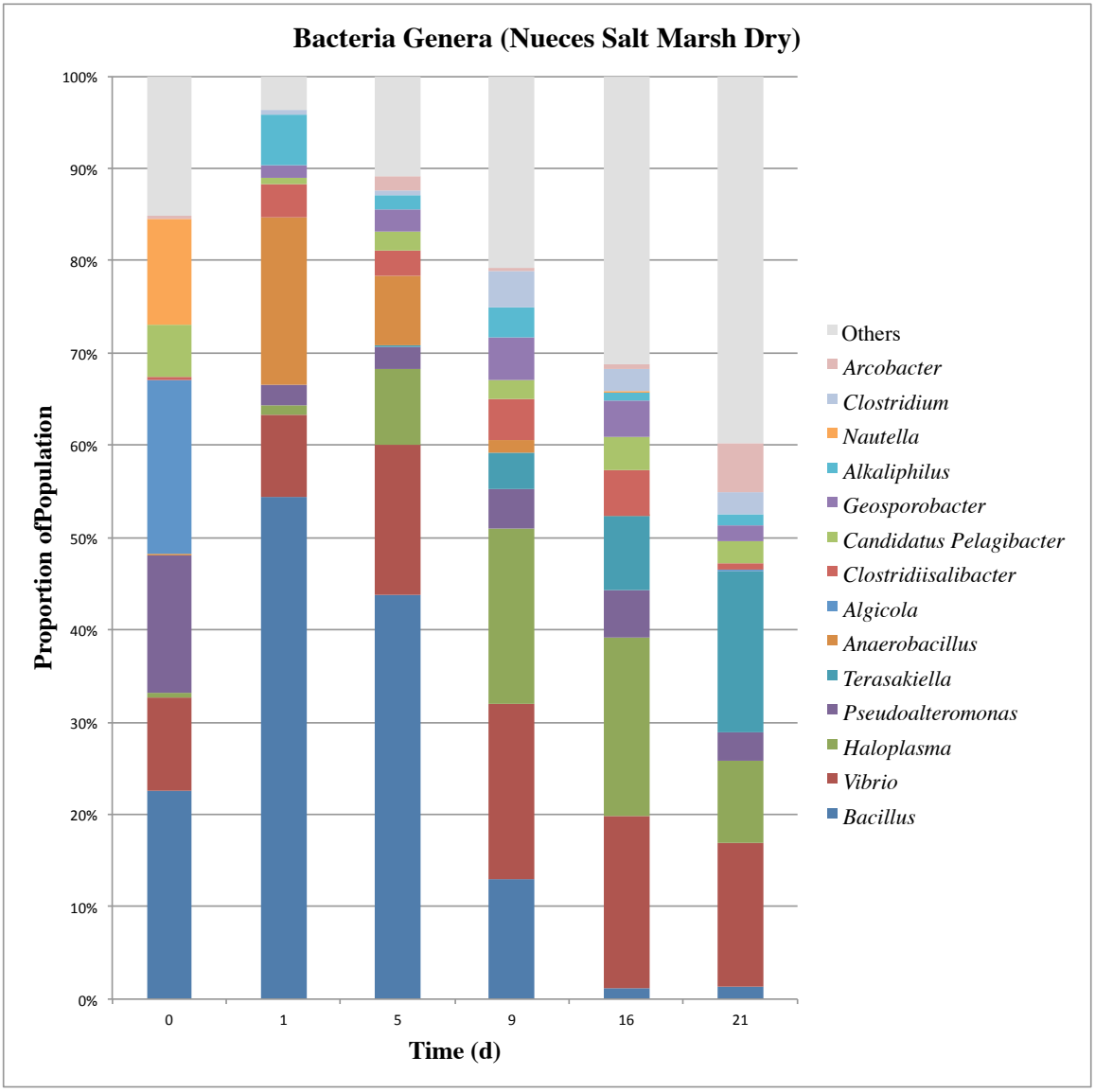

Figure 9: Bacterial community proportions at the genus level for Nueces Salt Marsh (dry treatment) sediment slurry supernatant. "Others" are those genera < 3% of the population.

