

Brief Report

Influence of local and regional drivers on spatial and temporal variation of ammonia-oxidizing communities in Gulf of Mexico salt marshes

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Summary

We characterized ammonia-oxidizing archaea (AOA) and bacteria (AOB) from salt marsh sediments in the Gulf of Mexico over 5 years to identify environmental drivers of nitrifying community patterns following the Deepwater Horizon oil spill. Samples were collected from oiled and unoled sites in July of 2012–2016 from 12 marshes spanning three regions on the Louisiana coast. No consistent oil effect was detected for either AOA or AOB abundance or community composition. At the local scale, abundance was correlated with changes in marsh elevation, suggesting that oxygen may be an important driver. Regional differences in abundance were best explained by salinity and soil moisture, while interannual variation may be more linked to changes in climate and Mississippi River discharge. Variation of AOA communities was correlated with organic sediment nutrients, while AOB communities were correlated with soil extractable nutrients. AOA and AOB diversity and AOB abundance decreased in 2014 in all regions, suggesting that broad-scale drivers, such as climate, may explain synchronous shifts throughout the coastal area. Our results provide insights about large-scale disturbances on nitrifying microbes in the Gulf of Mexico, and suggest that nitrogen cycling may be controlled primarily by local factors, but

large-scale drivers might override these localized differences at times.

Introduction

Nitrogen loading to the northern Gulf of Mexico (GoM) from the Mississippi River drives much of the microbial ecology of the region (e.g. Childs *et al.*, 2002; Mason *et al.*, 2016). The fate of nitrogen entering the GoM is of particular importance in coastal regions where nitrogen fuels high rates of primary productivity and contributes to a large seasonal hypoxic zone (Rabalais *et al.*, 2002). Nitrification, the sequential oxidation of ammonia to nitrate, plays a critical role in the fate of nitrogen, and, when coupled to denitrification, contributes to the loss of nitrogen from the system (Seitzinger, 1988). High nitrification rates have been reported in the northern GoM water column (Carini *et al.*, 2010) and, more recently, in GoM salt marshes (Marton *et al.*, 2015). Despite the importance of nitrogen to the ecology of the GoM, there are very few studies on nitrogen cycling in the adjacent salt marshes, and fewer still focused on the microbial communities involved.

In July 2010, oil from the Deepwater Horizon (DWH) wellhead blowout reached coastal salt marshes in Louisiana, impacting the microbial ecology of the marshes. Studies of total bacterial communities in the marshes reported major shifts following the introduction of oil (Beazley *et al.*, 2012; Engel *et al.*, 2017), with some communities showing signs of recovery several years later (Mahmoudi *et al.*, 2013; Atlas *et al.*, 2015). Studies on nitrification and ammonia-oxidizing archaea (AOA) and bacteria (AOB) in Louisiana salt marshes 2 years post-spill, however, reported little effect of the oil on either nitrification rates (Marton *et al.*, 2015) or nitrifying communities (Bernhard *et al.*, 2016). Whether such a large disturbance might lead to longer-term impacts on nitrification and nitrifiers in the marsh remains uncertain.

Furthermore, few studies of ammonia oxidizers have reported multi-year patterns in coastal systems (but see Bouskill *et al.*, 2011 and Newell *et al.*, 2014), information

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that is critical to understanding how these communities respond to changes in local- and regional-scale conditions, as well as future disturbances. Factors controlling nitrifying communities in estuaries often vary spatio-temporally, leading to variable responses in the nitrifying communities. As a result, studies of ammonia oxidizers in salt marshes and estuaries have identified multiple factors that shape their communities, including salinity (Francis *et al.*, 2003; Bernhard *et al.*, 2005; Mosier and Francis, 2008; Santoro *et al.*, 2008), pH (Moin *et al.*, 2009), oxygen (Santoro *et al.*, 2008), temperature (Sahan and Muyzer, 2008), sulphide (Caffrey *et al.*, 2007), and substrate availability (Bouskill *et al.*, 2011; Vipindas *et al.*, 2018). Understanding how nitrifying communities in GoM salt marshes might be impacted by changes in local or regional conditions, including major disturbances, is critical to build better predictive models for salt marsh N cycling dynamics.

In this study, we build on our previous studies (Marton *et al.*, 2015; Bernhard *et al.*, 2016) that reported patterns of nitrification and ammonia-oxidizers 2 years after the DWH spill. Here, we evaluate interannual patterns of ammonia oxidizers in Louisiana marshes over 5 years. We set out to answer the following questions: (i) are there predictable spatial or temporal patterns in AOA or AOB communities in Louisiana salt marshes? (ii) are there long-term effects of oil exposure on nitrifier abundance or community composition in the marshes? Answering these questions should help us to better understand how large-scale disturbances may impact nitrifiers, and thus, nitrification, in the GoM, and to understand whether nitrogen cycling across the region may be controlled by different drivers in different areas.

