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The Effects of Drought on Ammonia-oxidizing Archaea in Salt Marshes

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TABLE OF CONTENTS

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ABSTRACT

THE EFFECTS OF DROUGHT ON AMMONIA-OXIDIZING ARCHAEA IN SALT MARSHES

by Hayley McMahon

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Severe droughts are expected to occur with increasing frequency under current climate change models. Droughts conditions lead to changes in the chemistry of salt marsh sediment by raising salinity, decreasing pH, and shifting conditions from anoxic to oxic. These changes have the potential to impact salt marsh microbial communities, including those involved in the nitrogen cycle. Ammonia-oxidizing archaea (AOA) carry out the oxidation of ammonia (NH_3) to nitrite (NO_2^-), the first and most rate limiting step of nitrification. I analyzed community composition and abundance of AOA in sediment samples collected from a Connecticut salt marsh during 2016, a severe drought year, and two non-drought years (2014 and 2017). Samples were collected from sites dominated by short-form *Spartina alterniflora* (SSA), tall-form *Spartina alterniflora* (TSA), *Spartina patens* (SP), and the microbial mat (MAT). Results revealed a significant difference in overall AOA community composition, but not abundance, during the drought year. A significant shift in community composition during the drought year was seen in SSA and TSA sites, but not SP or MAT sites. This change in AOA community composition may signify differences in nitrogen cycling at the marsh.

INTRODUCTION

Nitrification

The availability of nitrogen in an ecosystem is controlled by the nitrogen cycle, a complex process mediated primarily by microorganisms. Nitrification is a step of the nitrogen cycle in which ammonia is oxidized to nitrite, which is then oxidized to nitrate. Because nitrate is the primary form of nitrogen taken up by plants, nitrification serves as an important regulator of primary productivity. Additionally, nitrification is often coupled with denitrification (Jenkins and Kemp 1984), a microbially facilitated process in which nitrate is converted to nitrogen gas and ultimately moved out of the system. Because nitrification plays a vital role in determining the fate of environmental nitrogen, it is important to understand how changes in environmental conditions may impact nitrification.

Ammonia-Oxidizing Archaea

The first and most rate limiting step of nitrification, the oxidation of ammonia to nitrate, is carried out by ammonia oxidizers. Ammonia oxidizers are chemolithoautotrophic archaea (AOA) and bacteria (AOB) present in the soil (Kowalchuk et al. 2001, Könneke et al. 2005). At this time, AOA and AOB are the only groups of organisms known to carry out this first step of nitrification. Ammonia oxidizers are a difficult group to culture, which previously made them very difficult to study. However, advancements in molecular techniques have made it possible to study ammonia oxidizers using culture-independent approaches based on functional genes. The ammonia monooxygenase subunit A gene (*amo*A) codes for a portion of ammonia monooxygenase, an enzyme used by AOA and AOB to catalyze the oxidation of ammonia to nitrite (Kowalchuk et al. 2001). Because this gene is unique to ammonia oxidizers, it is often used as a molecular marker to identify and quantify ammonia oxidizers in a sample.

Archaea were initially classified as a distinct domain in 1977 (Woese and Fox). At the time, they were believed to exist exclusively as extremophiles that can tolerate harsh environments such as hot springs. This view of archaea has since shifted as the advancement of molecular techniques has allowed for the identification of archaea in a diverse range of terrestrial and aquatic environments. In 2005, the first ammonia-oxidizing archaeon was isolated from a marine aquarium (Könneke et al. 2005). Prior to this discovery, ammonia oxidation was believed to be carried out exclusively by AOB. Since their discovery, ammonia-oxidizing archaea have been described in a number of terrestrial and aquatic environments, including rice paddies (Gao et al. 2018), grassland meadows (Fuchslueger et al. 2014), the Sargasso Sea (Newell et al. 2013) and salt marshes (Moin et al. 2009).

Salt marshes as nitrogen sinks

Salt marshes are highly productive coastal ecosystems that serve as a barrier between terrestrial and marine environments. Salt marshes provide vital ecosystem services such as storm surge protection, pollutant filtration, and habitat for many commercially important species of fish (Vernberg 1993). An understanding of salt marsh nitrogen cycling is especially important because these ecosystems are known to serve as nitrogen sinks. Because primary production in salt marshes is nitrogen limited, salt marshes are able to take up much of the bioavailable nitrogen that enters them (Vernberg 1993). The addition of nitrogen to salt marshes has been shown to increase primary productivity significantly (Morris 1991), supporting that primary productivity in salt marshes is nitrogen limited.

The anthropogenic introduction of nitrogen into coastal ecosystems is a growing problem. Increased human output of nitrous oxide and ammonia leads to atmospheric deposition, in which various forms of bioavailable nitrogen are deposited from the atmosphere and into natural

systems through precipitation or interactions between atmospheric nitrogen and surfaces. In 1991, it was estimated that 0.23 grams of nitrate and 0.14 grams of ammonium per meter squared were deposited in Massachusetts salt marshes per year (Morris 1991). Anthropogenic nitrogen additions to coastal and aquatic environments can lead to eutrophication and vegetation shifts (Bertness et al. 2002). Due to their ability to serve as sinks for added nitrogen, salt marshes may play an important role in reducing problems caused by increased nitrogen outputs. Nitrification is vital to this process since, in addition to converting nitrogen into a form that can be readily taken up by plants, nitrification in salt marshes is often followed by denitrification, the microbially facilitated conversion of nitrate into atmospheric nitrogen gas that ultimately leads to the removal of nitrogen from the system (Jenkins and Kemp 1984). Denitrification is an anaerobic process and is therefore favored in the waterlogged, anoxic sediment of salt marshes (Morris 1991).

Due to its importance, the fate of nitrogen introduced to salt marshes has been studied in depth. Vanzomeren et al. (2012) tracked the fate of nitrate in vegetated (*Spartina patens*) cores collected from a brackish marsh in Louisiana. They added 15N labeled potassium nitrate to the cores for three months and then analyzed labeled nitrogen concentrations in the soil, aboveground biomass and belowground biomass. Sixty-eight percent of the added 15N was not recovered at the end of the experiment, suggesting that it was lost via denitrification. Of the recovered nitrogen, the majority was incorporated into aboveground biomass. Nitrification is vital to this demonstrated ability of salt marshes to remove bioavailable nitrogen from the sediment since the nitrate produced via nitrification is what can be taken up by plants and used as fuel for denitrification.

Nitrification in salt marshes

Because nitrification is so important in controlling the availability of nitrogen, the factors that regulate this process have been studied extensively in salt marshes. Factors that are believed to regulate nitrification in salt marsh sediment include bioturbation caused by macrofaunal burrowing and Fe(III) concentrations (Dollhopf et al. 2005). Macrofaunal burrowing has been positively correlated with nitrification rates in salt marsh sediments, likely because this activity leads to increased oxygenation of the sediment. Fe(III) concentrations have also been positively correlated with salt marsh nitrification rates, likely because high Fe(III) concentrations reduce the inhibition of nitrification by sulfide (Dollhopf et al. 2005). Additionally, salt marsh nitrification has been shown to be driven by temporal patterns, with highest rates occurring during warmer months, and spatial patterns, with higher rates recorded in the interior of the marsh compared to the edge (Marton et al. 2015).

