

# **Mangrove Expansion Alters Sediment and Water Quality and Affects Biodiversity in Texas Wetlands**

Final Report

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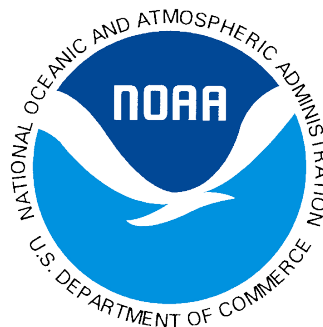
Prepared by:

Brandi Kiel Reese, Principal Investigator  
Texas A&M University-Corpus Christi  
6300 Ocean Dr., Unit 5800  
Corpus Christi, Texas 78412  
Phone: 361-825-3022  
Email: brandi.reese@tamucc.edu

Submitted to:  
**Texas General Land Office**  
1700 Congress Ave.  
Austin, TX 78701-1495

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## Executive Summary

This project investigates geochemical and microbial shifts in a mangrove marsh ecosystem within Mission Aransas National Estuarine Research Reserve. With mangrove populations encroaching along the Texas coast, more needs to be understood about the differences between geochemistry and microbial communities within mangrove, *Spartina*, and seagrass-dominated sediments. Samples were collected across spatial (i.e. vegetation type, depth within the sediment column) and temporal (i.e. seasonal, diurnal) scales. Sample collection occurred in November of 2017, June 2018, and August 2018. These sampling collections coincided with temperature and precipitation variations. Sediment samples were collected for geochemical analyses such as porewater chemistry (i.e., nitrate, ammonium, sulfide, sulfate, chloride), bulk sediment analyses (i.e., total carbon, total organic carbon, total nitrogen, grain size, porosity), and methane concentrations. DNA from the sediment was extracted and sent for sequencing to determine the microbial community structure. Methane and carbon dioxide gas were measured at each site using benthic flux chambers. There was evidence that day and night porewater samples from mangrove-dominated sediment collected in November, June, and August were statistically different in concentrations of both sulfide and ammonium ( $p < 0.05$ ). There was evidence that there was a statistically significant difference between day and night time flux chamber deployments for mangrove and *Spartina*-dominated sediment ( $p < 0.05$ ). There was a significant difference in methane and sulfide emissions between aerially vegetated sediment and submerged or bare sediment. Vegetation type also affected carbon dioxide flux from day to night. Comparing all sites, samples collected at night in August 2018 from mangrove and *Spartina*-dominated sediments were the most geochemically similar. These samples were also significantly different in concentrations of methane and sulfate. We observed the presence of DNA assigned to sulfate reducing bacteria, alkane degrading bacteria, and methane producing Archaea in each of the sediment samples we analyzed for microbial community structure. Overall, future work needs to be done to elucidate how these communities shift in spatial and temporal scales and how they overall will affect coastlines during periods of mangrove expansion.

## Outreach Efforts

Education and outreach were vital components of this study. One high school intern (photos shared separately), two M.S. students and two Ph.D. students participated in the study. Results from this study were presented in several venues including classroom presentations, public seminars, and scientific conferences. In collaboration with NERR staff, we also created an education module for elementary and for middle school that includes background information on climate change, estuarine ecology, assessing biodiversity, and measuring sediment parameters.

Below is a complete list of outreach and education efforts that were undertaken as part of this study. Supporting documents will be provided to GLO separately.

### *Presentations (public, local):*

- Kaffie Middle School's STEM night 10/01/19
- Texas branch of the American Society of Microbiology
- Earth Day Bay Day 04/20/19
- Kaffie Middle School's STEM night 09/26/2018
- Me by the Sea conference 06/15/2018
- Earth Day Bay Day 04/08/18
- Kaffie Middle School's STEM night 10/16/2017
- Teen STEM Café at the Texas State Aquarium 10/02/2017
- Me by the Sea conference 06/16/2017
- Collaborator in Art-Science Exhibit – TAMU-CC/Texas State Aquarium 06/09/2017
- Earth Day Bay Day 04/08/2017

### *Presentations (university and high school classes):*

Reese presented findings in a class she teaches at TAMU-CC, “Marine Organisms and Processes” in October 2019, and “Microbial Diversity and Ecology” in November 2019.



## **Acknowledgements**

First and foremost, we thank the many volunteers who dedicated their time and resources to the collection of this sediment and water quality data in the NERR mangroves. This study would not have been possible without their efforts. We are also grateful to the Texas Sea Grant and the Department of Energy Joint Genome Institute for funding. Finally, we thank our partners at the Coastal Bend Bays & Estuaries Program for their ongoing support and interest.

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## Introduction

Mangrove ecosystems play a valuable role in coastal processes including shoreline protection, fish habitats and nurseries, and carbon storage. Mangrove sites only cover 0.5% of coastal regions, but more than 37% of coastal carbon is stored within the sediment of these systems (Saunois et al 2016). The dense carbon storage is a direct result of their greater efficiency than terrestrial systems (e.g., temperate, tropical, and boreal forests) and other aquatic systems (e.g., salt marshes, seagrass) because they can trap suspended organic matter during tidal fluctuations (McLeod 2011). These mangrove systems are expanding in size because there are fewer winter freezes as a result of climate change (Montagna 2011) and are encroaching into salt marsh communities (Armitage et al 2015). This leads to the important point that with increasing mangrove size, and thereby the increase in carbon storage, microbial respiration will also increase resulting in potentially adverse water quality effects. This study simultaneously investigates shifts in geochemical signals and microbial communities over a spatial and diurnal scale. There is a substantial knowledge gap about how methane flux will change with vegetation type and how the associated microbial community structure and function will correspond to this shift, which makes the basis of this study vitally important.

The structure and function of microbial communities are driven by available chemical species (e.g., nitrate, sulfate, carbon dioxide). The availability of these substrates can fluctuate with seasons (e.g., precipitation and temperature), and between day and night as respiration rates change (Livesley 2012). Seasons with high precipitation can cause methane producing Archaea (i.e., methanogens) to be more abundant (Laanbroek 2010). As mangroves encroach into salt marshes, they alter the physical coastline and shift the sediment from being nitrogen and phosphorus rich to carbon rich. Alterations to other terminal electron acceptors could shift the microbial community and therefore shift methane production and eventual emissions (Kelleway et al 2017). Additionally, shifts in salinity can alter the microbial community and their respective geochemical cycles. In shifts from freshwater to estuarine wetlands, methane production initially decreased but then rebounded with a respective shift in hydrogenotrophic methanogens (Dang et al 2019). Prolonged anoxic conditions will also favor the utilization of available carbon and methanogen populations will become more abundant. This will result in increased methane flux to the atmosphere. By investigating the geochemical signals (within the sediment and flux out of the sediment) and microbial community we can advance our understanding of methanogen

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activity, which is integral to accurately determining the rate and overall abundance of methane emissions from the sediment.

Simultaneous collection of geochemical data and sediment cores for microbial analyses will connect how microbial communities are affected by geochemical processes, and how, in turn, they affect these cycles. Combining the geochemical signature with the active microbial population will help us understand the activity of the system. By understanding what drives microbial function, and therefore methane emissions, future predictions can be made regarding methane cycling in mangrove ecosystems.

## **Methods**

### *Sampling*

Sediment from all vegetation types was collected using a percussion corer targeting the top 30 cm of the sediment column. Cores were stored at -20 °C for geochemical analysis and -80 °C for molecular analysis. Fresh sediment cores were sectioned in 2 cm increments for methane gas, geophysical analyses (e.g., grain size, porosity), total carbon, total organic carbon, and total nitrogen. A total of 1 g of sediment was preserved with NaOH in a glass and stored at 4 °C for total methane. An aliquot of gas was withdrawn from the headspace and measured on a gas chromatograph (Iversen and Jorgensen 1985). Grain size was measured using the following wet sieves: 4,760 µm (gravel and roots), 74 µm (sand), 38 µm (silt), and < 38 µm (silt and clay) (Guggenberger et al 1994). Porosity was measured by comparing the weight of the sediment when dried and when wet (Comeaux et al 2012, Folk 1980). Frozen sediment cores were subsectioned into 2 cm sections and porewater was extracted using 0.2 µm Rhizons (Rhizosphere, Wageningen, The Netherlands). Porewater was immediately placed into an anoxic chamber to prevent oxidation.

### *Geochemical analyses*

Sediment porewater was analyzed for sulfide, sulfate, nitrate/nitrite, ammonium, and chloride. Sulfide was measured with the modified methylene blue method in an anoxic chamber (Reese et al 2011). Reactive nitrogen in the forms of nitrate and nitrite were measured using colorimetric methods (Cataldo et al 1975, Strickland and Parsons 1972). All colorimetric methods required

standards to be made fresh on days of analysis. Sulfate and chloride concentrations were determined using an ion chromatograph.

Previously subsectioned sediment will be analyzed for total organic carbon (TOC), total carbon (TC), and total nitrogen (TN). The TOC sediment samples were acid fumigated to remove of inorganic carbon. These samples were measured using a combustion analyzer (Elemental Analyzer, Pennsauken, NJ, USA).

### *Infaunal Sampling*

Infaunal organisms were collected from 4 sites on San Jose Island, Texas during August of 2018. Sites had paired stands of marsh (*Spartina alterniflora*) and mangrove (*Avicennia germinans*) vegetation and were approximately 2m apart. Infauna were sampled with 2 replicate cores using a 30 cm PVC corer to a depth of 6 cm within each vegetation type in each site (n=8 per vegetation type). Additionally, 2 replicate cores were extracted from a nearby location (~ 5.0 m away) that was devoid of vegetation. Samples were sieved in the field with 500 µm meshed and placed in seawater buffered formalin with rose Bengal for two weeks and then transferred to 70% isopropyl alcohol for processing (sorting, identifying, counting). Samples were processed at the Dauphin Island Sea Lab from October 2018 through November 2019. Samples were exceptionally difficult to process due to large amounts of detritus in the samples.

After identification, species richness and total abundance of organisms were compared among vegetation types using Welch's ANOVA in JMP Pro 14. Welch's ANOVA was used because variances were unequal. Permutational Analysis of Variance (PERMANOVA) in PRIMER™ was employed to evaluate differences in infaunal communities between marsh, mangrove, and bare substrates. A canonical analysis of principal coordinates (CAP) plot was created to illustrate community differences between infaunal communities collected from marsh, mangrove, and bare substrate habitats. A second CAP plot was created to present infaunal community differences between marsh and mangrove areas.

### *Microbial DNA extraction and sequencing*

Cores stored at -80 °C were sectioned in 2 cm increments. Sediment from 2, 12, and 20 cm were targeted for DNA extractions because these regions had varying methane concentrations within the sediment. DNA from the sediment was extracted using the Qiagen RNeasy Kit with DNA

elution accessory kit. The Zymo Clean and Concentrator kit was used to clean and concentrate extractions (Zymo Research, Irvine, CA). DNA samples were sent to the DOE Joint Genome Institute (Walnut Creek, CA) for library preparation using Illumina NovaSeq and sequencing using Illumina Nexterra platform.

### *Bioinformatics*

Using BARRNAP, assembled metagenomes (metaSPAdes individual assemblies) were annotated using each of the databases (e.g., Archaea, Bacteria, Eukaryote, mitochondria). Samples were assigned taxonomy and compared at the order level.