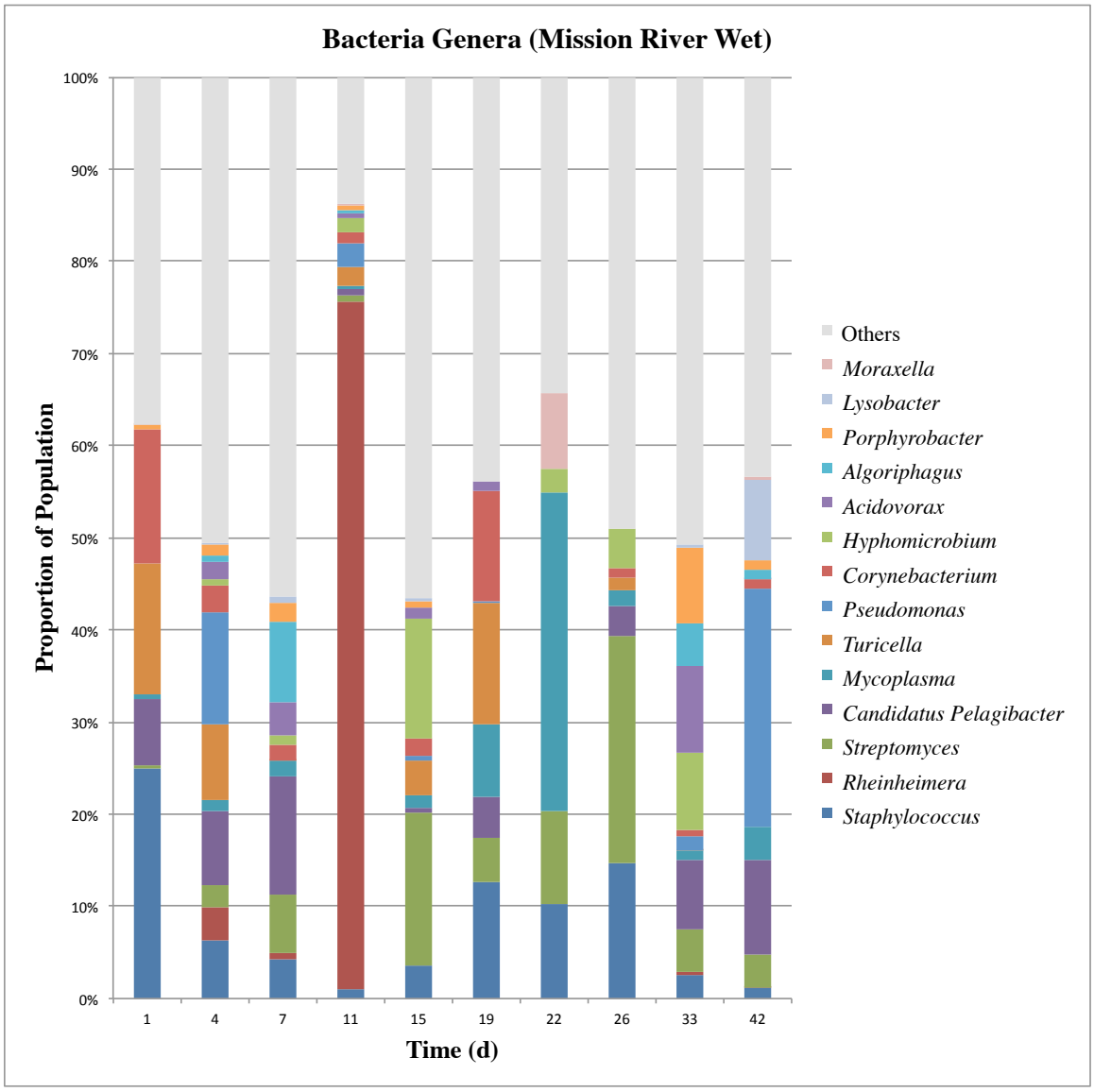

Figure 10: Bacterial community proportions at the genus level for Mission River (wet treatment) supernatant of sediment slurry of incubation. "Others" are those genera < 3% of the population.