Results and discussion

We analysed archaeal and betaproteobacterial *amoA* genes, representing AOA and AOB respectively, in 240 surface marsh sediment samples collected over 5 years along the Louisiana coast. In each of three regions, Terrebonne Bay (TB), west Barataria Bay (WB) and east Barataria Bay (EB), sites that received oil from the Deepwater Horizon oil spill were paired with unoiled sites for comparison. Detailed sampling and site descriptions have been previously published (Marton and Roberts, 2014; Marton *et al.*, 2015; Bernhard *et al.*, 2016). Details of the oiling of the sites can be found in Turner *et al.* (2014).

Except for AOB at EB in 2016, no significant differences in abundances between oiled and unoiled sites were detected over the study period (Table 1), so we combined data from both oiled and unoiled sites to analyse local, regional and interannual patterns of abundance.

Within-marsh variation

AOA and AOB abundance and AOA:AOB ratios varied significantly with distance from the marsh edge (5–20 m) in all three regions, except for AOB at EB, but showed opposite patterns at TB compared to WB and EB (Fig. 1). However, in all regions, abundance and ratios increased as marsh elevation increased (Fig. S1).

Differences in sediment chemistry in relation to distance from marsh edge were detected for some variables, but no variable showed consistent patterns across all three regions (Table S1). Organic C, total N and soil moisture varied with distance from marsh edge in TB, while only soil moisture varied in WB, and only salinity in EB.

Based on multiple regression analyses of AOA and AOB abundance in each region versus sediment chemistry, up to 73% of the within-marsh variation in AOA abundance was explained by sediment variables, but only as much as 41% of the variation in AOB abundance (Table 2). Total N and distance from marsh edge were the variables that had the most explanatory power within regions.

Increases in AOA abundance related to increased marsh elevation may reflect a greater sensitivity of AOA to conditions that change with elevation, such as oxygen (Armstrong *et al.*, 1985). Although AOA have generally been found to dominate in low oxygen environments such as oxygen minimum zones (OMZ) (Newell *et al.*, 2011; Bouskill *et al.*, 2012), including in the GoM (Bristow *et al.*, 2015), there are also reports of low oxygen environments favouring AOB (Fitzgerald *et al.*, 2015). Since it has been shown that AOA tend to have habitat-specific distributions (Biller *et al.* 2012), it is likely that the AOA in our study are not only phylogenetically distinct from those in the OMZ, but may also have different oxygen sensitivities.

Community composition, based on terminal restriction fragment length polymorphism (TRFLP) analysis of *amoA* genes, revealed little variation within each region, and unlike abundance patterns, did not vary significantly with distance from the marsh edge (data not shown). Since next-generation sequencing attempts for *amoA* genes from these samples did not yield quality data, we were only able to assess community composition based on TRFLP data. We previously published *amoA* sequence data from a subset of these samples, and showed that operational taxonomic unit distribution correlated strongly with TRFLP patterns for both AOA ($r = 0.93$) and AOB ($r = 0.83$) (Bernhard *et al.*, 2016).

Potential drivers of community composition within each region varied for AOA and AOB, suggesting that the two groups respond to different environmental cues (Table 3). AOA communities were more strongly correlated with

Table 1. *P* values for comparisons of AOA and AOB gene abundance and community composition between oiled and unoiled sites over 5 years in the three regions.

	Year	Gene Abundance				Community Composition			
		TB	WB	EB	All regions	TB	WB	EB	All regions
AOA	2012	0.55	0.36	0.67	0.69	0.67	0.09	0.06	0.66
	2013	0.44	0.54	0.60	0.93	0.22	0.61	0.32	0.30
	2014	0.85	0.56	0.72	0.57	0.07	0.23	0.25	0.50
	2015	0.36	0.95	0.33	0.50	0.36	0.12	0.03*	0.13
	2016	0.40	0.17	0.45	0.19	0.05*	0.64	0.01*	0.007*
	All years	0.54	0.60	0.17	0.37	0.78	0.07	0.04*	0.18
AOB	2012	0.81	0.07	0.53	0.17	0.88	0.38	0.15	0.24
	2013	0.85	0.98	0.26	0.69	0.89	0.02*	0.61	0.34
	2014	0.50	0.63	0.28	0.57	0.50	0.09	0.76	0.12
	2015	0.42	0.75	0.18	0.41	0.83	0.47	0.38	0.67
	2016	0.33	0.35	0.001*	0.02*	0.70	0.93	0.99	0.98
	All years	0.98	0.25	0.63	0.31	0.92	0.02*	0.03*	0.04*

Gene abundances were compared using Student's *t*-tests, and community composition was compared by a multi-response permutation procedure (MRPP). Bold numbers with asterisks indicate that abundances or community compositions were significantly different between oiled and unoiled sites.

sediment parameters such as organic C and total N, while AOB communities were correlated more strongly with soil extractable parameters, such as nitrate and ammonium. It is possible that this division reflects a potential reliance on organic C of AOA, since they have been shown to be mixotrophic in some instances (Mußmann *et al.*, 2011; Qin *et al.*, 2014), while AOB are strictly chemoautotrophs relying on inorganic substrates.

Regional variation

Comparisons between regions revealed significant differences for both AOA and AOB abundance (Fig. 2) and community composition (Figs 3 and S2). The 5-year average AOA abundance was approximately twice as high at EB than at TB and WB, and the differences were significant (Fig. 2A–C). Conversely, AOB abundances were significantly lower at EB, compared to TB and WB sites (Fig. 2D–F).