### *Statistical analysis*

Students T-test was conducted on the day and night samples to look for significant differences ( $\alpha = 0.05$ ). Principle Components Analysis (PCA) and Hierarchical clustering were conducted using all porewater data from the following sampling events: November 2017, June 2018, August 2018. During these events, cores were taken in triplicate during the day and night at mangrove (*Avicennia germanins*), Spartina (*Spartina alterniflora*) and sediment that was bare or ephemerally covered in seagrass. For the purpose of this analysis, the average of the triplicates were taken to incorporate the accompanying TOC/TC data, methane data, grain size, and porosity.

Principle Component Analyses were conducted with concentrations of nitrate, ammonium, sulfate, methane, and a sulfate:chloride ratio. These models were made by separating day samples (n=44) and night samples (n=66). Hierarchical clustering was conducted to compare all 18 sampling events incorporating concentrations of total carbon, nitrate, ammonium, sulfide, methane, a sulfate to chloride ratio, and percent sand using R Studio.

## **Results and Discussion**

### *Geochemical variation*

Measured methane flux (in ppm) from each flux chamber deployment has been summarized to show the differences between multiple trials in Spartina, mangrove, and seagrass-dominated sediment (Figures 7 & 8). There was a statistically significant difference between day and night time flux chamber deployments for mangrove and Spartina-dominated sediment ( $p < 0.05$ ).



No significant difference ( $\alpha=0.05$ ) was noted between  $\text{CH}_4$  and  $\text{CO}_2$  concentrations within mangrove and seagrass-dominated sediment based on ANOVA statistical analysis. However, there was evidence of a significant difference between samples read during the day versus night. With this model, an interaction between the vegetation and the time of day was noted. This means that while the vegetation is not significantly different from each other, it does have an effect on how much  $\text{CO}_2$  will vary from day to night.

One notable result from this study was the significant difference in gas emissions on a diurnal scale. While some studies measure gas emissions on a 24 hour scale, the microbial community and geochemical signal within the sediment are often not analyzed in tandem (Call et al 2015). Incorporating all three aspects together can provide a better idea on when we should expect increased emissions and under what conditions.

Differences between the three vegetation types can begin to address the larger mangrove marsh ecosystem as a whole. Seagrass-dominated sediment, regardless of temporal scales, was consistent throughout all flux chamber deployments. While there was no significant difference between mangrove and *Spartina*-dominated sediment, the interaction between the vegetation and time of day for carbon dioxide emissions may hint at a bigger story. The observed interaction can be elucidated by investigating the activity of the microbial community within these sediments. By investigating pathways for methanogenesis and methanotrophy, more can be understood about the gases within the sediment and the relative amount that fluxes into the atmosphere. Understanding shifts in these microbial communities and respective shifts in gas emissions will be beneficial to coastal management as we see a shift in mangrove marsh vegetation along coastlines.

It is important to note that these flux chamber trials were variable within sites. This may be because of a variety of factors including bioturbation, and microsites within the sediment. Bioturbation in these mangrove marsh systems could be due to the benthic macrofauna and their accompanying burrows (Cameron et al 2019). Within the chamber for each deployment, individual burrows were observed but not quantified. These burrows can expose deeper sediment to oxic conditions as well as allow for gas to escape into the atmosphere at a quicker rate. Future studies should quantify these burrows and incorporate these data into their statistical models. Within the sediment, there is evidence for microsites seen within cores at the geochemical level. This can also be observed by seeing changes in coloration from sampled sediment. When

deploying the flux chambers, the chamber area encompasses the top four to six centimeters of the sediment column. Within this area, one would assume there are microsites that have different access to nutrients (i.e. root mass, trapped detritus), inundation, and oxygen levels (Goreau and de Mello 2007, Welte et al 2017). This will go hand in hand with a varying microbial community, potentially leading to altered methane and carbon dioxide emissions. When deploying flux chambers, this is not apparent. Even though chambers are deployed in what seems to be the same area, this can lead to variation within flux chamber readings. Future studies should examine these microsites further by ensuring adequate sampling to address variation in flux measurements.

Nitrate/nitrite concentrations for the 18 sampling events were statistically different between day and night sampling events in the November 2017 mangrove-dominated samples and sediment that was bare or ephemerally covered in seagrass in June 2018 ( $p < 0.05$ ). These results did however show that the amount of nitrate/nitrite in November (max 200  $\mu\text{M}$ ) was greater than the concentrations in June and August 2018 (max 50  $\mu\text{M}$ ).

There was evidence that day and night samples from mangrove-dominated sediment collected in November, June, and August were statistically different in concentrations of both sulfide and ammonium ( $p < 0.05$ ). There was evidence that day and night samples from mangrove and *Spartina*-dominated sediment collected in June, and August were statistically different in concentrations of sulfate ( $p < 0.05$ ). The concentrations of sulfate were not statistically significant ( $p < 0.05$ ) between June and August for the mangrove and *Spartina*-dominated sediment. There was evidence that the November *Spartina*, November base/seagrass, and June *Spartina*-dominated sediment were significantly different between day and night in concentrations of sulfate.

#### *Infaunal organism variation*

Twenty-nine infaunal species were collected from cores. Nematodes were the most abundant organisms collected, followed by copepods and seed shrimp (*Ostracoda*) (Table 3, Figure 11). SIMPER analysis revealed that bare sites were more than 89% dissimilar from both marsh and mangrove sites, and marsh and mangrove sites were 54% dissimilar from one another (Table 3). Species richness was significantly different among marsh, mangrove, and bare sites, with richness highest in marsh sites ( $F_{2,7.5} = 5.18$ ,  $p = 0.039$ , Figure 2a). The total abundance of organisms was highest in mangrove sites and driven by large nematode abundance ( $F_{2,9.36} = 36.3$ ,

$p < 0.001$ , Figure 12b). Infaunal communities collected were significantly different among bare/seagrass, marsh, and mangrove habitats (Pseudo  $F_{2,17} = 3.32$ ,  $p = 0.006$ , Figure 13a). Bare sites were more different than both vegetated sites, but communities were different among marsh vs. mangrove locations (Figure 13b) exceeded 65. Based on the limited one-year nature of this study, we can only correlate the cause of this difference, which we attribute to the emission of reduced gases and the quality of the sediment. A prolonged sampling period is required to verify these initial findings.

#### *Microbial population variation*

Outside the scope of this study, but relevant to the findings, we analyzed a total of 31 sediment samples for microbial community structure (Figure 18). These samples were from all 18 sampling events at 2, 12, and 20 cm below ground surface. These depths were selected to target the top of the sediment column, and areas of interest based on methane production.

Methanogens present at the order level were the *Methanosarcinales* which are a class II methanogen known for using methanol, methylamine, and acetate ( $H_2 + CO_2$ ) as their substrates for methanogenesis (Holmes and Smith 2016, Liu 2010). Previous studies have shown that methanogenesis can occur in oxygenated soils. Acetoclastic methanogenesis are most commonly found in these oxidized sediments (Angle et al 2017). Other methanogens present included the candidate taxa Methanofastidiosales, which are related to Class I and II methanogens (Borrel et al 2019, Vanwonterghem et al 2016).

Relating to methane production, *Anaeroliniales*, were also found in each sample. These bacteria are known for breaking down alkane chains crucial to starting methanogenesis (Liang et al 2015, Liu et al 2019). The presence of these Bacteria shows the potential for carbon sources to be available to methanogens in all samples. Potentially interfering with methanogenesis, *Desulfobacterales* were shown as a large portion of all samples. These sulfate reducing bacteria use acetate, an electron acceptor also used by methanogens (Chuang et al 2016, Das et al 2018). This competition could therefore effect methane production and overall emissions.

In order to truly understand more about the microbial community and its relation to the cryptic carbon cycle, more work needs to be done addressing the total and active community. Pathways regarding methanogenesis, methanotrophy, and sulfate reduction should be

investigated for completeness, and relative abundance with respect to other cycles. These directions can help elucidate the cryptic carbon cycle.

### *Statistical network modeling*

A Principle Component Analysis was conducted to compare day (n=44) and night (n= 75) sampling events. Concentrations of sulfide, methane, nitrate, ammonium, and a sulfate:chloride ratio were used to build this model. Samples collected during the day can be explained by concentrations of methane (PC1 – 42.30%) and sulfide (PC2 – 70 %). Samples collected during the night can be explained by concentrations of methane (PC1 – 89.99 %) and sulfide (PC2 – 70.66%). Both figures showed groupings of vegetation regardless of time of year sampled. Sulfide had the biggest effect on *Spartina* night samples in November 2017. Methane had the biggest effect on mangrove day samples from June of 2018. This is where we also observed the greatest flux of methane via benthic flux chamber measurements.

Hierarchical clustering was used to compare all 18 sampling events using concentrations of nitrate, ammonium, sulfide, methane, and a sulfate:chloride ratio (Figure 15). This model also incorporated the percent sand and the total carbon within each sample. Night samples in August 2018 for mangrove and *Spartina*-dominated sediments served as the outgroup for this model. These samples were significantly different in concentrations of methane and sulfate. Alternatively, the day samples in August 2018 for mangrove and *Spartina* were shown to be similar to each other and grouped with other mangrove samples (June night and November night). Submerged bare sediment that was near seagrass samples collected in August 2018 grouped together and was shown to be more similar to the seagrass June and November night samples.

### **Conclusions**

The Texas coastal mangroves are expanding at an alarming rate overtaking saltmarsh habitats. This purpose of this study was to determine the effect of this expansion on sediment and water quality, and therefore the subsequent effect on the benthic organisms residing therein. Unique to this study, we saw distinctions in microbial populations, porewater chemistry, and methane emissions on a diurnal scale (midnight and noon sampling). Bare sites were more different than both vegetated sites, but benthic organism communities were also different among marsh vs.

mangrove locations. Based on the limited one-year nature of this study, we could only correlate the cause of this difference, which we attribute to the emission of reduced gases and the quality of the sediment. A prolonged monitoring study is required to verify these initial findings.

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## Tables







		<b>Total Carbon</b>
Vegetation vs Vegetation	Mangrove vs. Spartina	Significant $p < 0.05$
	Spartina vs. Seagrass	not significant
	Mangrove vs. Seagrass	Significant $p < 0.05$
Month vs Month	November vs. June	not significant
	June vs. August	not significant
	November vs. August	Significant $p < 0.05$

**Table 1:** P-values and significance of percent carbon when comparing vegetation types (including types for that mall months for that type of vegetation) and when comparing months (including all vegetation month).





## Daily flux ( $\mu\text{M cm}^{-2} \text{ day}^{-1}$ )

		November	June	August
<b>Mangrove</b>	<b>Day</b> 	-	-125.18	-72.81
	<b>Night</b> 	-122.30	-40.63	-317.35
<b>Spartina</b>	<b>Day</b> 	-	-32.02	-92.99
	<b>Night</b> 	-66.99	-10.57	-289.10
<b>Seagrass</b>	<b>Day</b> 	-	-16.72	-12.62
	<b>Night</b> 	-16.40	-14.26	-9.28

**Table 2.** Calculated flux from the three sampling events. Negative values denote flux out of the sediment into the atmosphere.