AOA as nitrifiers in salt marshes

The discovery of AOA led to questions of niche differentiation between AOA and AOB, both of which require the same resources (Bernhard and Bollmann 2010). One possible explanation for the coexistence of these two groups is that AOA have a higher substrate affinity. Martens-Habbena et al. (2009) reported an extremely high affinity for reduced nitrogen in a strain (SCMIO) of *Nitrosopumilus maritimus*, a common marine AOA, suggesting that *N. maritimus* could successfully compete with AOB and phytoplankton. AOA are typically more abundant than AOB across most marine (Newell et al. 2013) and terrestrial ecosystems (Prosser et al. 2008), including salt marshes (Bernhard et al. 2010). In salt marshes, the distribution is determined by differences in microsites. Moin et al. (2009) found that AOA at Barn Island had a higher species richness (number of species per sample) than AOB in plots dominated by the salt

marsh grasses tall *Spartina alterniflora* and *Spartina patens* but that AOA and AOB richness was similar under short *Spartina alterniflora*.

Although AOA are typically more abundant than AOB in salt marshes, the overall contribution of these two groups to nitrification is still not fully known. Functional gene abundance does not necessarily translate to protein abundance or activity, and attempts to correlate the abundance of bacterial and archaeal *amo*A genes to potential nitrification rates in salt marshes have yielded varying results. Caffrey et al. (2007) found that archaeal *amo*A abundance was positively correlated with potential nitrification rates in estuaries, while bacterial *amo*A abundance was not correlated with nitrification rates. These results suggest that AOA, but not AOB, are major nitrifiers in these estuaries. However, Dollhopf et al. (2005) found that AOB abundance correlated with nitrification potential in a different estuary. Additionally, AOB abundance was shown to be more strongly correlated with nitrification rates than AOA abundance at Plum Island estuary (Bernhard et al. 2007, Bernhard et al. 2010). Overall, the role of AOA and AOB in carrying out nitrification in estuaries seems to vary from site to site.

Patterns that regulate salt marsh AOA communities

Salt marshes are arranged in patches of grasses that are largely monospecific. New England marshes are dominated by short and tall forms of *Spartina alterniflora,* and *Spartina patens* (Bertness and Ellison 1987). These species are typically found in monospecific patches, the locations of which are determined primarily by elevation. Tall *S. alterniflora* tends to dominate the low marsh, which is flooded daily. *S. patens* and short *S. alterniflora* are found higher up in the marsh, where flooding occurs less frequently. Additionally, salt marshes have microbial mats, or unvegetated patches characterized by multiple layers of bacteria. The factors

that regulate the monospecific vegetation communities in salt marshes are also likely to impact the distribution of microbial communities.

Community composition and abundance of salt marsh AOA have been shown to vary by vegetation site. Moin et al. (2009) found that AOA diversity at Barn Island salt marsh in Connecticut, measured in terms of distinct operational taxonomic units (OTUs), or groups of closely related individuals, was lowest at short *S. alterniflora* sites and highest at *S. patens* sites, but that AOA abundance was significantly higher at *S. patens* and tall *S. alterniflora* sites than short *S. alterniflora* sites. Moin et al. (2009) also found highest AOA abundance at *S. patens* sites and lowest at short *S. alterniflora* sites. This research suggests that salt marsh AOA communities vary by vegetation.

Distinct ammonia oxidizer communities have been described along salt marsh salinity gradients, although the role that salinity plays in shaping salt marsh AOA community abundance is not entirely clear. Caffrey et al (2007) found a positive correlation between salinity and AOA abundance in estuarine sediment while Mosier and Frances (2008) found a negative correlation. Bernhard et al. (2010) found highest AOA abundance at intermediate salinity sites in an estuary. Studies on the impact of salinity on nitrification rates in estuaries have also yielded contradictory results. Caffrey et al. (2007) found a positive correlation between salinity and nitrification rates in estuarine sediments, while other studies have found negative correlations (Bernhard and Bollmann 2010). These results suggest that AOA abundance and nitrification rates in salt marshes are regulated by factors other than salinity.

Sediment salinity may impact ammonia oxidizer community composition. There is evidence that increased salinity leads to less diverse AOB communities in both salt marshes (Bernhard et al. 2005) and aquarium biofilters (Grommen et al. 2005). Distinct communities of

AOA have also been found across estuarine salinity gradients, with distinct clusters of AOA being found at low salinity areas (Francis et al. 2005). This research suggests that high salinity serves as a stressor that limits the types of ammonia oxidizers that can survive.

Drought

Drought is an environmental stressor with the potential to greatly impact soil microbial communities. Tian et al. (2012) found that drought conditions led to a decrease in abundance and a shift in community composition of total archaeal communities in a freshwater wetland. There is also research demonstrating the effect of drought on AOA specifically. Fuchslueger et al. (2014) found that drought conditions led to a decrease in AOA abundance in managed grassland meadows. Based on these findings, it is possible that communities of AOA in salt marshes are impacted by drought.

The impact of drought may vary within the marsh depending on the specific site. Research has shown variation in the effect of drought on microbial communities, including nitrogen cyclers, in the rhizospheres of different grassland plants (Sanaullah et al. 2010), suggesting that vegetation type has a significant impact on how soil microbes will be impacted by drought. Since salt marsh AOA communities have been shown to vary under different grasses (Moin et al. 2009), these distinct communities may differ in their tolerance to environmental stressors such as drought.

Drought conditions impact salt marsh sediment by shifting the sediment conditions from anoxic to oxic (Palomo et al. 2011). Because denitrification is an anaerobic process and nitrification is aerobic, this shift might lead to a shift towards favoring nitrification over denitrification. In salt marsh sediments, drought has been shown to cause a decrease in pH and an increase in denitrification fueled by increased ammonia oxidation rates (Palomo et al. 2013).

This decrease in sediment pH during drought conditions may have significant implications for ammonia oxidizer communities, as AOA community composition has been shown to vary across pH gradients in an agricultural field (Nicol et al. 2008). Current climate change models predict increased droughts in the upcoming century (Dai 2012). Therefore, it is important to understand how ammonia oxidizer communities respond to droughts, as this could help to predict potential changes in nitrogen cycling in salt marshes.

Purpose

In 2016, Connecticut experienced a severe drought. The Connecticut Department of Public Health issued a drought advisory in June 2016, which was officially lifted in June 2017. Sediment samples were collected from Barn Island salt marsh in Stonington, Connecticut in July of 2016 for ongoing study of microbial communities at the marsh. Analysis revealed that sediment salinity in the 2016 samples was significantly higher compared to samples collected in June 2014 and June 2017 (Figure 2).

This research addresses the following questions:

1. How are community composition and abundance of salt mash AOA populations impacted by drought?
2. Does the drought effect vary by different vegetation types and sediment depths?