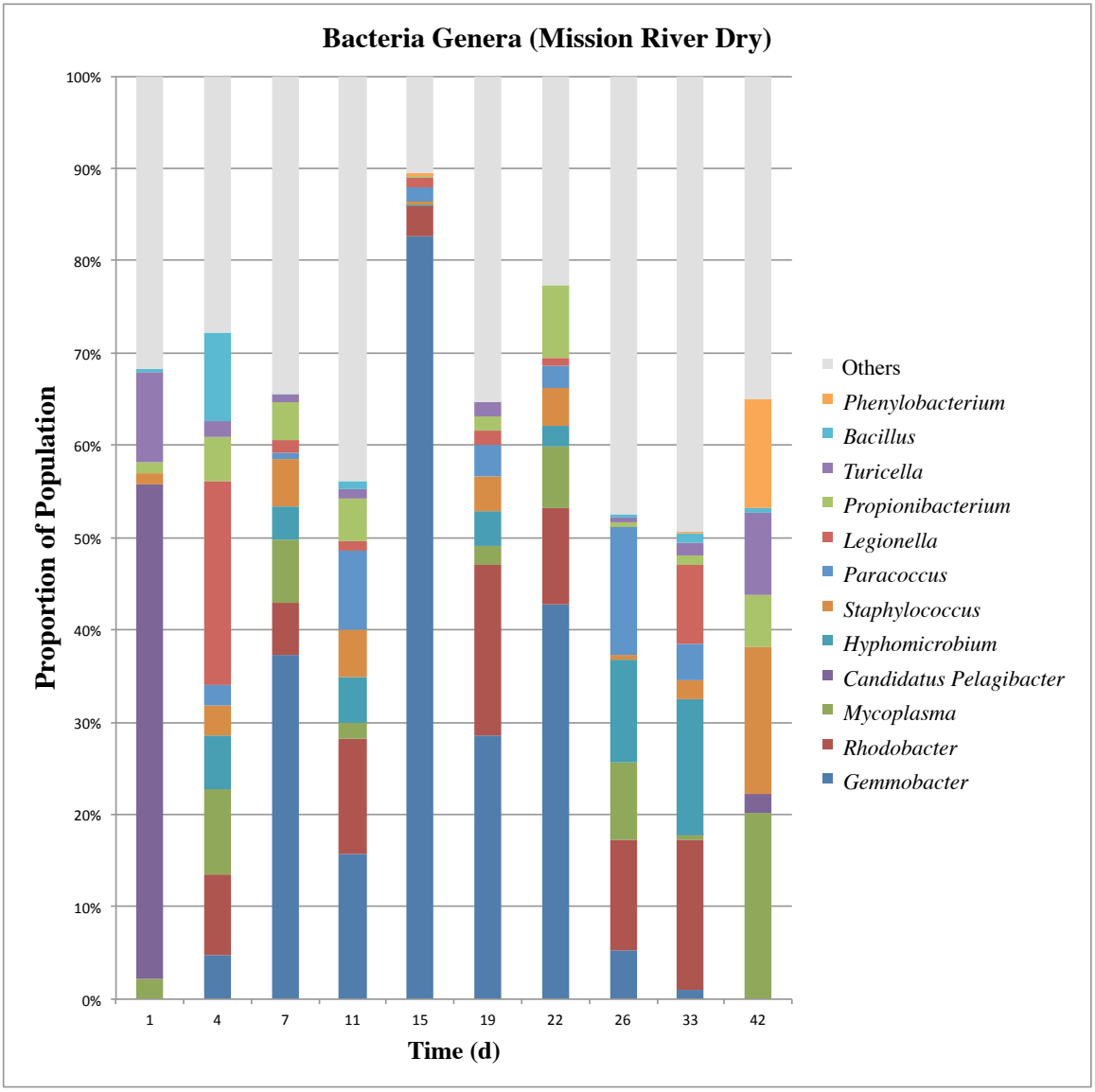

Figure 11: Bacterial community proportions at the genus level for Mission River (dry treatment) supernatant of sediment slurry of incubation. "Others" are those genera < 3% of the population.