<b>Taxa</b>	<b>Vegetation</b>
Arachnida	Spartina
Ascidacea	Spartina
Brachyura, Zoea	Spartina
Capitellidae	Mangrove, Spartina, Unvegetated
Centropagidae	Mangrove, Spartina
Cirratulidae	Spartina
Crepidula sp.	Spartina
Cumacea	Mangrove, Spartina
Cyclopoida	Mangrove, Spartina, Unvegetated
Diptera, larvae	Mangrove, Spartina,
Foramifera	Mangrove, Spartina, Unvegetated
Hesionidae	Spartina
Hydrobiidae	Mangrove, Spartina, Unvegetated
Insecta	Spartina
Lepidoptera, larvae	Spartina
Nematoda	Mangrove, Spartina, Unvegetated
Oligochaeta	Mangrove, Spartina, Unvegetated
Onuphidae	Mangrove, Spartina
Orbiniidae	Mangrove, Spartina, Unvegetated
Ostracoda	Mangrove, Spartina, Unvegetated
Paraonidae	Unvegetated
Pseudopolydora, larvae	Spartina
Sipuncula	Spartina
Spionidae	Mangrove, Spartina, Unvegetated
Tanaidacea	Mangrove, Spartina

**Table 3.** Taxa identified in mangrove, Spartina, and seagrass samples in August 2018.

Table 4. SIMPER analysis level of dissimilarity among bare, mangrove, and marsh communities and the types of organisms contributing to observed differences.

**Bare vs. Mangrove** Average Dissimilarity = 90.16

Order	Common Name	Mean Abundance Bare	Mean Abundance Mangrove	Average Dissimilarity	% Dissimilarity Contribution
Nematoda	Nematodes	9.0	544.57	52.9	58.71
Ostracoda	Seed Shrimp	29.0	66.3	1.46	11.97

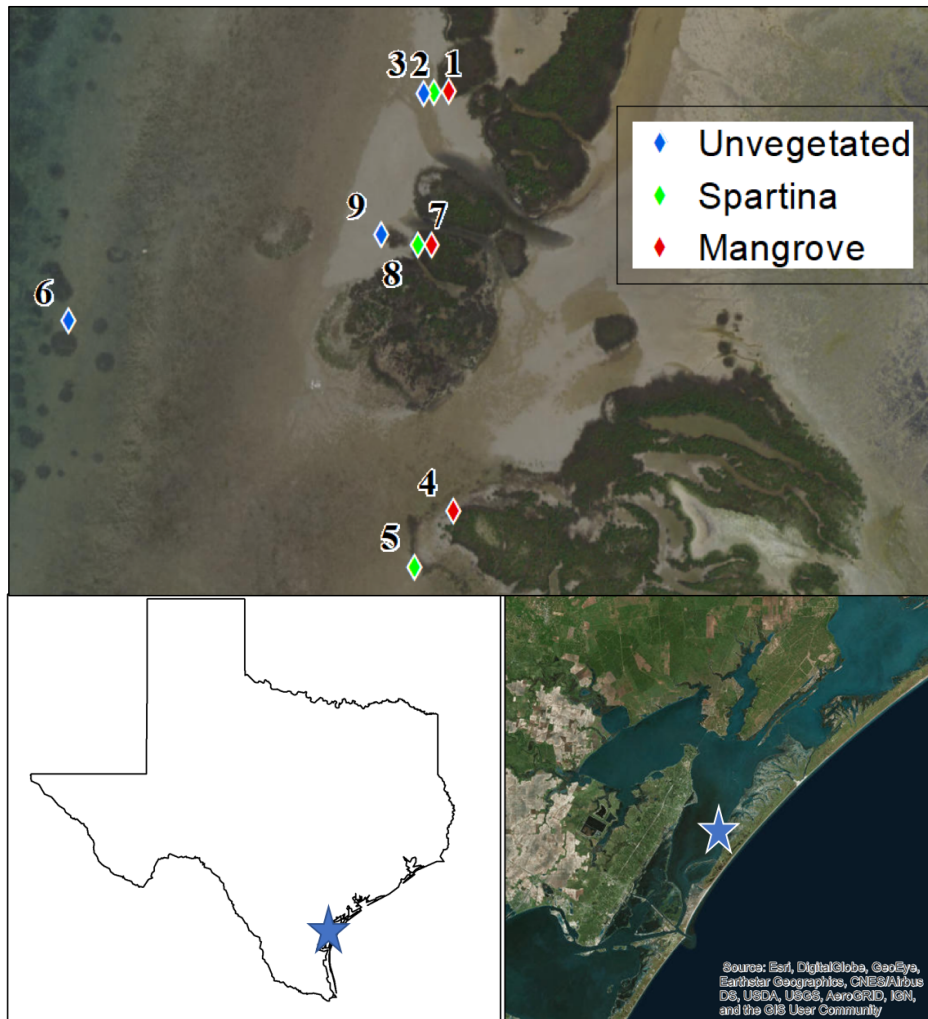
**Bare vs. Marsh** Average Dissimilarity = 89.22

Order	Common Name	Mean Abundance Bare	Mean Abundance Mangrove	Average Dissimilarity	% Dissimilarity Contribution
Nematoda	Nematodes	9.0	365.75	45.29	50.76
Cyclopoida	Copepods	0.50	112.13	13.97	15.66
Foramifera	Foramiferans	3.0	102.75	13.45	15.08

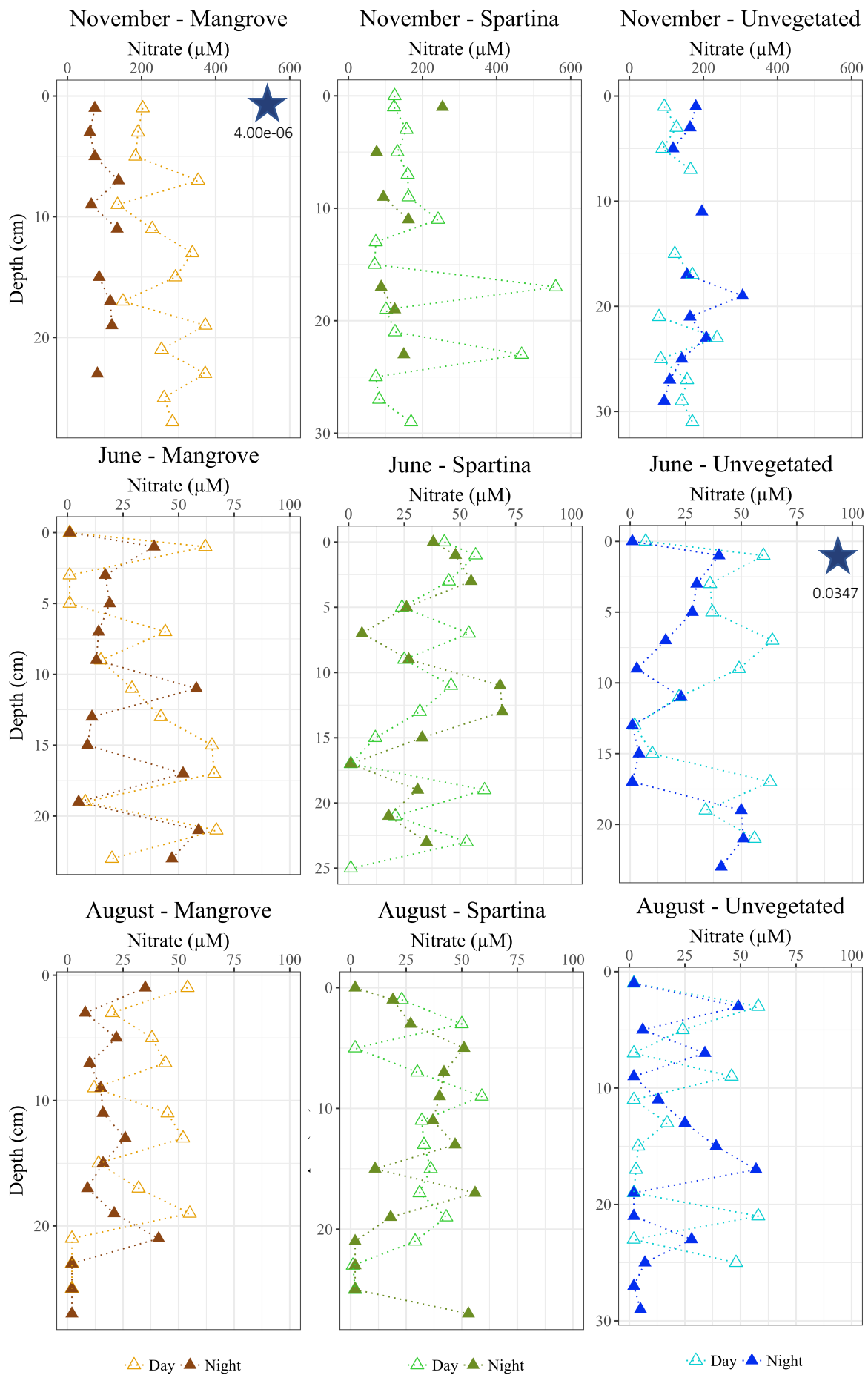
**Mangrove vs. Marsh** Average Dissimilarity = 54.62

Order	Common Name	Mean Abundance Mangrove	Mean Abundance Marsh	Average Dissimilarity	% Dissimilarity Contribution
Nematoda	Nematodes	544.57	365.75	26.61	48.72
Foramifera	Foramiferans	79.86	102.75	8.38	15.34
Cyclopoida	Copepods	81.57	112.13	6.46	11.83