Analyzing sediment AOA communities from four different vegetation sites will allow us to determine whether the impact of drought on salt marsh AOA communities differs depending on the vegetation. By comparing AOA communities from different vegetation sites across multiple non-drought years, we can also assess whether the vegetation affects the variability of

the AOA communities during non-drought years. If there are differences in AOA communities between the different vegetations, these differences could provide insight into the potential future of nitrogen cycling in New England salt marshes. For example, New England salt marshes were previously dominated by *S. patens*. However, since the mid-1900s, patches of *S. patens* at these marshes have been shifting to *S. alterniflora* (Warren and Niering 1993). *S. patens* has been shown to be especially vulnerable to increases in inundation and raised salinity (Watson et al. 2016), which may increase as sea levels continue to rise. Any differences seen in AOA communities at different vegetation sites could be relevant as plant communities at these marshes continue to shift. This research could help predict the impact that climate change will have on nitrification in New England salt marshes.

Hypothesis

Due to research reporting changes in the chemistry of salt marsh sediment during drought conditions (Palomo et al. 2011) and sensitivity of total archaeal (Tian et al. 2012) and AOA (Fuchslueger et al. 2014) communities to drought, I hypothesized that there would be a significant drought effect for AOA community composition and abundance. Based on the findings showing distinct AOA communities at different salt marsh vegetation sites (Moin et al. 2009) and research suggesting that drought effect varies for grassland microbial communities based on vegetation (Sanaullah et al. 2011), I hypothesized that drought effect on AOA community composition and abundance would vary by site.

METHODS

Sample Collection

Samples were collected from Headquarters salt marsh, which is part of the Wequetequock-Pawcatuck tidal marshes (referred to as Barn Island) in Stonington, Connecticut. Samples were collected from plots dominated by short-form *S. alterniflora* (SSA sites), tall-form *S. alterniflora* (TSA sites), *S. patens* (SP sites), and microbial mat (MAT sites). Samples were collected in June 2014 (pre-drought), July 2016 (drought year), and June 2017 (post-drought) (Figure 1). These samples were collected in cores, which were then separated into surface (0-2 cm) and sub-surface (6-8 cm) sediment samples. In 2014 and 2017, three sediment cores were collected at each site aside from TSA in 2014, from which 6 cores were collected. In 2016, 2 cores were collected for SSA and TSA, 4 cores were collected for SP, and 1 core was collected for the MAT (Table 2).

Figure 1. The microbial mat of Headquarters salt marsh in June 2014, a non-drought year (left) and July 2016, the drought year (right). Both of these photographs were taken during low tide.

DNA extraction

To isolate DNA from the sediment samples, I used the PowerSoil DNA Isolation kit and followed the manufacturer's instructions. I then assessed the 260/280 ratio and concentration of the DNA in ng/µl through spectrophotometry using a NanoDrop (BioRad). DNA concentrations ranged from 6.5 to 221.1 ng/µl.

AOA Community Composition

AOA community composition for each sample was determined using terminal restriction fragment length polymorphism (TRFLP) on the archaeal *amo*A gene. First, the archaeal *amo*A gene was amplified for each sample using PCR with a forward primer labeled at the 5' end with the fluorescent dye 6-FAM. Each 20 µl PCR reaction was run with 1 µl template DNA, 1X AmpliTaq Gold™ 360 Master Mix (ThermoFisher), 0.5 µM each forward (Arch26F-FAM) and reverse (ArchA417R) (Park et al. 2008) primers and 1 µl enhancer provided by the manufacturer of the master mix.

The PCR was run at 95°C for 10 minutes, followed by 35 cycles of 95°C for 30 seconds, 56°C for 30 seconds, 72°C for 30 seconds. This was followed by a final elongation time of 72°C for 7 minutes. To confirm the amplification of archaeal *amo*A, the PCR products were run on a 1% agarose gel that contained 5 µl GelRed dye and compared to a 1 Kb ladder gel to confirm that the products were the proper length.

Restriction enzyme digest was performed using about 15 µl PCR product and 1 µl of restriction enzyme (*Aci*I), 2 µl buffer, and 2 µl water. The samples were incubated overnight at 37°C. Ethanol precipitation was then performed to prepare the samples for analysis. Samples were then resuspended in 5 µl water, 10 µl formamide, and 0.2 µl GeneScan 500-ROX standard.

These digests were sent to Biotechnology Resource Center at Cornell University for analysis on an ABI 3730XL Sequencer.

TRF size and relative abundance were analyzed using GeneMarker software version 1.5 (SoftGenetics, State College, PA, USA). Relative abundance was determined by peak height. For analysis of T-RFLP data, we only included TRFs that correspond to a published sequence. Only peaks with a height of at least 50 relative fluorescence units were included in order to reduce artifacts and background noise.

AOA Abundance

AOA abundance was measured using QPCR to quantify the number of copies of the archaeal *amo*A gene. Most samples were run undiluted. However, all three of the 2017 SP samples from surface sediment and one of the 2017 sample from SSA from surface sediment were run diluted 1:10 prior to amplification because inhibitors were present that caused these samples to not amplify when run undiluted. Each 20 µl reaction was run using 1 µl of DNA template, 1X SYBR Advantage QPCR Premix (Clontech), 0.5 µM each of archamoAF forward and archamoAR reverse primers (Francis et al. 2005), and 0.008% BSA. The QPCR was run at 95°C for 1 minute, followed by 50 cycles of 95°C for 5 seconds, 54°C for 20 seconds, and 72°C for 20 seconds. This was followed by melt curve analysis to confirm the identity of the product. Results were compared to a standard curve that was made using known concentrations of a plasmid with a cloned archaeal *amo*A gene. Template concentrations for the standard curve were 1 pg/µl, 0.1 pg/µl, 0.01 pg/µl, 0.001 pg/µl, and 0.0001 pg/µl.

Abiotic Factors

Porewater was extracted from sediment by centrifugation one day after sample collection. This porewater was used to obtain salinity, pH, nitrate, and ammonium values. Porewater salinity

was measured for each core at each depth using a refractometer one day after sample collection as samples were processed. pH was measured from pore water using a pH100 meter with a piercing probe (YSI, Yellow Springs, OH). Porewater ammonium concentrations were measured using the phenol-hypochlorite method described by Strickland et al. (1972). Porewater nitrate was measured using the enzymatic reduction of nitrate to nitrate (Campbell et al. 2004), followed by the colorimetric determination of nitrite in seawater described by Stricklane et al. (1972). Ammonium and nitrate values are available for 2017 samples and 14 2016 samples because the remaining 2016 samples were too dry to extract pore water from.

Analysis

QPCR data were analyzed using one-way ANOVAs to compare AOA abundance between years and vegetation types. Independent T-tests were used to test for drought effects by comparing abundance between the drought year and the non-drought years.

Analysis of T-RFLP data was done using PC-ORD v.6 (McCune and Mefford 2011). Prior to analysis, the data was transformed by an arcsine square root function. Ordinations were created using the Non-Metric Multidimensional Scaling (NMS) technique (Kruskal 1964) using Sørensen's distance measure. Ordinations were created using autopilot mode and were set to slow and thorough. Monte-Carlo tests confirmed that results were significantly better than results that could be obtained from randomized data. Coefficients of determination for the correlations between ordination distances and distances in the original n-dimensional space were used to calculate the proportion of variance explained by each axis on the ordinations. Differences between groups were tested using Multi-Response Permutation Procedures (MRPP) with Euclidean distance measures. MRPP is a non-parametric used to test for differences between user-defined groups (McCune and Grace 2002).