All the sediment chemistry variables tested, except total P, varied significantly between the regions (Table S2), with WB having lowest nutrient concentrations and ratios, and highest salinity. TB and EB had similar nutrient concentrations, but TB had higher pH and lower redox (consistent with less flooding). Salinity and soil moisture were the only two variables that differed significantly in all three regions, with WB having highest salinity and lowest water content.

When all three regions were analysed together, environmental factors explained 60% of the regional variation of AOA abundance, but only 11% of AOB abundance, with salinity and soil moisture explaining the most variation for AOA (Table 2). AOA abundance was also significantly correlated with precipitation at TB (Table S3). Salinity has frequently been implicated as a major driver

of AOA and AOB diversity and abundance in many different estuaries (see review by Bernhard and Bollmann, 2010), yet it remains uncertain if the effect is direct physiological stress on the organisms, or if it is due to other factors correlated with changes in salinity, such as available NH_4^+ , or a combination of both. Soil moisture has also been identified as an important driver of AOB community composition (Martiny *et al.*, 2011), and is likely related to oxygen availability, which would be expected to have a strong impact on aerobic ammonia-oxidizing communities.

We detected significant regional differences for both AOA and AOB community composition. For AOA, all three regions harboured distinct communities (Fig. 3A, Table 4), although they were all dominated by a common TRF, AOA170 (Fig. S2). This TRF represents *amoA* genes that are >95% similar to the *Nitrosopumilus maritimus amoA* gene, based on *in silico* analysis of *amoA* sequences (Bernhard *et al.*, 2016). However, two distinct subclusters of sequences closely related to *N. maritimus* (Fig. S3) showed region-specific patterns. TRF170 was significantly higher at TB compared to WB and EB, while TRF296 was significantly greater at WB and EB compared to TB. Region-specific distribution of closely related populations suggests there may be ecotypes of *N. maritimus*-related AOA that may respond to edaphic differences, such as pH and redox that differed significantly at TB compared to WB and EB (Table S2). Previous studies have found pH to be an important driver for AOA community composition (Li *et al.*, 2018).

AOB communities at TB and EB differed significantly from those at WB but not from each other (Fig. 3B, Table 4). TB and EB regions were dominated by TRF AOB336, which represents sequences related to *Nitrosomonas*, determined previously (Bernhard *et al.*,