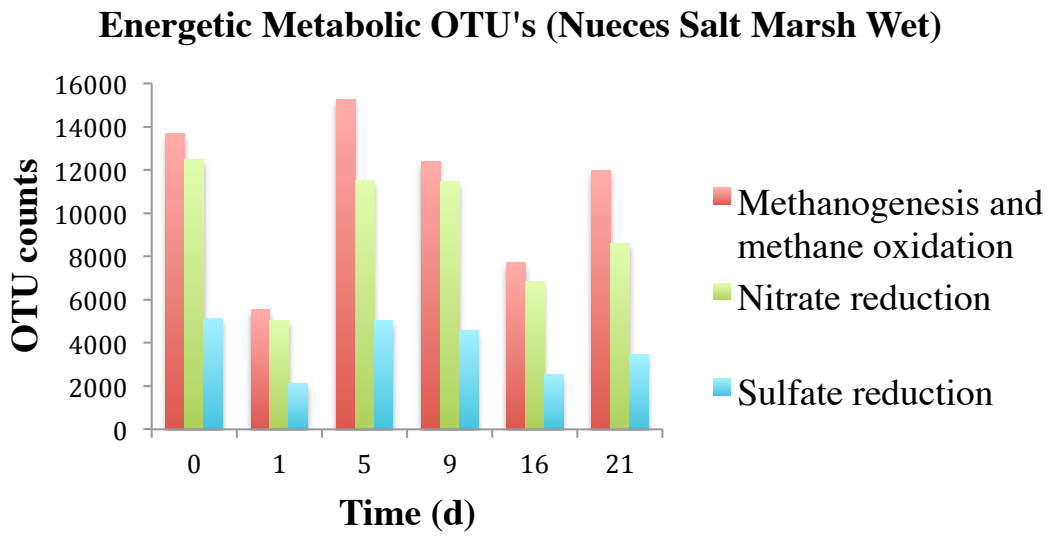

Figure 12: Operational taxonomic unit (OTU) counts for NSM wet treatment metabolic functional genes.

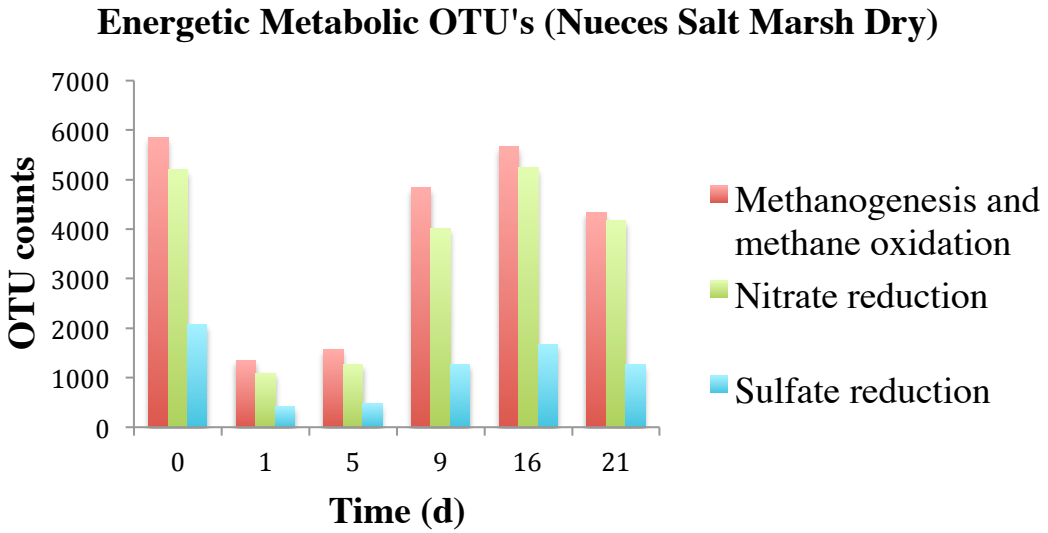

Figure 13: Operational taxonomic unit counts for NSM dry treatment metabolic functional genes.

Appendix

Dissolved Oxygen (DO %) of Supernatant

Nueces Salt Marsh			Mission River		
Day	Wet	Dry	Day	Wet	Dry
0	8.32	49.5	1	7.41	14.2
1	0.30	-0.10	4	1.2	-0.44
5	0.00	-1.27	7	1.1	0.76
7	-1.74	-4.51	11	-1.5	-0.2
9	-0.60	-2.10	15	ND	ND
14	1.06	-0.21	19	-0.71	1.3
16	ND	ND	22	0.15	0.34
18	ND	ND	26	-0.34	-1.22
21	ND	ND	33	ND	ND
			42	-1.67	-0.45

Appendix 1: Table for DO concentrations for both NSM and MR sediment treatments. Negative values are the result of the calibration equation. ND = No Data.

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