Because there was only one core taken from the MAT site in 2016, there are only two available samples for the mat during the drought year, one from surface sediment and one from sub-surface sediment. Therefore, the microbial mat samples were only included when analyzing total drought effects with vegetation sites combined and were not analyzed for significant differences only within the mat.

RESULTS

Because there were no significant differences in AOA community composition ($T=0.1115$, $A=-0.00073$, $p>0.43$) or abundance ($t=0.15579$, $p>0.87$) between surface and sub-surface sediment, these two depths were combined for analysis.

Environmental Variables

Rainfall one month prior to sampling was lowest in 2016 and highest in 2017 (Table 1).

Table 1. Total rainfall in inches starting 1 month prior and 1 year prior to each sampling date. Data was collected from theweathercollector.com using the Groton/New London station (station ID: USW00014707).

	Total Inches Rainfall (Inches)	
Sampling date	**1 month prior**	**1 year prior**
June 24th 2014	1.43	18.19
July 13th 2016	0.34	23.06
June 28th 2017	4.21	42.91

ANOVA results reveal that porewater salinity was significantly different between the three sample years ($F= 34.13$, $p<0.001$, Figure 2). Salinity was highest in 2016 and lowest in 2017. This salinity pattern is generally negatively correlated with rainfall 1 month prior to sampling.

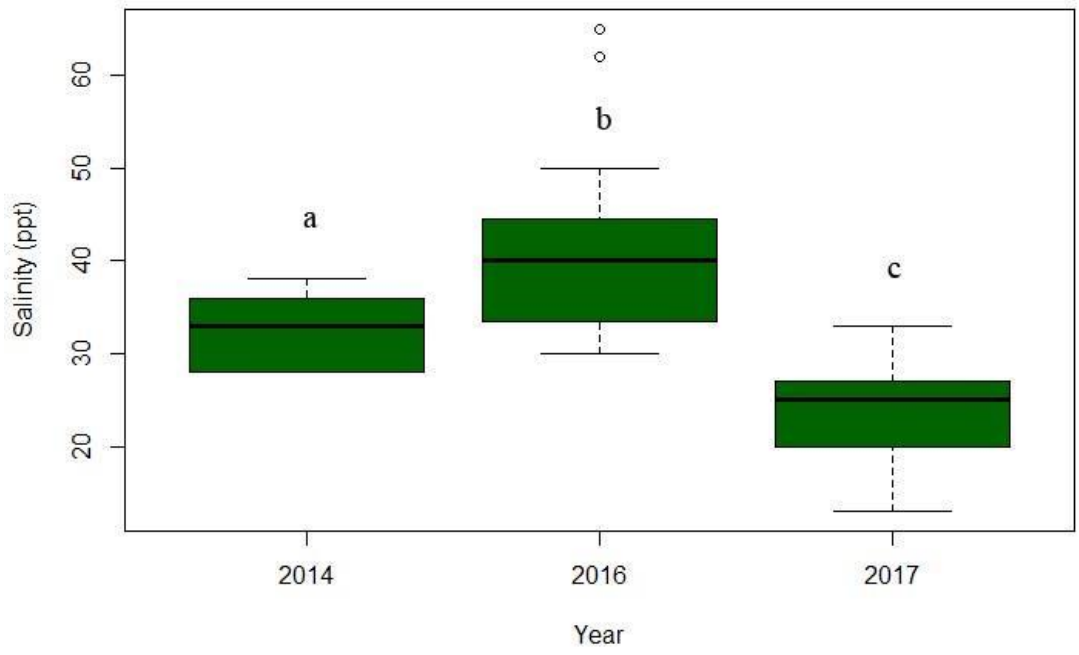

Figure 2. Porewater salinity at Headquarters salt marsh. 2016 (n=15) was a drought year and 2014 (n=24) and 2017 (n=24) were non-drought years. Different letters indicate significant differences in salinity between years.

There was a significant effect of drought on salinity for SSA sites ($t = -6.0573$, $p<0.0001$, Figure 3) and SP sites ($t=-2.3657$, $p<0.05$). There were not enough salinity values for TSA sites in 2016 due to a lack of porewater to test for drought effect.

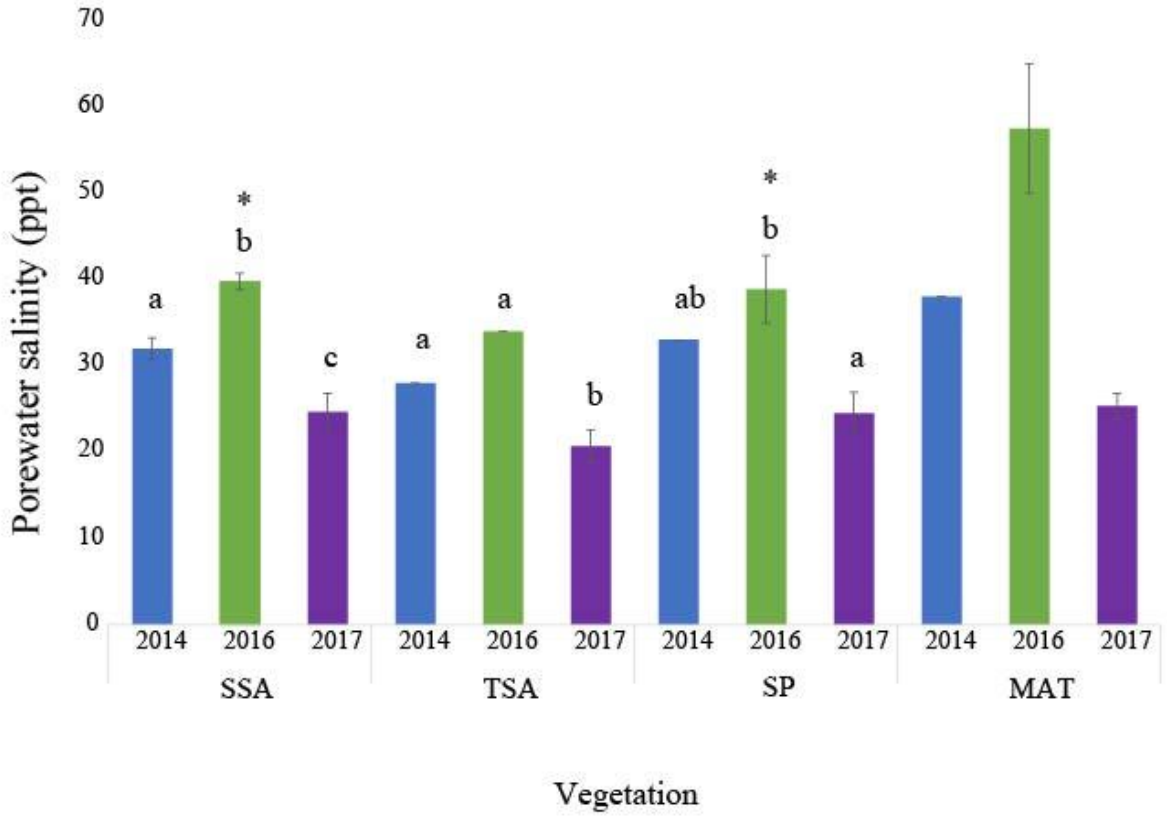

Figure 3. Average porewater salinity at combined depths in plots dominated by short *Spartina alterniflora* (SSA), tall *Spartina alterniflora* (TSA), *Spartina patens* (SP), and the microbial mat (MAT). Error bars represent 1 standard error. Different letters indicate significant differences in salinity between years within each vegetation site. Stars indicate significant drought effect for that site.