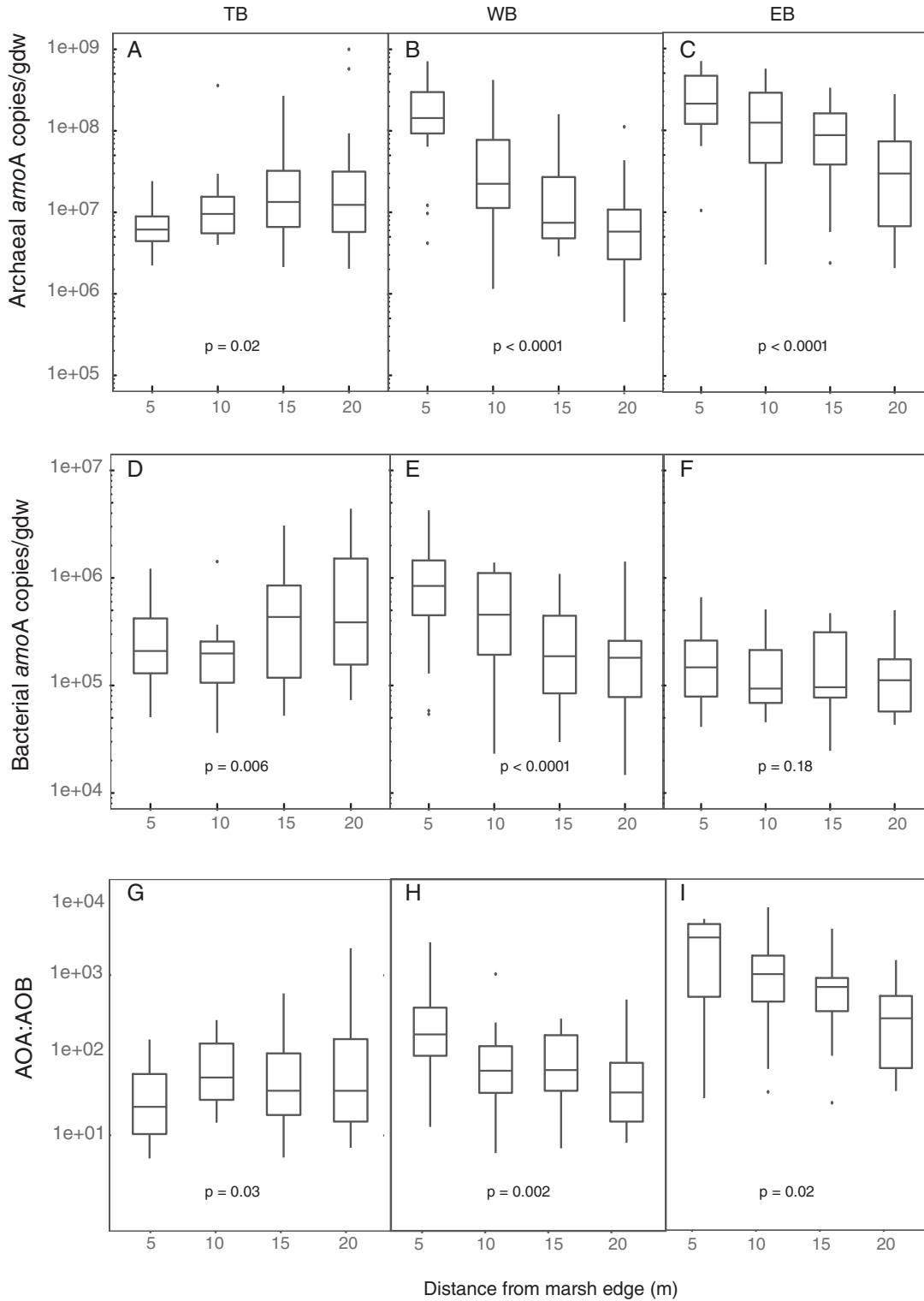


Fig. 1. Changes in abundance of AOA (panels A–C), AOB (panels D–F), and AOA:AOB ratios (panels G–I) by region with distance from marsh edge. Significance values for linear regression analysis of abundance versus distance from marsh edge are shown for each site. Data are based on abundance of *amoA* genes. Sample collection and processing is described in detail in Marton *et al.* (2015) and Bernhard *et al.* (2016). Archaeal and betaproteobacterial *amoA* genes were amplified as described in Bernhard *et al.* (2016). PCR efficiencies were 95.7% and 89.8% for archaeal and betaproteobacterial *amoA* genes respectively.

Table 2. Results from multiple regression analysis of AOA or AOB abundance with environmental variables.

Gene	Region	Adj. r^2	P value	Significant predictors
AOA	TB	0.73	<0.0001	Total N, Eh
	WB	0.38	0.008	Distance from edge
	EB	0.43	0.002	Distance from edge
	All	0.49	0.001	Soil moisture, salinity
AOB	TB	0.41	0.016	Total N
	WB	0.24	0.05	Distance from edge
	EB	-0.02	0.52	na
	All	0.11	0.23	na
AOA:AOB	TB	0.43	0.0003	Total N
	WB	0.37	0.01	Total N
	EB	0.29	0.03	Total P
	All	0.57	0.0004	Salinity, soil moisture

Adjusted r^2 , level of significance (P values), and variables with the most explanatory power are reported for the best-fit model for each region and gene.

Table 3. Correlation coefficients of environmental variables with the axes of the NMDS ordination for AOA and AOB community composition.

	AOA TRFLP				AOB TRFLP			
	TB	WB	EB	All	TB	WB	EB	All
pH				(0.15)				
Org C	(0.36)	0.17	(0.39)					0.18
Total N	(0.23)	0.23	(0.32)					0.23
Total P	(0.17)	0.20						
C:N				(0.19)	0.21	(0.25)	(0.29)	
N:P								0.21
PW salinity				0.34				(0.44)
Soil NO_3^-					(0.22)	(0.21)		
Soil NH_4^+								(0.22)
Soil PO_4^{2-}					(0.20)	(0.47)		
Soil moisture	(0.20)			(0.16)			0.27	0.36

Only coefficients >0.15 are shown. Negative correlations are indicated parenthetically. Statistical analyses were performed as previously described (Bernhard *et al.*, 2016). Sediment for chemical analyses were processed and analysed as described in Marton and Roberts (2014) and Marton *et al.* (2015).

2016). WB was dominated by TRF AOB278 representing *Nitrosospira*-like *amoA* sequences (Bernhard *et al.*, 2016).

Regional differences in AOB communities followed predicted patterns based on salinity and organic matter. *Nitrosospira*-related AOB are typically associated with higher salinity and lower organic matter (Francis *et al.*, 2003; Bernhard *et al.*, 2005; Zhang *et al.*, 2015), which were the conditions found at WB where *Nitrosospira*-related AOB dominated. Conversely, *Nitrosomonas*-related AOB are typically associated with higher organic matter and lower salinity (Urakawa *et al.*, 2006; Dang *et al.*, 2010; Peng *et al.*, 2013) and, as expected, these AOB dominated at TB and EB.

Some of the patterns we detected for AOA and AOB communities are similar to those detected in sediments

from the eastern GoM (Flood *et al.*, 2015), with AOA communities dominated by a few *N. maritimus*-related sequences, and AOB showing more variation between sites. Similar to abundance patterns, salinity was a major factor influencing regional differences in AOA community composition, but unlike AOB, the relative abundances of the dominant TRFs did not correspond to differences in salinity between the regions. Others have reported significant correlations between salinity and AOA in estuaries, although the relationship is not always robust (as reviewed in Bernhard and Bollmann, 2010).