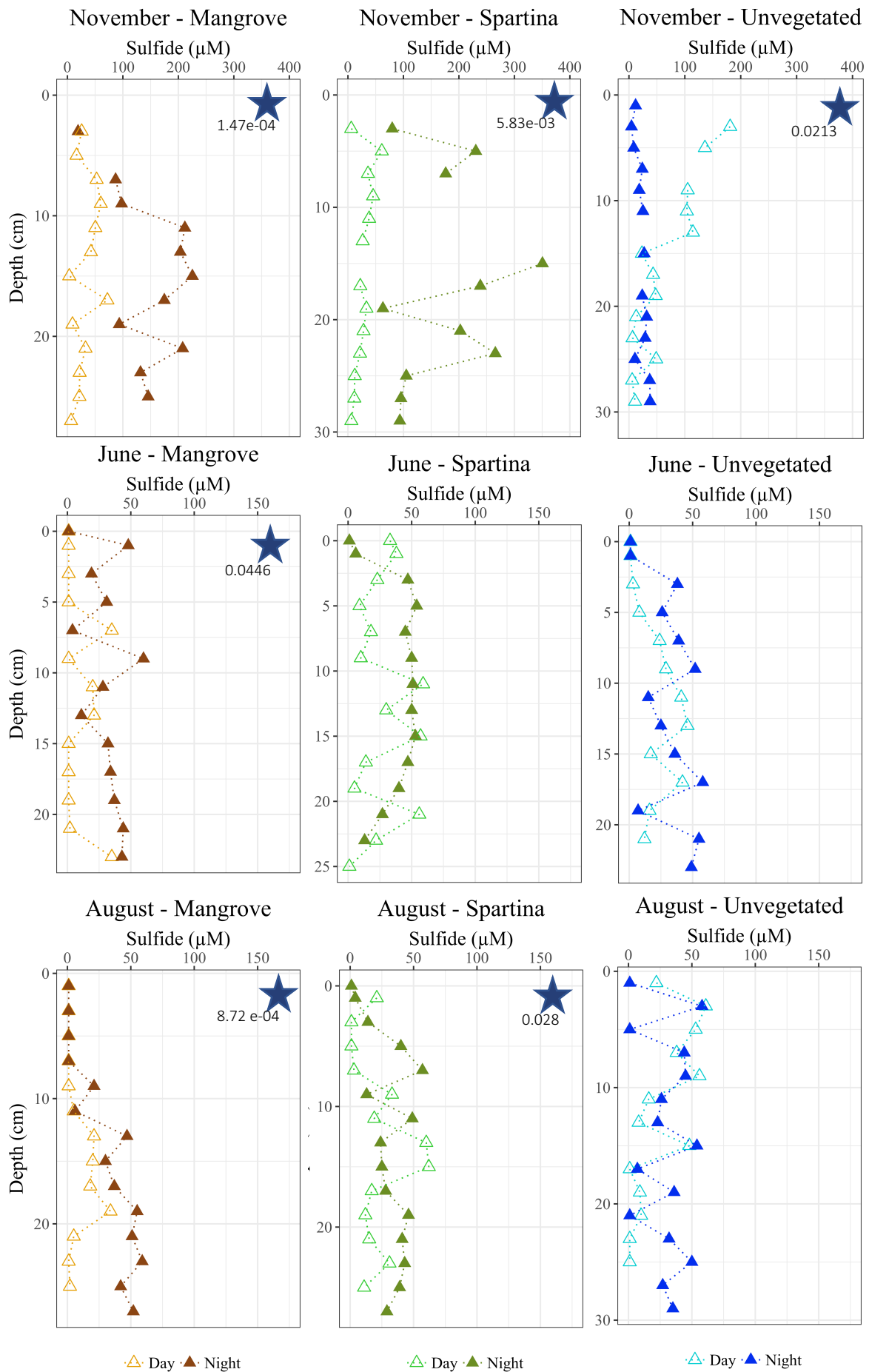
## Figures



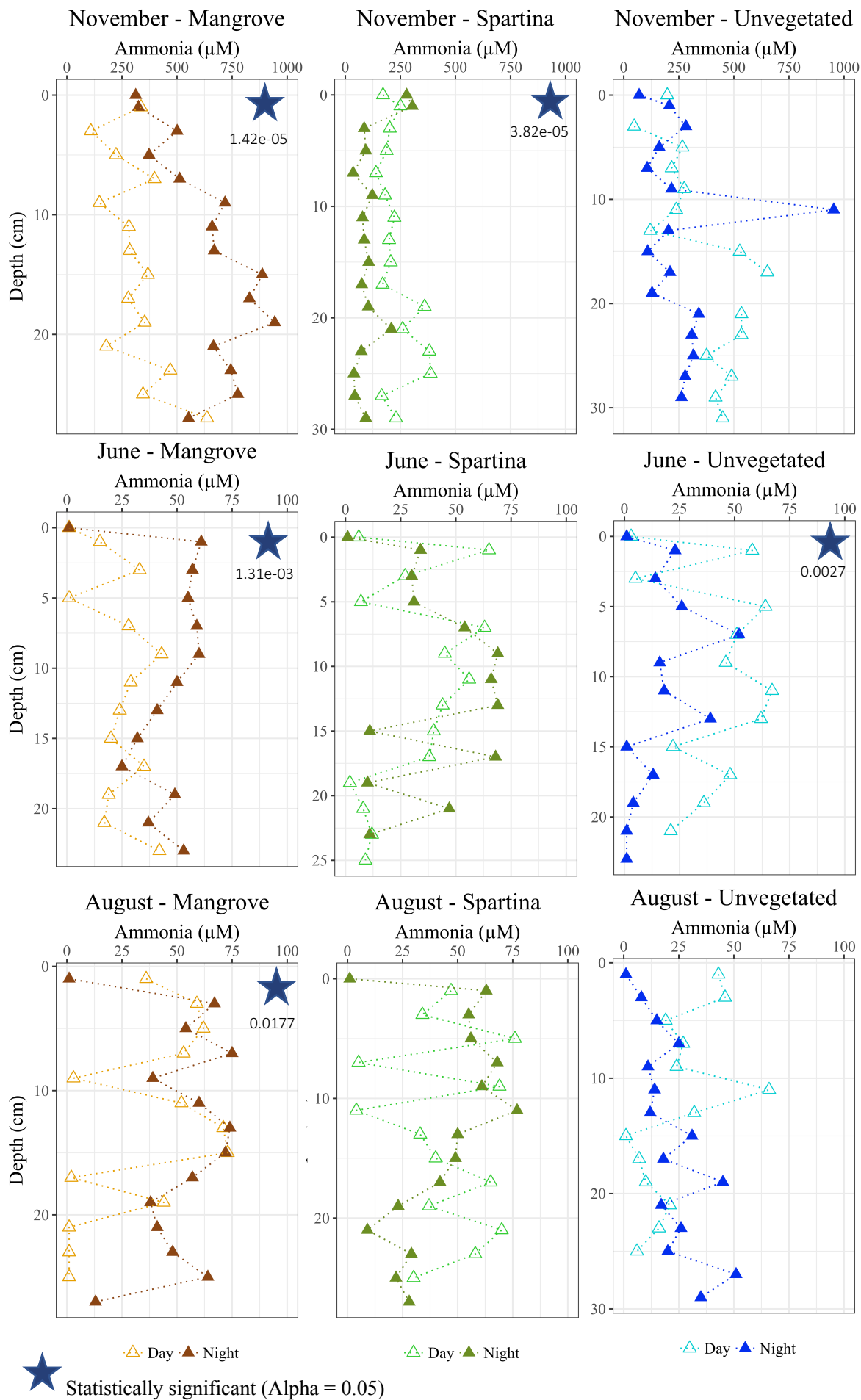
**Figure 1.** Map of sample sites from Mission-Aransas National Estuarine Research Reserve. Numbers 1-3 denote November 2017, 4-6 denote June 2018, and 7-9 denote August 2018.