Porewater ammonium and nitrate concentrations were only available for 2016 and 2017 samples, and the majority of the 2016 concentrations were below the limit of detection. In 2017, there was a significant effect of vegetation on ammonium concentrations ($F=14.98$, $p<0.001$, Figure 4), with TSA being significantly different from both SP ($p<0.001$) and SSA ($p<0.001$). There was no significant effect of vegetation on nitrate.

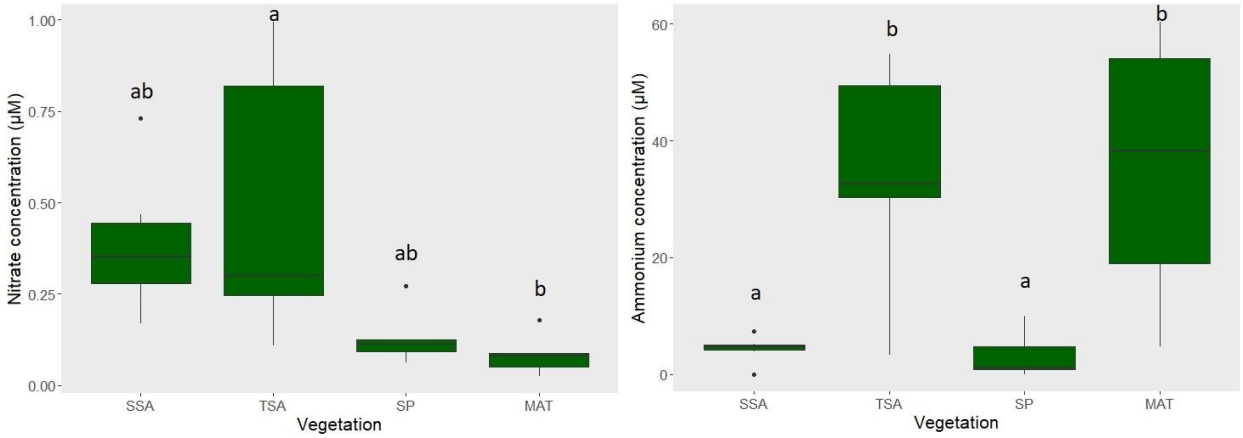

Figure 4. Porewater nitrate (left) and ammonium (right) in 2017 at combined depths in plots dominated by short *Spartina alterniflora* (SSA), tall *Spartina alterniflora* (TSA), *Spartina patens* (SP), and the microbial mat (MAT). Error bars represent 1 standard error.

Abundance of ammonia-oxidizing archaea

Overall, 72 samples were used for analysis of AOA abundance (Table 2).

Table 2. The number of samples at each site used for analysis of AOA abundance and community composition. When a different number of samples were used for analysis of community composition, this number is indicated in parentheses. Certain samples amplified for QPCR but not TRFLP, most likely due to the use of different primers for these tests.

	Depth	2014	2016	2017
SSA	Surface	3	2	3
	Sub-surface	3	2	3 (2)
TSA	Surface	6	2	3
	Sub-surface	6	2	3
SP	Surface	3	4	3
	Sub-surface	3	4 (3)	3(2)
MAT	Surface	3(1)	1	3
	Sub-surface	3(2)	1	3

Across all vegetation types and depths, AOA abundance ranged from $1.16 \times 10^1$to 5.33×10^6 copies/g wet sediment with an average of 3.23×10^5during the non-drought years. During the drought year, AOA abundance ranged from between 9.80×10^2 and 1.97×10^6 copies/g wet sediment with an average of 2.17×10^5.

There was no significant drought effect on AOA abundance for any of the vegetation sites or when vegetation sites were combined (Figure 5). AOA abundance was positively correlated with ammonium concentration ($r= 0.365$, $p<0.023$), but was not correlated with salinity, nitrate, or pH.

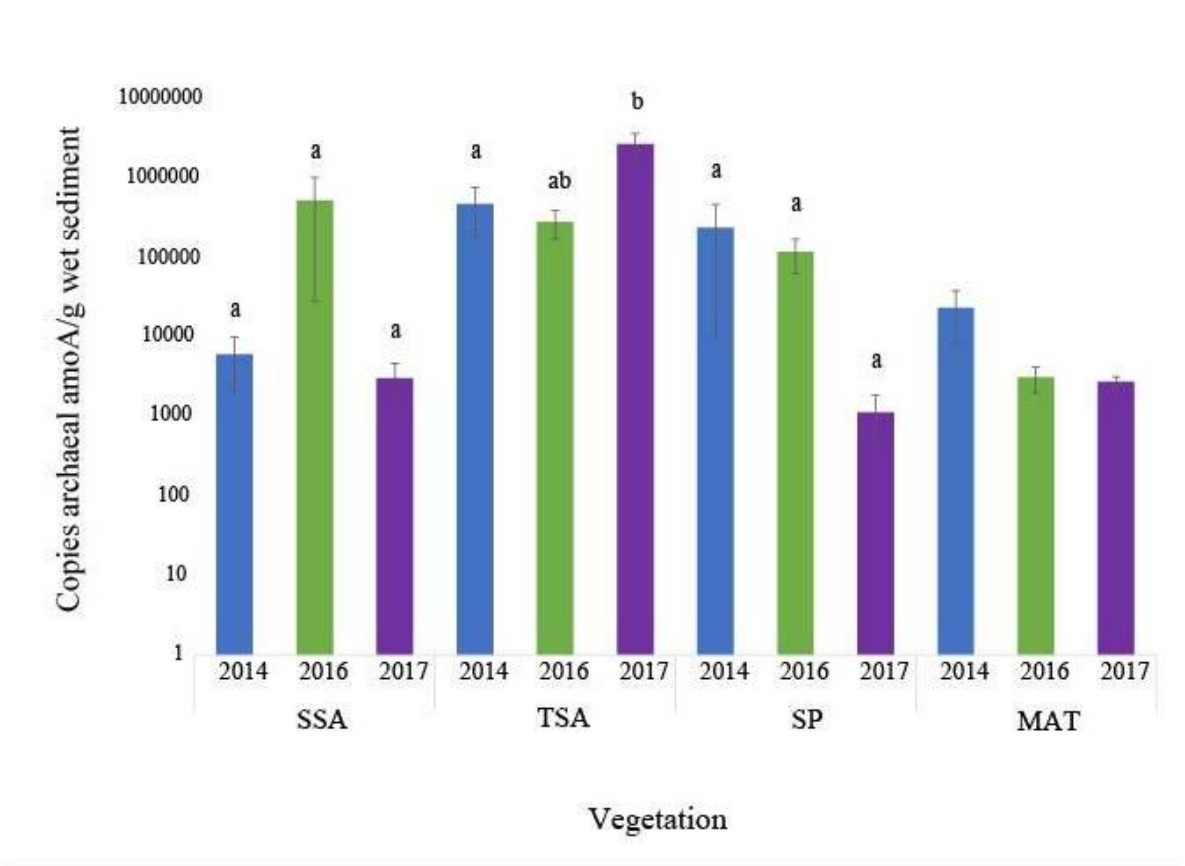

Figure 5. Average AOA abundance per gram of wet sediment across in sites dominated by short *Spartina alterniflora* (SSA), tall *Spartina alterniflora* (TSA), *Spartina patens* (SP), and the microbial mat (MAT). Error bars represent 1 standard error.