Interannual variation

There were significant interannual differences in AOA abundance at TB and WB, where abundance generally increased over the 5-year period (Fig. 2A–C). At EB, although differences between years were not significant, there was a trend of increasing abundance over the 5-year period, similar to TB and WB. AOB abundance was also highly variable, with significant interannual differences in all regions, but no clear trend over time (Fig. 2D–F). Interestingly, AOB abundance was lowest in 2014 and 2015 in all regions. Ratios of AOA:AOB also increased significantly over time at TB ($p < 0.001$) and EB ($p = 0.027$). Interannual variations in sediment chemistry were also detected, with total N, C:N and soil extractable phosphate varying significantly over time in all three regions (Table S2).

AOA community composition showed significant interannual differences in all three regions (Fig. S2, Table 4). In most cases, AOA communities tended to fluctuate between years, with communities in 2012 and 2014 more similar to each other, and 2013 and 2015 more similar. AOB community composition at TB and EB differed each year from 2012 to 2014, and then remained stable from 2014 to 2016 (Fig. 3B). At WB, however, AOB communities fluctuated over the 5-year period, with different communities from 2013 to 2015, then reverting back in 2016 to communities similar to 2012–2013. Diversity of AOA and AOB decreased significantly in all three regions in 2014, and then generally recovered by 2016 (Fig. S4).

We think the high interannual variability is likely a reflection of a highly dynamic system, possibly driven by the dynamics of the discharge from the Mississippi River. Analysis of monthly river discharge and nitrate concentrations obtained from USGS (<https://waterdata.usgs.gov/usa/nwis>) showed that both parameters increased significantly over the 5-year period ($p < 0.001$), and mean yearly discharge was significantly correlated with average AOA abundance at EB ($r = 0.92$, $p = 0.02$), and was nearly significantly correlated with AOA abundance ($r = 0.84$, $p = 0.07$), and AOA:AOB ratios ($r = 0.85$, $p = 0.06$) at TB. Annual dynamics of the system are also