**Figure 2.** Nitrate concentrations changing with depth

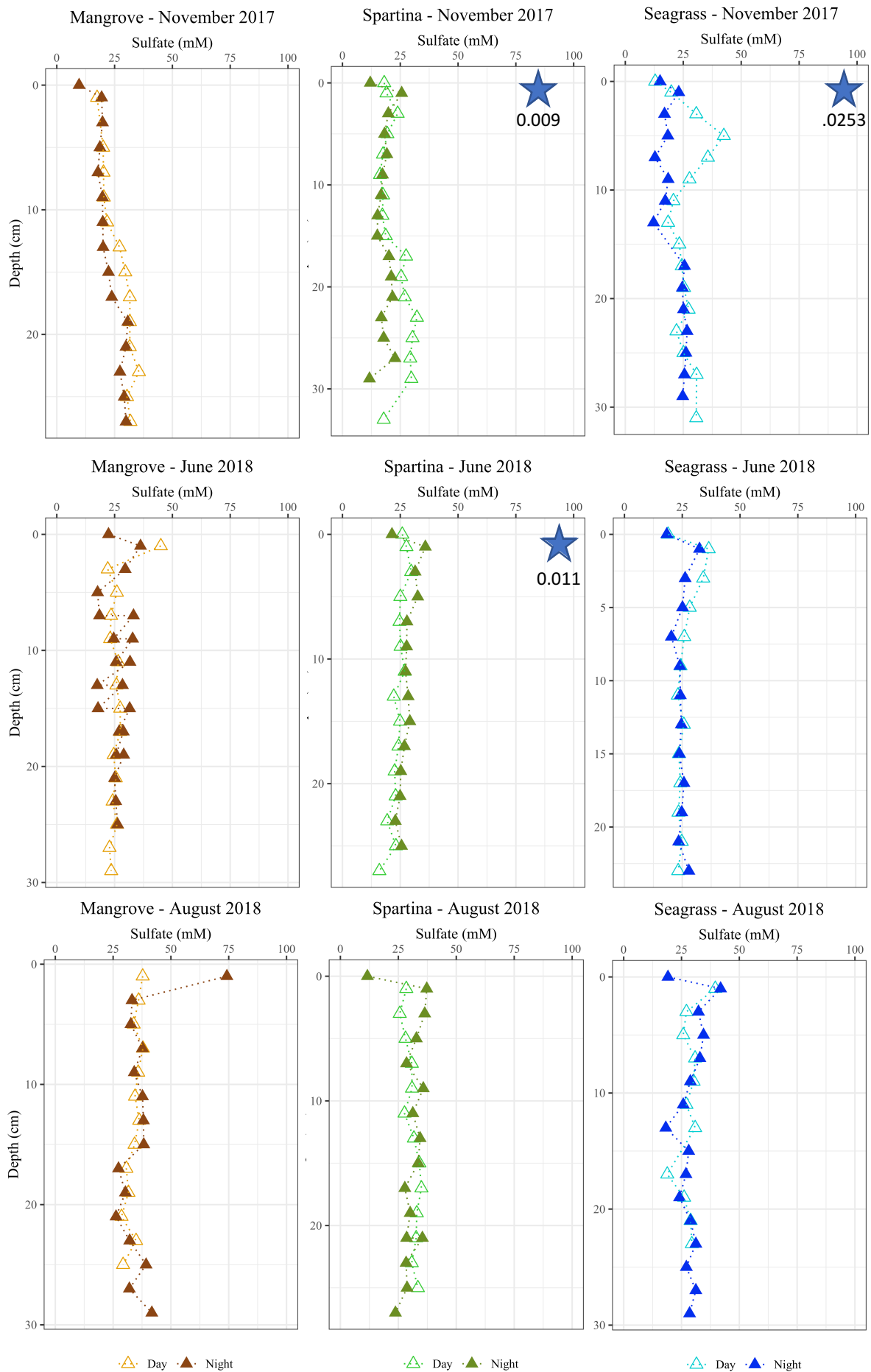


**Figure 3.** Sulfide concentrations changing with depth

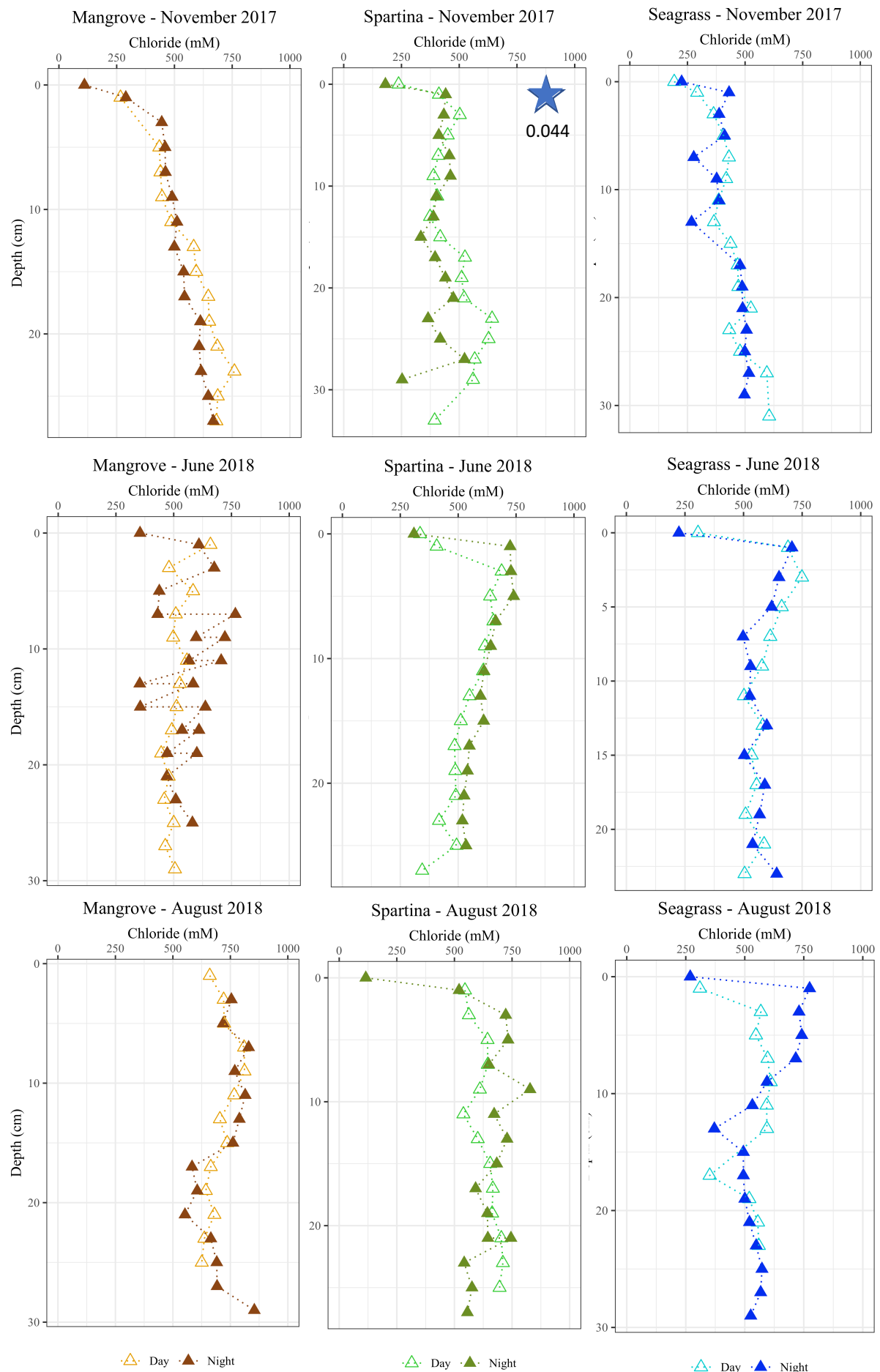


**Figure 4.** Ammonium concentrations changing with depth.

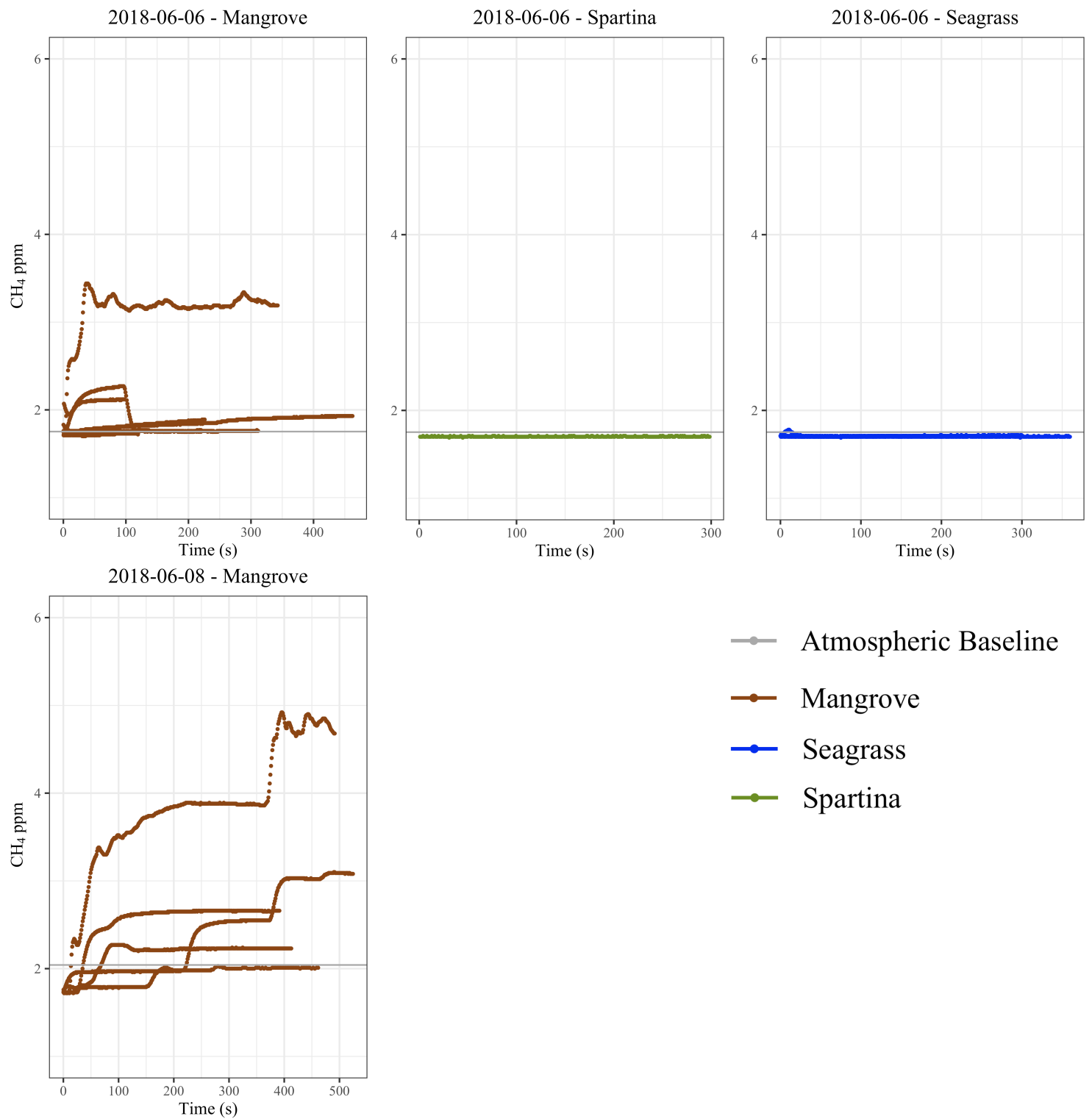




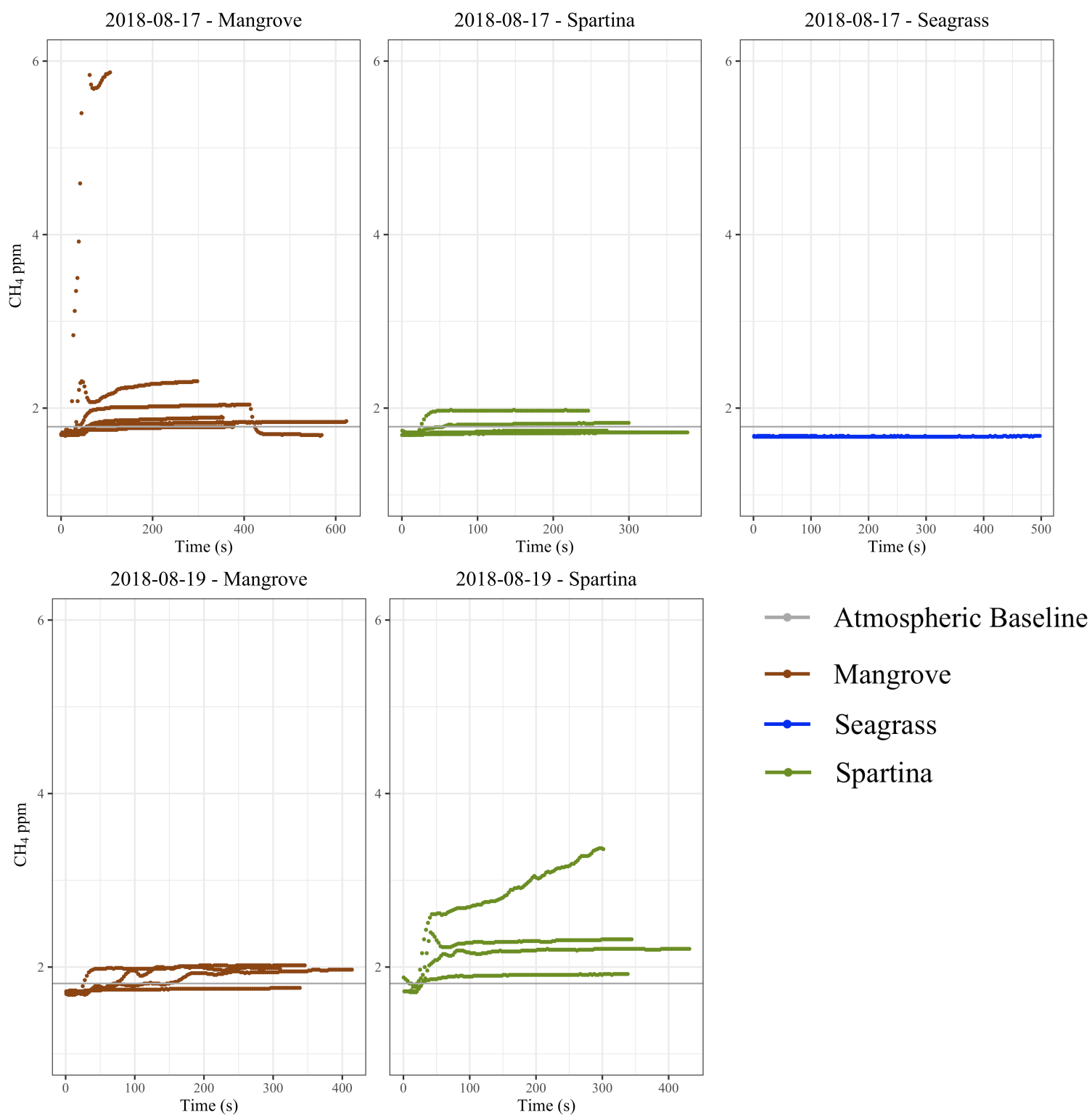
**Figure 5.** Sulfate concentrations changing with depth



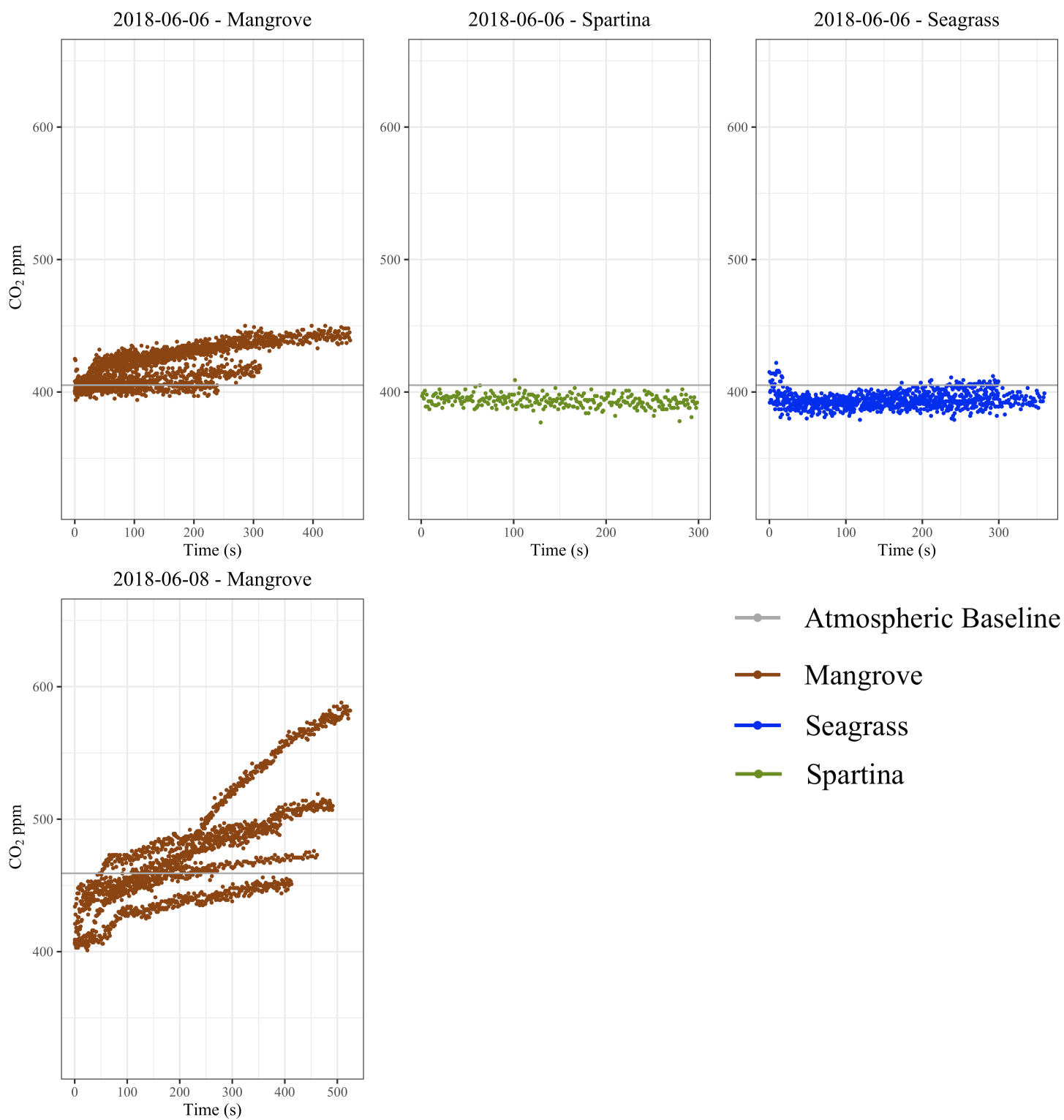
**Figure 6.** Chloride concentrations changing with depth