Community Composition

Overall, 66 samples were used for analysis of AOA community composition. There were six samples that amplified in QPCR but not in the T-RFLP analysis (Table 2), so these samples were excluded from analysis of community composition. These differences are likely due to the fact that different primer sets were used for QPCR and T-RFLP analysis.

When vegetation sites were combined, MRPP revealed a significant drought effect on AOA community composition ($T=-2.152$, $A= 0.0138$, $p<0.05$, Figure 6).

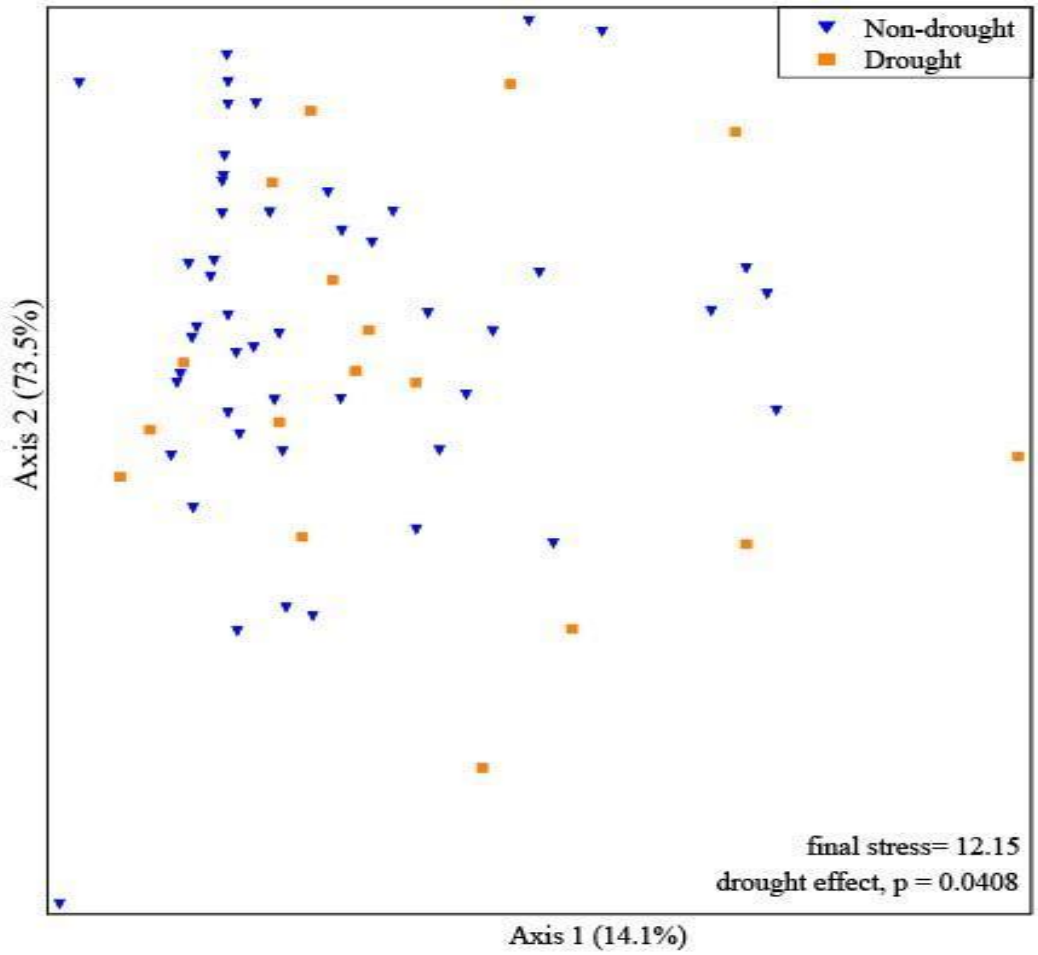

Figure 6. NMS ordination for AOA community composition based on TRF composition for the drought year (2016) and non-drought years (2014 and 2017).

With vegetations and depths combined, there was also a significant effect of year on AOA community composition (T=-3.178, A=0.0289, p<0.02,), with pairwise comparisons revealing that 2017 was significantly different from both 2014 (p<0.03) and 2016 (p<0.02), but 2014 and 2016 were not significantly different. With all three years and both depths combined, there was a significant effect of vegetation (T=-2.58 4, A=0.0291, p<0.02). TSA samples were significantly different from both SSA (p<0.03) and microbial mat samples (p<0.02).

There was a significant effect of drought on AOA community composition for SSA sites (T=-2.097, A=0.0666, p<0.045, Figure 9) and TSA sites (T=-2.400, A=0.05394, p<0.031), but not for SP sites.