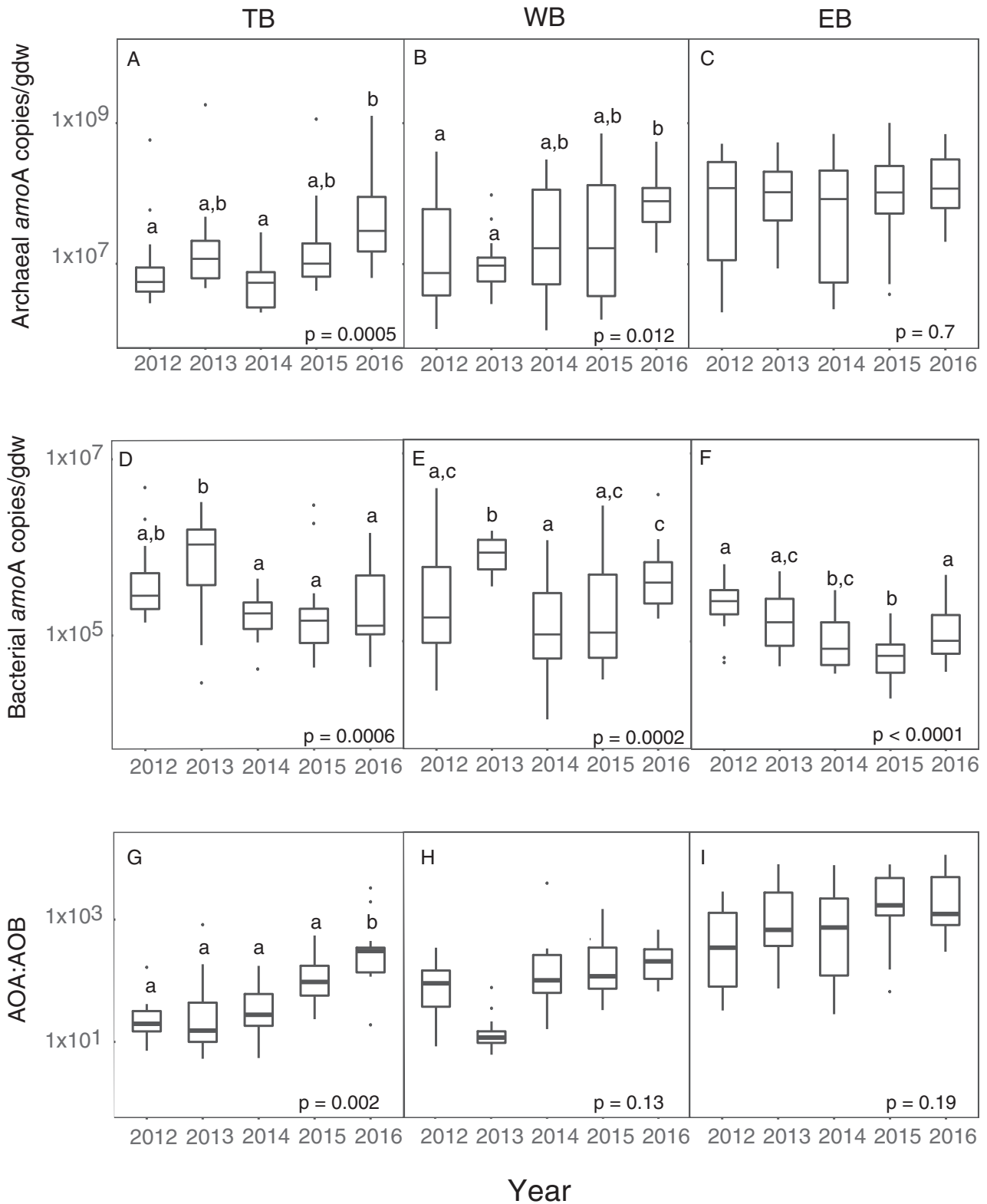


Fig. 2. Abundance of archaeal (panels A–C) and betaproteobacterial (panels D–F) *amoA* genes, and AOA:AOB ratios (panels G–I) in the three regions in July 2012–2016. Different letters indicate that significantly different values based on year in each region for each gene. *P* values from the ANOVA (performed in Prism v. 6.0) analysis with year as the variable are shown in each panel.

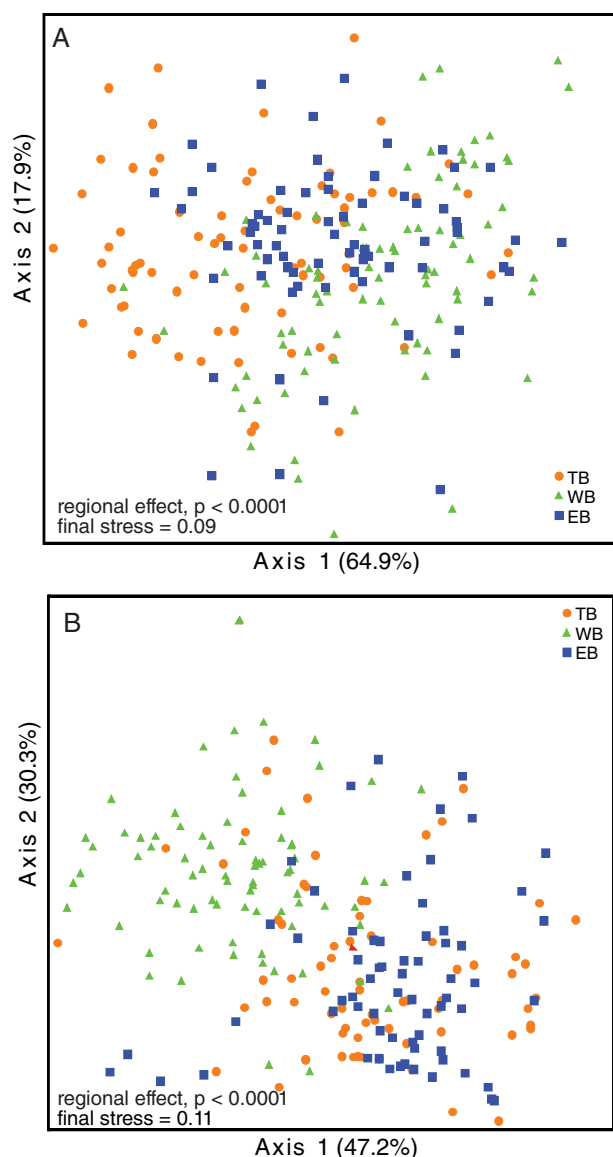


Fig. 3. Nonmetric multidimensional scaling ordinations of AOA (panel A) and AOB (panel B) communities by region based on TRFLP analysis of *amoA* genes. TRFLP analysis was performed as previously described (Bernhard *et al.*, 2016) and analysis was performed using PC-Ord v. 6. TRFs were based on peak height, and only those with known sequence representatives were included in the final analysis.

Table 4. *P*-values from MRPP analyses of TRFLP data combining all 5 years using oil, region, and year as grouping variables. Values indicating significant effects are bold and italic.

Gene	Region	Oil Effect	Region Effect	Year Effect
AOA	All regions	0.18	<i><0.0001</i>	<i><0.0001</i>
	TB	0.78	na	<i><0.0001</i>
	WB	0.07	na	<i><0.0001</i>
	EB	<i>0.04</i>	na	<i><0.0001</i>
AOB	All regions	<i>0.04</i>	<i><0.0001</i>	<i><0.0001</i>
	TB	0.92	na	<i><0.0001</i>
	WB	<i>0.02</i>	na	<i><0.0001</i>
	EB	<i>0.03</i>	na	<i><0.0001</i>

echoed in many of the sediment chemistry variables, suggesting that nitrifiers are intimately linked to the variation in sediment chemistry. Studies have shown high annual variability in nutrients and sediments from the Mississippi River, with much of the variation attributed to changes in climate (Justic *et al.*, 2003; Donner and Scavia, 2007).

Additional regional data on changes in marsh elevation and water level, obtained from the Coastwide Reference Monitoring System, indicated large interannual fluctuations (Fig. S5). From 2013 to 2014, average water level dropped 57% in WB, 72% in TB and 84% in EB. Although water levels began to increase again in 2015 and 2016, they still had not returned to levels measured in 2012 and 2013 in any of the regions. From September to October 2013, marsh elevation dropped 23.6% at TB, 33.2% at WB and 69.1% at EB, and remained low for the duration of the study. Tropical storm Karen moved through the region in October 2013 and likely contributed to the observed changes in water levels and marsh elevation. The sudden changes in late 2013 could be related to the decreases in AOB abundance and AOA and AOB diversity in 2014, particularly since we detected significant relationships between diversity and precipitation in some cases (Table S3).