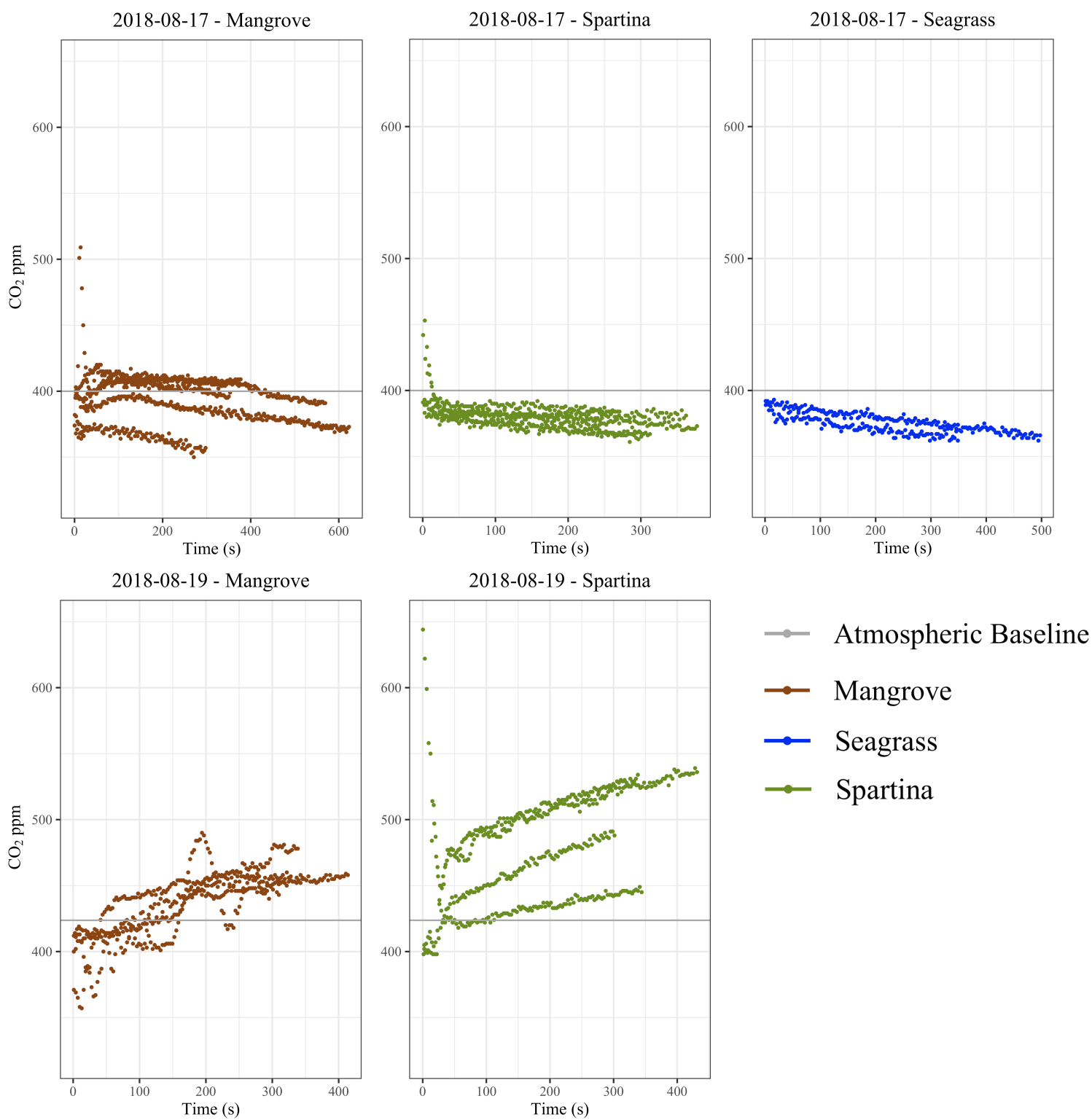
**Figure 7.** Methane concentrations in mangrove, Spartina, and seagrass dominated sediment during the day and night of June 2018.



**Figure 8.** Methane concentrations in mangrove, Spartina, and seagrass dominated sediment during the day and night of August 2018.



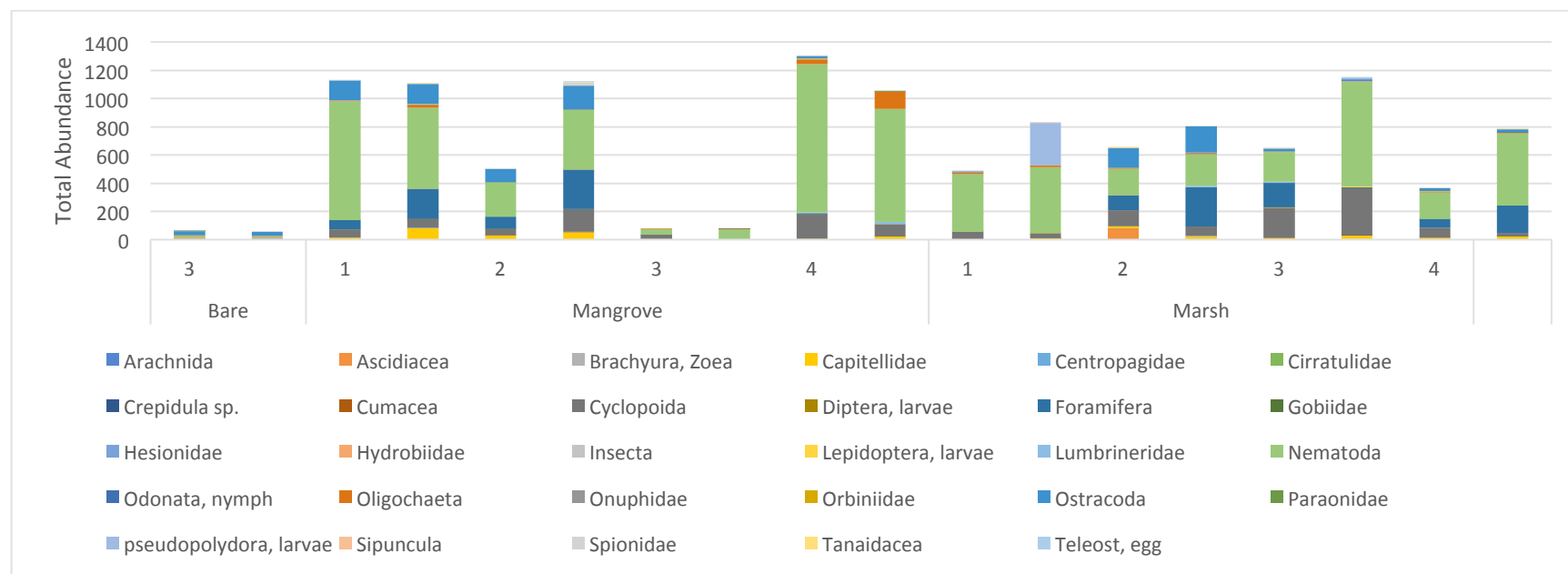
**Figure 9.** Carbon dioxide concentrations in mangrove, Spartina, and seagrass dominated sediment during the day and night of June 2018.



**Figure 10.** Carbon dioxide concentrations in mangrove, Spartina, and seagrass dominated sediment during the day and night of August 2018.

Figure 11. A) Total abundance of infauna collected in bare, mangrove, and marsh sites. B) Percentage of organisms in each sample.

**A**



**B**

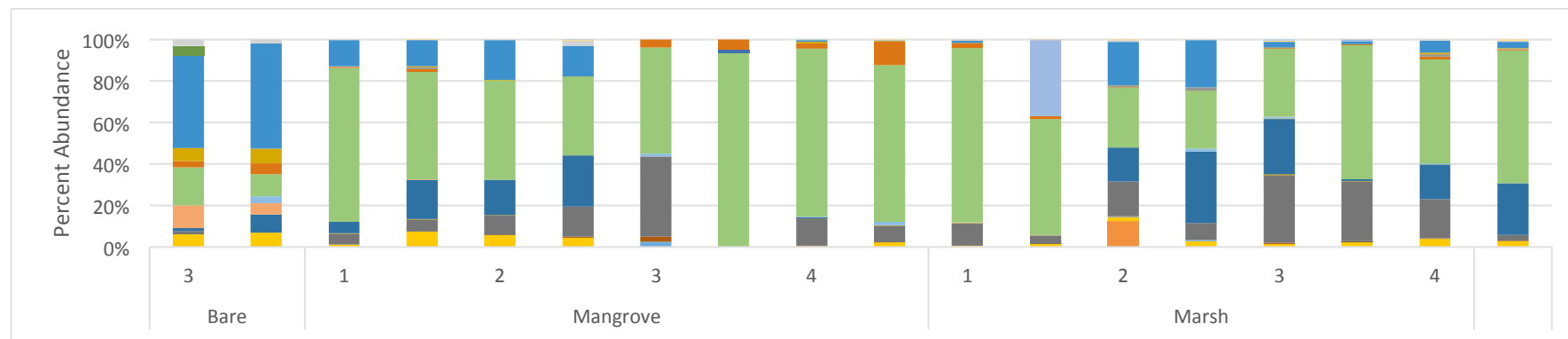
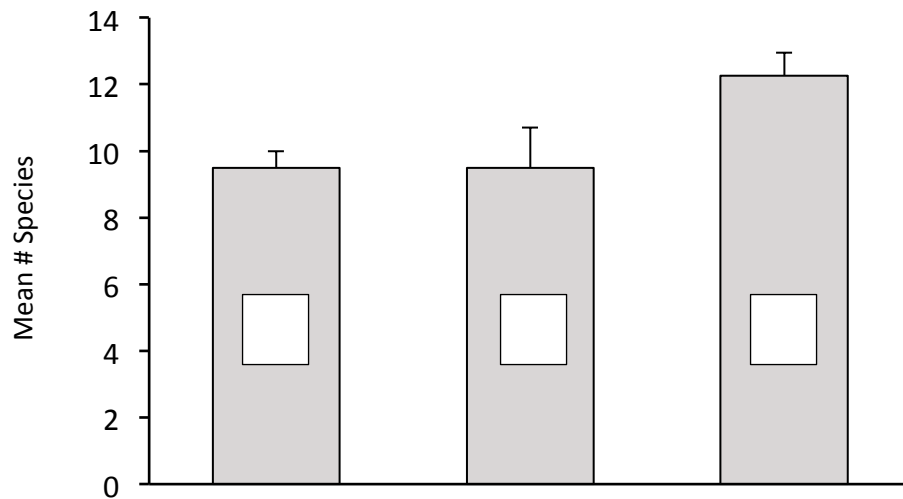


Figure 12. Mean + SE A) species richness and B) species abundance in marsh and mangrove habitats. Values are significantly different, with richness higher in marsh sites and abundance higher in mangrove sites. Letters indicate pairwise differences.