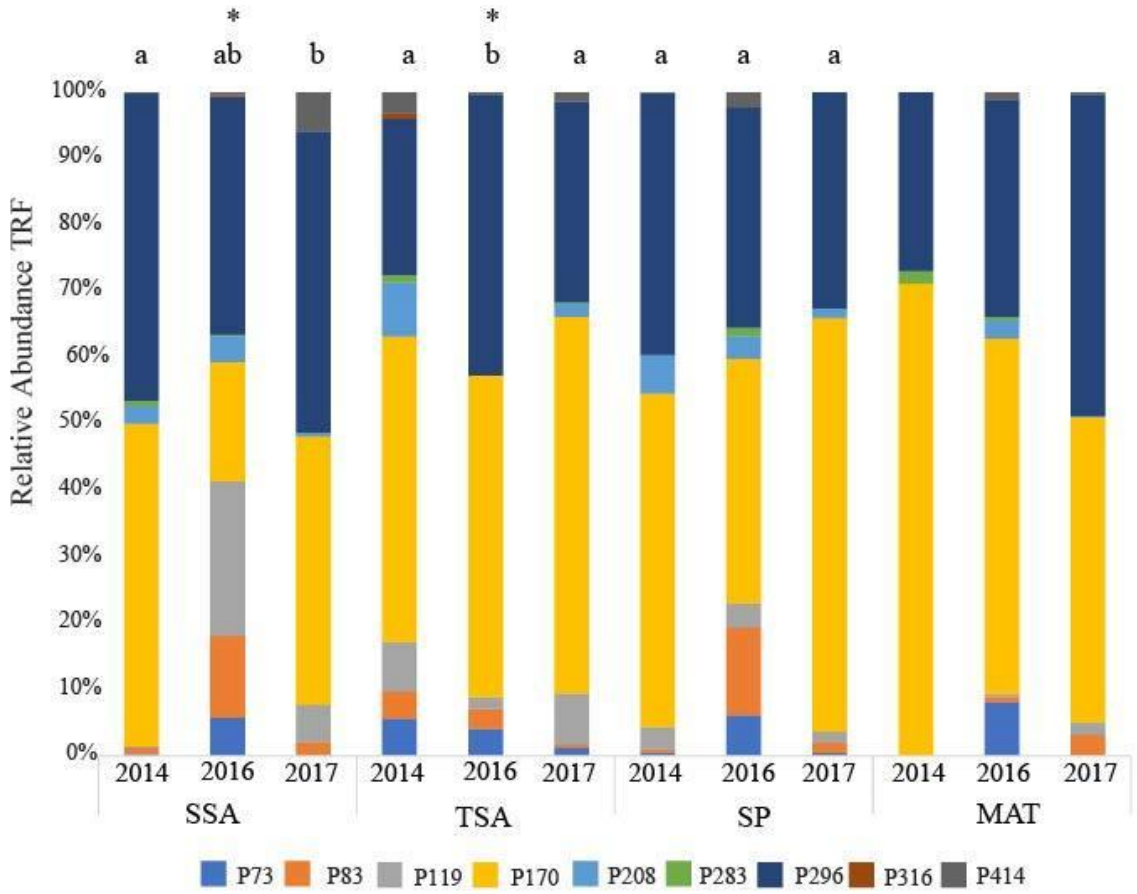

Figure 7. Relative abundance of each TRF for AOA in plots dominated by short *Spartina alterniflora* (SSA), tall *Spartina alterniflora* (TSA), *Spartina patens* (SP), and the microbial mat (MAT). Different letters indicate significant differences in AOA community composition between years within each vegetation site. Stars indicate a significant drought effect in that vegetation site. No statistics were performed on the MAT samples due to insufficient sample size in 2016.

The only TRF for which there was a nearly significant drought effect was P73 (t=-2.0557, p<0.051, Figure 8). This TRF accounted for a higher percentage of TRFs identified in 2016 compared to the non-drought years. There were no significant drought effects for any TRF when separated by vegetation or with vegetations combined.

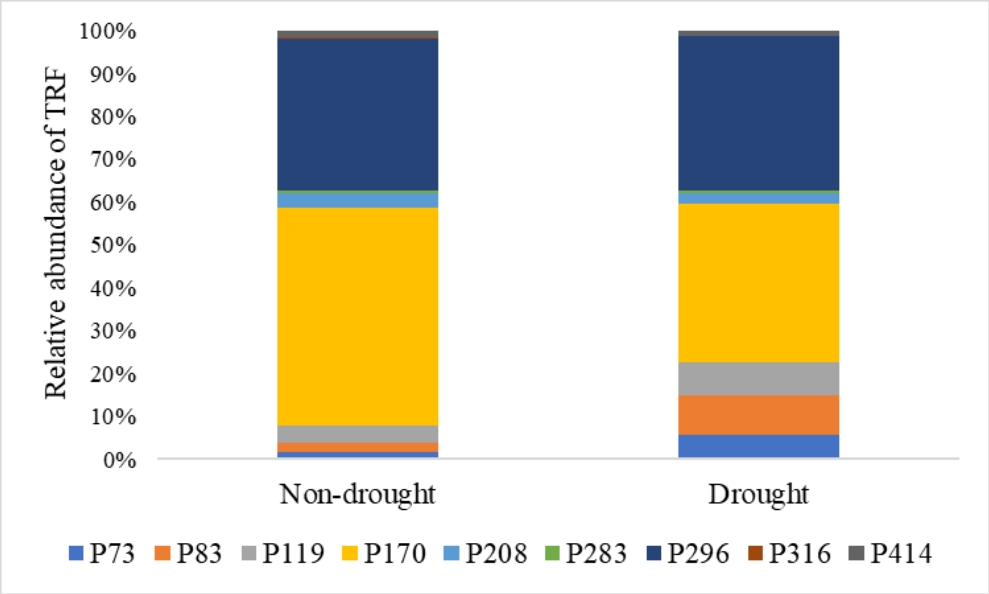

Figure 8. Relative abundance of each TRF for AOA during the drought year (2016) and non-drought years (2014 and 2017).

There was no significant effect of drought or year on TRF richness, evenness, Shannon diversity, or Simpson's diversity.

DISCUSSION

Results of this study suggest that the composition, but not abundance, of AOA communities at Headquarters marsh of Barn Island was significantly impacted by the 2016 drought in some areas of the marsh. These results partially support my initial hypothesis that the 2016 drought would have a significant impact on AOA communities.

Drought effect on AOA community composition

With depths and vegetation sites combined, non-drought years differed from the drought year significantly in terms of AOA community composition (Figure 6). It is possible that this shift in community composition was driven by drought-related changes in the chemistry of the sediment. During the drought year, precipitation one month prior to sampling was four times lower than it was in 2014 and twelve times lower than it was in 2017. Drought conditions have been shown to alter chemical processes in salt marsh sediment by shifting conditions from anoxic to oxic, decreasing pH (Palomo et al. 2013), and increasing salinity (Hughes et al. 2012).

The significant difference in AOA community composition during the drought year compared to the non-drought years may indicate differences in community function. Finding patterns between community composition and community function is an important goal of microbial ecology, and there is research indicating that the function of ammonia oxidizer communities may be impacted by community structure. Horz et al. (2004) observed that shifts in grassland AOB communities induced by simulated global change were associated with increased nitrification rates. Bernhard et al. (2007) found that site specific differences in salt marsh AOB community structure and abundance were correlated with differences in nitrification rates. Nitrification rates in salt marshes have also been linked to AOA community composition. Bernhard et al. (2016) reported that certain AOA TRFs correlated with nitrification potential in

Louisiana salt marshes, but that which TRFs were correlated varied depending on site. This research suggests that certain AOA communities are correlated with nitrification in salt marshes, but that these communities are not constant between sites. These studies suggest that the composition of ammonia oxidizer communities impacts the function of these communities in terms of nitrification rates. These differences could play an important role in determining the fate of environmental nitrogen in the ecosystem.

As predicted, AOA communities at different vegetation sites responded differently to the drought. AOA communities at both TSA and SSA sites were significantly different during the drought year compared to the non-drought years, while communities in SP dominated sites showed no significant drought effect (Figure 7). Vegetation specific response of microbial communities to drought was also seen in a study by Sanaullah et al. (2011), which found that microbial biomass varied with drought depending on the vegetation site. The lack of drought effect seen at SP sites could suggest that AOA communities at these sites are more stable or more resistant to disturbance. *S. patens* is a high marsh grass that is intolerant to frequent inundation and anoxia but has been shown to be tolerant of different precipitation regimes, including drought (Watson et al. 2016). AOA communities that live at SP sites might be similarly adapted and able to tolerate different levels of precipitation.