Oil effects

AOA and AOB abundances and community composition were not different between oiled and unoled sites in 2012, 2 years after the DWH oil spill (Marton *et al.*, 2015; Bernhard *et al.*, 2016). This pattern continued through 2014, but some differences in community composition were detected in 2015 and 2016 (Table 1).

There are several possible explanations for these unexpected results in oiled sites. First, the communities may have been impacted when initially exposed to oil in 2010 and 2011, but since our study did not begin until 2012, we may have missed the initial impacts. Newell *et al.* (2014) reported significant shifts in AOA communities in GoM sediments that were still stable 1 year later. It is possible that by the time of our sampling, communities may have recovered, but because of the initial disturbance, some communities may have become more vulnerable to other stressors in the environment, such as hurricanes, climate change, or other broad-scale perturbations. Others have reported increased vulnerability of microbial communities exposed to multiple stressors (Birrer *et al.*, 2018; Calderón *et al.*, 2018).

A second explanation is that AOA and AOB were not impacted by the oil initially, but that other components of the ecosystem that were impacted are now having a trickle-down effect on the microbial community. For example, various components of the food web in the

Louisiana marshes have been significantly impacted by the oil (McCann *et al.*, 2017), and as food web dynamics change, nutrient availability may also change, resulting in shifts in the microbial communities. It is also possible that the differences we detected are not related to oiling history at all, but we cannot distinguish between these hypotheses with our data set.

Recently, Tartariw *et al.* (2018) reported significant shifts in denitrifiers in salt marshes impacted by the DWH spill, even 6 years post-spill. It is possible that denitrifiers may be more sensitive to oiling since they rely on organic C, while most nitrifiers are chemoautotrophs, and would not be expected to be affected as much by changes in organic C due to oiling.

Conclusions

Our 5-year study of AOA and AOB during summer in Louisiana salt marshes is one of very few long-term studies of nitrifiers in coastal systems, and points to several overarching patterns. First, variation between the marshes appears to be driven by factors different from those that drive variation within each marsh, suggesting that there are local and region-wide forces that act on nitrifying communities at these different scales. Second, AOA and AOB appear to respond to different environmental conditions, suggesting that controls on nitrification are highly complex, and that understanding the interplay between the two groups of nitrifiers will require more sophisticated approaches that can identify activity of specific populations under specific conditions. Third, although we did not detect a convincing long-term oil effect, we cannot rule out the possibility that nitrifiers may be more vulnerable than our study suggests. Controlled experimental manipulations will be required to more fully understand the response of nitrifiers to oiling.

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sediment chemistry), N7Z31WJF (2013-14 sediment chemistry), n7-0svp-nn22 (2015-16 sediment chemistry).

References

- Armstrong, W., Wright, E.J., Lythe, S., and Gaynard, T.J. (1985) Plant zonation and the effects of the spring-neap tidal cycle on soil aeration in a Humber salt marsh. *J Ecol* **73**: 323–339.
- Atlas, R.M., Stoeckel, D.M., Faith, S.A., Minard-Smith, A., Thorn, J.R., and Benotti, M.J. (2015) Oil biodegradation and oil-degrading microbial populations in marsh sediments impacted by oil from the Deepwater Horizon well blowout. *Environ Sci Technol* **49**: 8356–8366.
- Beazley, M.J., Martinez, R.J., Rajan, S., Powell, J., Piceno, Y.M., Tom, L.M., *et al.* (2012) Microbial community analysis of a coastal salt marsh affected by the Deepwater Horizon oil spill. *PLoS One* **7**: e41305.
- Bernhard, A.E., and Bollmann, A. (2010) Estuarine nitrifiers: new players, patterns and processes. *Estuar Coast Shelf Sci* **88**: 1–11.
- Bernhard, A.E., Donn, T., Giblin, A.E., and Stahl, D.A. (2005) Loss of diversity of ammonia-oxidizing bacteria correlates with increasing salinity in an estuary system. *Environ Microbiol* **7**: 1289–1297.
- Bernhard, A.E., Sheffer, R., Giblin, A.E., Marton, J.M., and Roberts, B.J. (2016) Population dynamics and community composition of ammonia oxidizers in salt marshes after the Deepwater Horizon oil spill. *Front Microbiol* **7**: 854. <https://doi.org/10.3389/fmicb.2016.00854>.
- Biller, S.J., Mosier, A.C., Wells, G.F., and Francis, C.A. (2012) Global biodiversity of aquatic ammonia-oxidizing archaea: Is partitioned by habitat. *Front Microbiol* **3**: 252. <https://doi.org/10.3389/fmicb.2012.00252>
- Birrer, S.C., Dafforn, K.A., Simpson, S.L., Kelaher, B.P., Potts, J., Scanes, P., and Johnston, E.L. (2018) Interactive effects of multiple stressors revealed by sequencing total (DNA) and active (RNA) components of experimental sediment microbial communities. *Sci Total Environ* **637–638**: 1383–1394.
- Bouskill, N.J., Eveillard, D., O'Mullan, G., Jackson, G.A., and Ward, B.B. (2011) Seasonal and annual reoccurrence in betaproteobacterial ammonia-oxidizing bacterial population structure. *Environ Microbiol* **13**: 872–886.
- Bouskill, N.J., Eveillard, D., Chien, D., Jayakumar, A., and Ward, B.B. (2012) Environmental factors determining ammonia-oxidizing organism distribution and diversity in marine environments. *Environ Microbiol* **14**: 714–729.
- Bristow, L.A., Sarode, N., Cartee, J., Caro-Quintero, A., and Thamdrup, B. (2015) Biogeochemical and metagenomic analysis of nitrite accumulation in the Gulf of Mexico hypoxic zone. *Limnol Oceanogr* **60**: 1733–1750.
- Caffrey, J.M., Bano, N., Kalanetra, K., and Hollibaugh, J.T. (2007) Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. *ISME J* **1**: 660–662.
- Calderón, K., Philippot, L., Bizouard, F., Breuil, M.-C., Bru, D., and Spor, A. (2018) Compounded disturbance chronology modulates the resilience of soil microbial communities and N-cycle related functions. *Front Microbiol* **9**: 2721. <https://doi.org/10.3389/fmicb.2018.02721>.