**A)**



**B)**

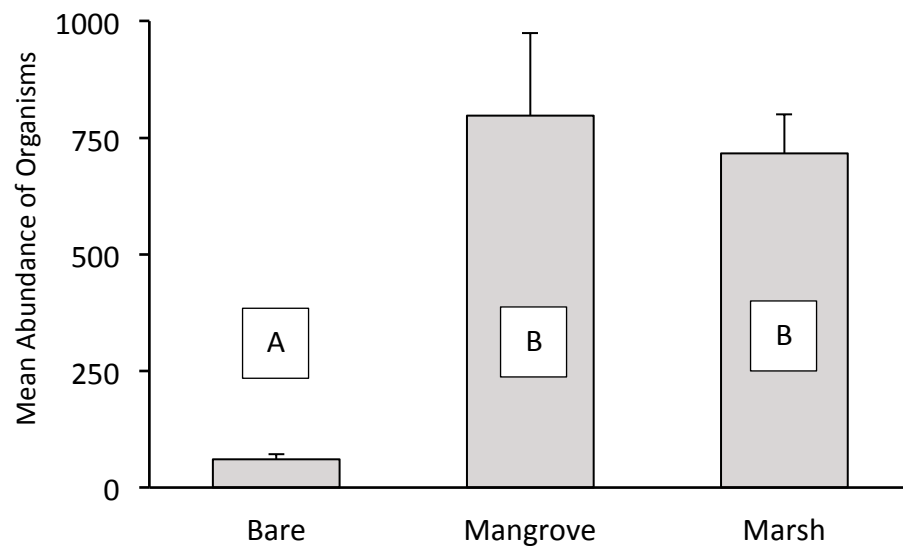
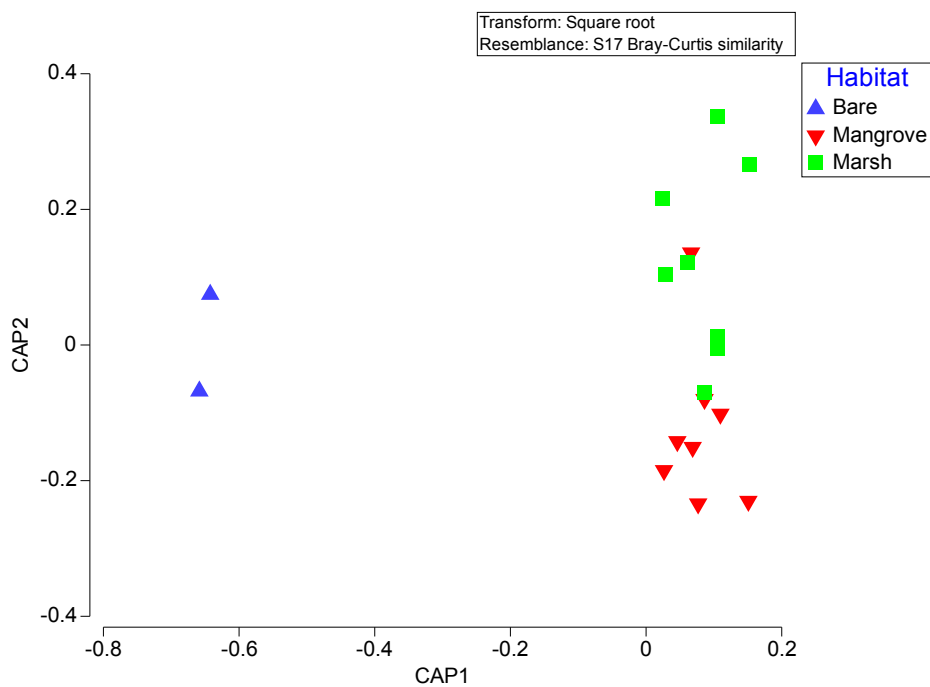


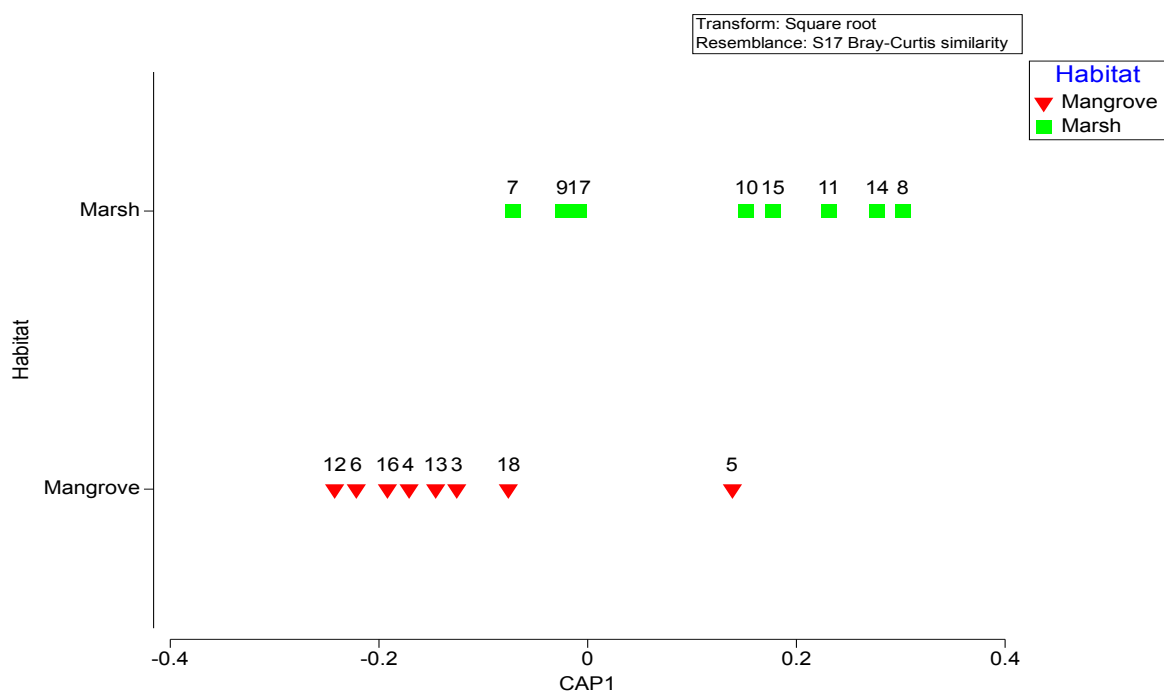


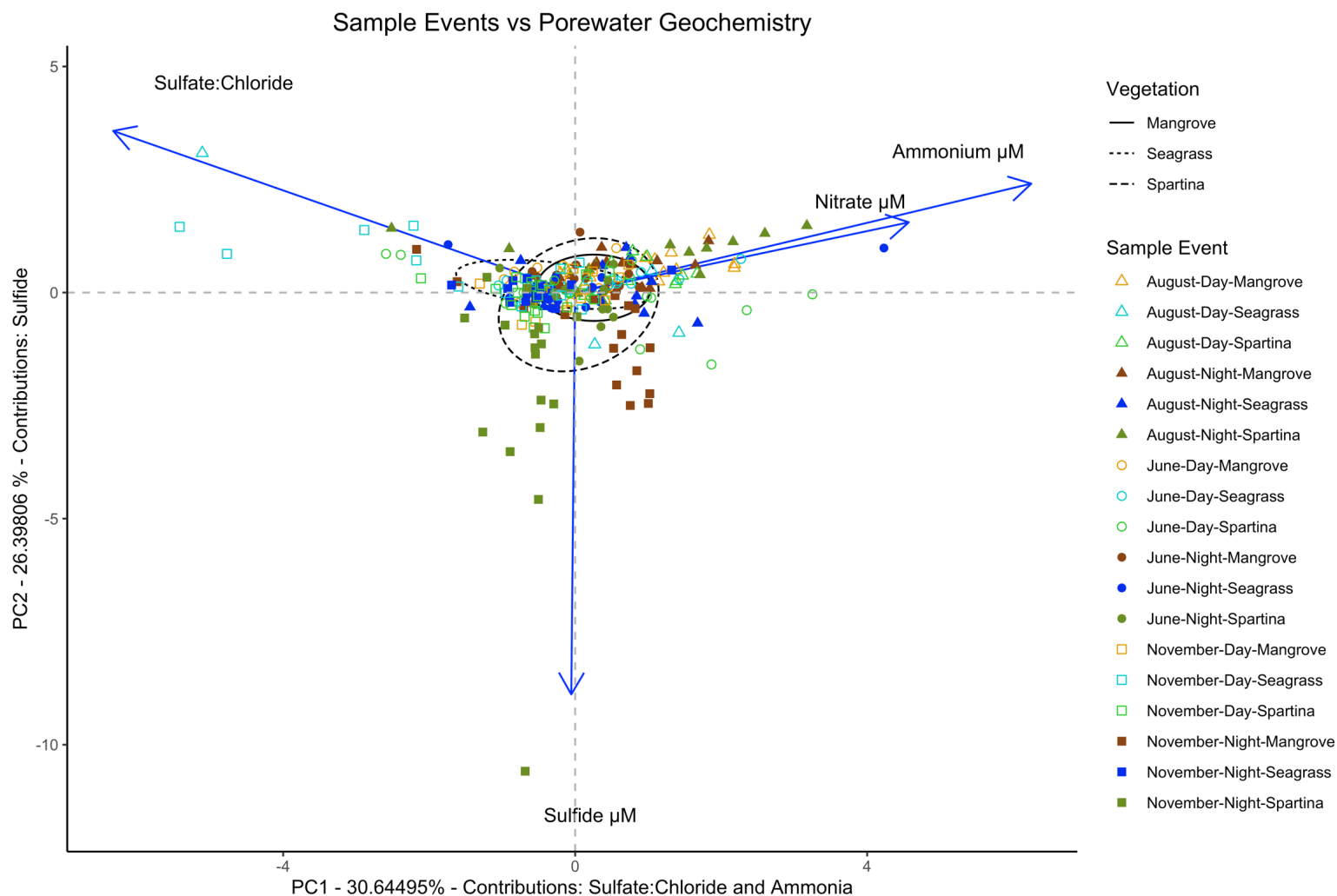
Figure 13. A) Canonical analysis of principle coordinates (CAP) plot comparing infaunal communities collected from areas with marsh vegetation, mangrove vegetation, or lacking vegetation (bare).