Drought effect on AOA abundance

AOA abundance detected in this study was very low compared to values published in previous studies at Headquarters salt marsh at Barn Island. Abundances cited in this study ranged from 1.39×10^2 to 5.60×10^9. Moin et al. (2009) reported values ranging from 1.6×10^6 to 5.7×10^9 at Barn Island using different primers. Bernhard et al. (2015) found AOA abundances between 8.9×10^5 to 1.2×10^9 using the same primers. Therefore, it is reasonable to believe that

the primers used to detect AOA abundance in this study did not target all of the AOA present in the samples. Actual AOA abundance in these samples may be higher than they are reported in this study and future analysis will focus on detecting AOA abundance using different primers to see if more comparable results are gathered.

Although salinity was significantly higher during the drought year compared to the non-drought years, salinity was not correlated with overall AOA abundance. This lack of correlation was not surprising since no clear, conclusive pattern between salinity and AOA abundance in salt marshes has been identified. Different studies have found positive (Caffrey et al. 2007) and negative (Mosier, Frances 2008) correlations between AOA abundance and salinity in estuarine sediments, suggesting that there is no direct, consistent relationship between salinity and AOA abundance. There was also no correlation between AOA abundance and pH, although previous research has found negative correlations between salt marsh sediment pH and AOA abundance (Moin et al. 2009). This lack of correlation may be due in part to only having pH values available for 2017. There was a positive correlation between AOA abundance and pore water ammonium concentrations, although this correlation was very weak. Previous studies have found no correlation between ammonium concentrations and AOA abundance (Moin et al. 2009).

For the data gathered in this study, there was no significant effect of drought on the abundance of ammonia-oxidizing archaea at Headquarters marsh across the four vegetation plots and two depths studied. This lack of significance may be due to high variability and low sample number. There were some trends in the abundance data despite this statistical insignificance. When vegetation sites were combined, average AOA abundance was similar in 2014 and 2016 and increased in 2017 (Figure 5). This increase could potentially be a response to changing sediment conditions in 2017, which had higher rainfall than the other two years (Table 1). Kaurin

et al. (2018) reported a significant decrease in total bacterial, total crenarchaeal, AOB, and AOA communities under drought conditions in agricultural soil. In this case, all of these communities returned to pre-drought conditions as soon as three weeks following the drought. The higher AOA abundance detected in the study could be due to AOA communities returning to a normal state following two years of lower than average rainfall.

Drought effect on AOB and total bacterial communities

Data was also collected for community composition and abundance of AOB using the bacterial *amo*A gene and total bacterial communities using the Bacterial 16S rRNA gene for the same samples (data not shown). For total bacterial communities, there was a significant drought effect for both community composition and abundance when vegetation sites were combined. For AOB, there was no overall drought effect on abundance with vegetation sites combined but there were significant drought effects for all individual vegetation sites except for MAT sites. Whether AOB abundance increased or decreased significantly during the drought year varied by vegetation site. Additionally, AOB communities were significantly different at TSA sites in surface sediment and at SP and SSA sites in sub-surface sediment. These results suggest that neither total bacterial communities nor ammonia oxidizers are entirely resistance to drought.

Based on previous research, it is not surprising that AOA and AOB communities responded differently to the drought. Previous research has also shown that AOA and AOB communities respond differently to environmental stressors such as copper and ether contamination (Wang et al. 2018), manure application on rice paddies (Gao et al. 2018), and long-term salt marsh NPK fertilization (Peng et al. 2013). The unique responses of AOA and AOB to drought support the idea that different ammonia oxidizer communities respond differently to disturbance.

Based on previous research, resistance and resilience of total bacterial and ammonia oxidizer communities seem to vary depending on the disturbance. Many studies have demonstrated that the compositions of microbial communities are frequently not resistant or resilient to disturbance that simulate global change (Allison and Martiny 2008). The lack of resistance and resilience of Barn Island bacterial communities was demonstrated by a study revealing significantly higher variability in bacterial communities at four Barn Island marshes in which tidal flow was restricted 30 years after tidal flow was restored compared to four similar marshes in which tidal flow was never restricted (Bernhard et al. 2012). At these same marshes, significant differences were found in community composition for AOB, but not AOA. Additionally, higher AOA and AOB abundance were found in sub-surface sediment of restored marshes (Bernhard et al. 2015). These results suggest that neither total bacterial nor ammonia oxidizer communities are fully resistant to disturbance, but that the responses of these groups to disturbance can vary. However, other studies have demonstrated the resistance of total bacterial and ammonia oxidizer communities to various forms of disturbance. Peng et al. (2013) found no consistent response of AOA community composition to long-term fertilization treatment. At this same marsh, Bowen et al. (2011) found no significant response of Bacterial 16S rRNA to fertilization. Additionally, Bernhard et al. (2016) found no significant differences in AOA or AOB community composition between marshes that were exposed to oil two years after the Deepwater Horizon oil spill compared to marshes that were not exposed to oil. These results suggest that microbial response to disturbance is variable depending on the disturbance and broad generalizations cannot be made about how these communities will respond to a given disturbance.

Implications

Widespread, severe droughts are predicted to occur at higher frequency under current climate change models (Dai 2012). Drought has been reported as a cause of acute marsh dieback, or the sudden, drastic decline in *S. alterniflora* (Hughes et al. 2012). Based on the results of this study, these droughts may also lead to differences in the composition of AOA communities, which could potentially indicate differences in nitrogen cycling. A shift in salt marsh nitrogen cycler communities in response to drought was also reported by Davis et al. (2018), who found significant shifts in communities of nitrogen fixing bacteria during drought conditions. These results suggest that drought may significantly impact the way salt marshes cycle nitrogen.

Future Study

It might also be interesting to look at the community composition and abundance of other microbial communities involved in the nitrogen cycle, such as denitrifiers and nitrogen fixers, for these samples. Since nitrification is an aerobic process and denitrification is anaerobic, these populations might respond differently to the increasingly aerobic conditions created by drought. Denitrifiers are another functional groups of microbes that may have been impacted by drought conditions. Additionally, a recent study by Davis et al. (2018) revealed differences in the community composition of salt marsh nitrogen fixers during drought conditions. Analyzing other nitrogen cyclers in these samples could provide a more complete picture of how drought impacts nitrogen cycling at the marsh.

Measuring changes in nitrification rates in salt marsh sediment under drought conditions could also provide meaningful information. Studying microbial communities using molecular markers provides information about what groups are present in the sediment, but it does not provide any information about how these communities are functioning. No clear, consistent

pattern has been identified between AOA community composition or abundance and nitrification rates (Caffrey et al. 2007, Bernhard et al. 2010). It would be interesting to see if the contribution of salt marsh AOA to nitrification varies with precipitation conditions.

Barn Island consists of a group of salt marshes with varying management histories. Headquarters marsh, the focus of this study, has never had its tidal flow restricted. Previous research at this marsh system has shown significantly higher AOA abundance in sub-surface sediment of marshes that have had their tidal flows restricted compared to marshes that have not (Bernhard et al. 2012). This suggests that AOA communities at these marshes may differ in their ability to respond to environmental stressors such as drought. Future research may focus on the impact of the 2016 drought on ammonia oxidizer communities at previously impounded Barn Island marshes.

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