A)



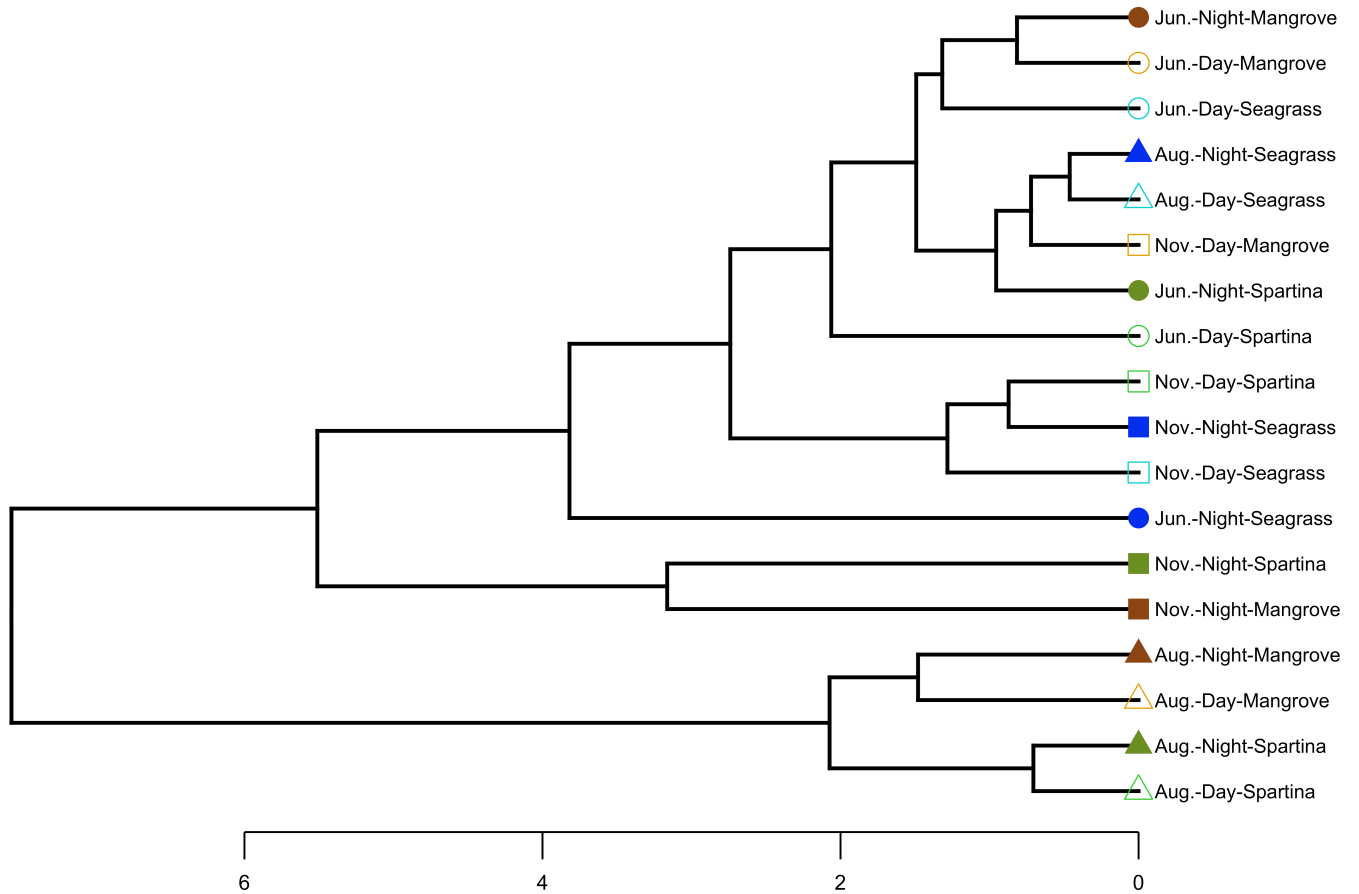
B)



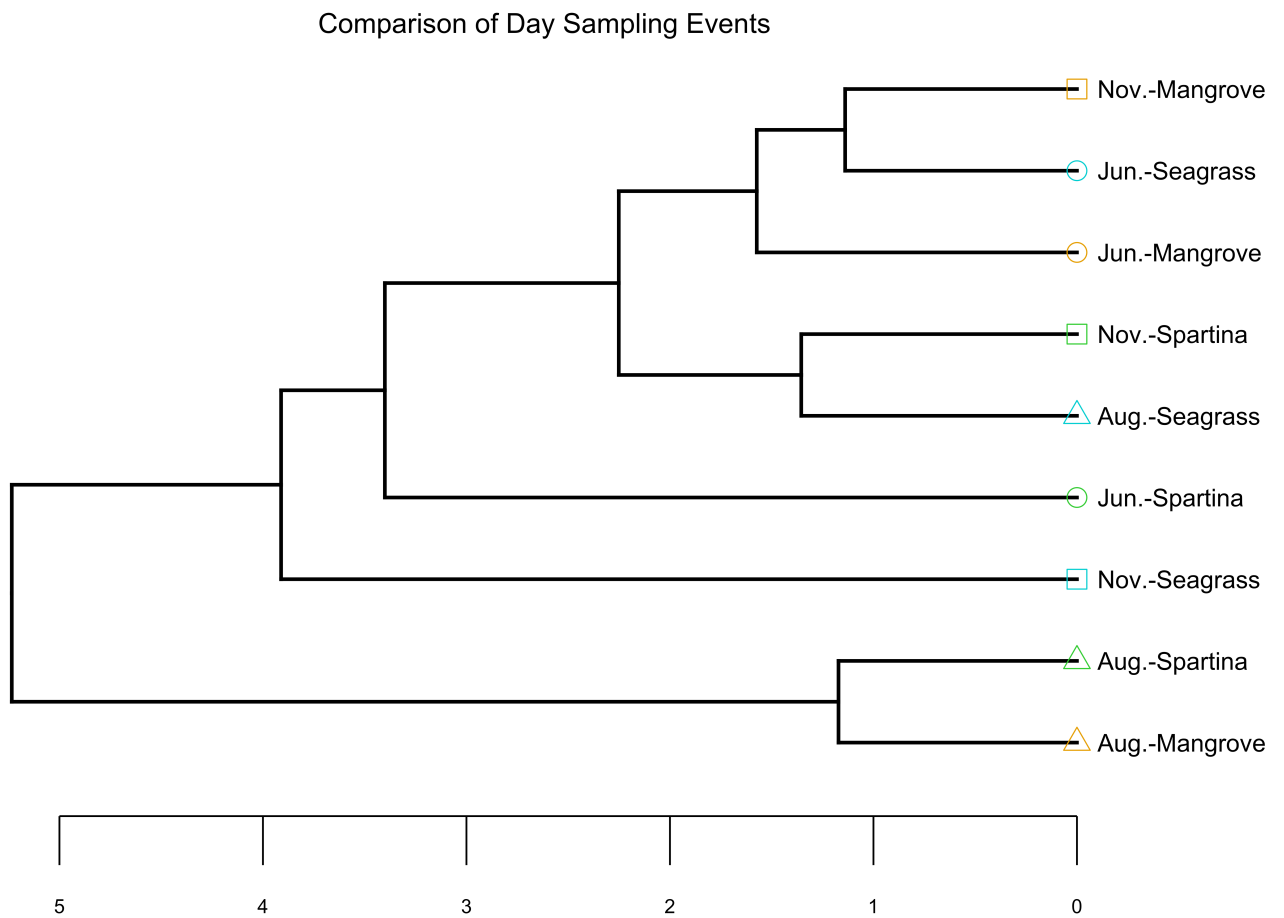


**Figure 14.** Principle components Analysis model investigating how porewater concentrations of nitrate, ammonium, a sulfate:chloride ratio, and sulfide affect our sampling events. Samples were collected during November 2017, June 2018, and August 2018 during the day and during the night for a total of 18 individual sampling events (3 months x 3 vegetations x 2 time points).

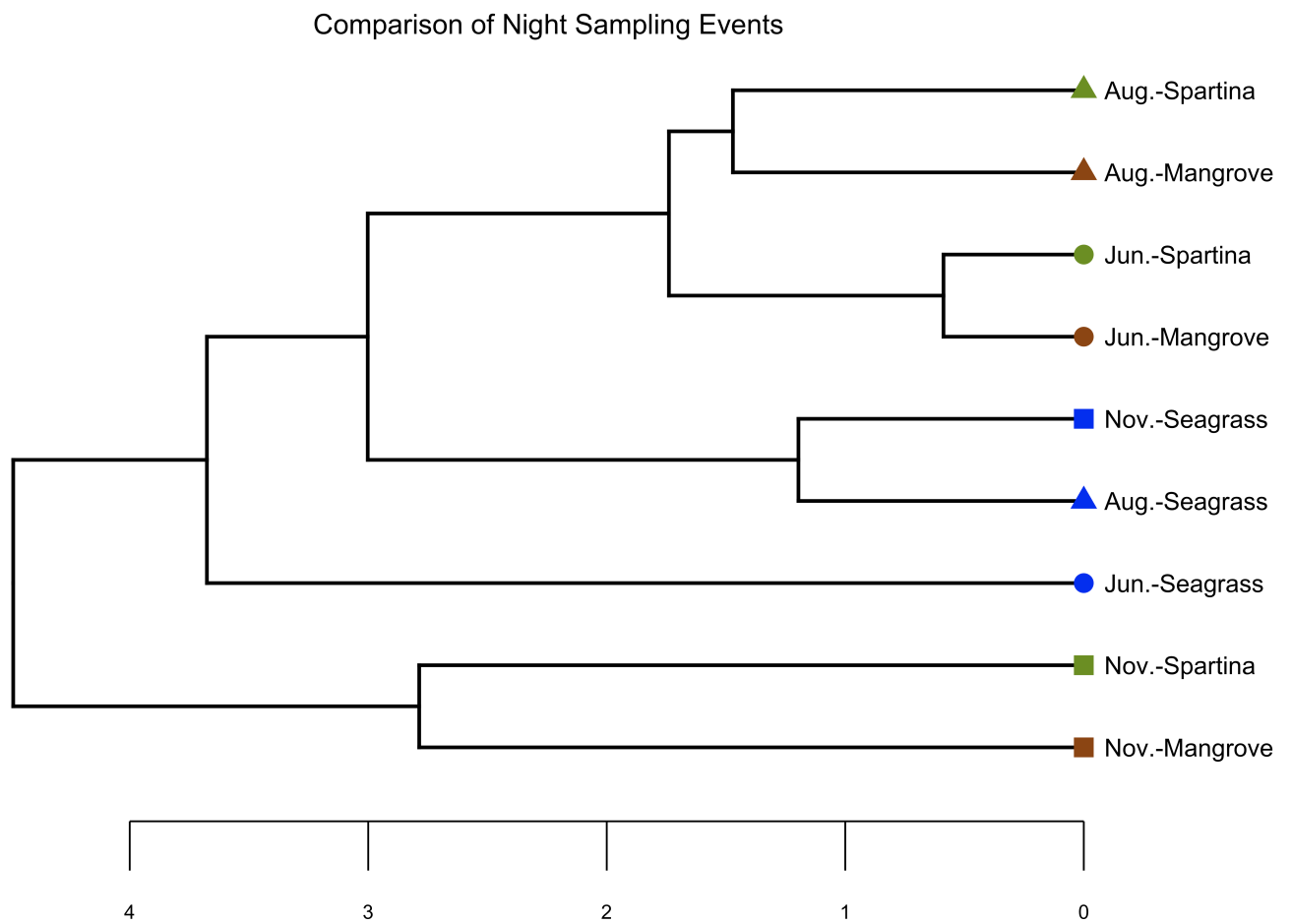
### Comparison of Sampling Events



**Figure 15.** Hierarchal clustering showing similarities between sampling events.



**Figure 16.** Hierarchical clustering showing similarities between all day time sampling events.



**Figure 17.** Hierarchical clustering showing similarities between all night time sampling events.