- Carini, S.A., McCarthy, M.J., and Garder, W.S. (2010) An isotope dilution method to measure nitrification rates in the northern Gulf of Mexico and other eutrophic waters. *Cont Shelf Res* **30**: 1795–1801.
- Childs, C.R., Rabalais, N.N., Turner, R.E., and Proctor, L.M. (2002) Sediment denitrification in the Gulf of Mexico zone of hypoxia. *Mar Ecol Prog Ser* **240**: 285–290.
- Dang, H., Li, J., Chen, R., Wang, L., Guo, L., Zhang, Z., and Klotz, M.G. (2010) Diversity, abundance, and spatial distribution of sediment ammonia-oxidizing betaproteobacteria in response to environmental gradients and coastal eutrophication in Jiaozhou Bay, China. *Appl Environ Microbiol* **76**: 4691–4702.
- Donner, S.D., and Scavia, D. (2007) How climate controls the flux of nitrogen by the Mississippi River and the development of hypoxia in the Gulf of Mexico. *Limnol Oceanogr* **52**: 856–861.
- Engel, A.S., Liu, C., Paterson, A.T., Anderson, L.C., Turner, R.E., and Overton, E.B. (2017) Salt marsh bacterial communities before and after the Deepwater Horizon oil spill. *Appl Environ Microbiol* **83**: e00784–17. <https://doi.org/10.1128/AEM.00784-17>.
- Fitzgerald, C.M., Camejo, P., Oshlag, J.Z., and Noguera, D. R. (2015) Ammonia-oxidizing microbial communities in reactors with efficient nitrification at low-dissolved oxygen. *Water Res* **70**: 38–51.
- Flood, M., Frabutt, D., Floyd, D., Powers, A., Ezegwe, U., Devol, A., and Tiquia-Arashiro, S.M. (2015) Ammonia-oxidizing bacteria and archaea in sediments in the Gulf of Mexico. *Environ Technol* **36**: 125–135. <https://doi.org/10.1080/09593330.2014.942385>.
- Francis, C.A., O'Mullan, G.D., and Ward, B.B. (2003) Diversity of ammonia monooxygenase (*amoA*) genes across environmental gradients in Chesapeake Bay sediments. *Geobiology* **1**: 129–140.
- Justic, D., Rabalais, N.N., and Turner, R.E. (2003) Simulated responses of the Gulf of Mexico hypoxia to variations in climate and anthropogenic nutrient loading. *J Mar Syst* **42**: 115–126.
- Li, Y., Chapman, S.J., Nicol, G.W., and Yao, H. (2018) Nitrification and nitrifiers in acidic soils. *Soil Biol Biochem* **116**: 290–301.
- Mahmoudi, N., Porter, T.M., Zimmerman, A.R., Fulthorpe, R. R., Kasozi, G.N., Silliman, B.R., and Slater, G.F. (2013) Rapid degradation of Deepwater Horizon spilled oil by indigenous microbial communities in Louisiana saltmarsh sediments. *Environ Sci Technol* **47**: 13303–13312.
- Martiny, J.B.H., Eisen, J.A., Penn, K., Allison, S.D., and Horner-Devine, M.C. (2011) Drivers of bacterial β -diversity depend on spatial scale. *Proc Natl Acad Sci U S A* **108**: 7850–7854.
- Marton, J.M., and Roberts, B.J. (2014) Spatial variability of phosphorus sorption dynamics in Louisiana salt marshes. *J Geophys Res Biogeosci* **119**: 451–465.
- Marton, J.M., Roberts, B.J., Bernhard, A.E., and Giblin, A.E. (2015) Spatial and temporal variability of nitrification potential and ammonia-oxidizer abundances in Louisiana salt marshes. *Estuaries Coasts* **38**: 1824–1837. <https://doi.org/10.1007/s12237-015-9943-5>.
- Mason, O.U., Canter, E., Gillies, L.E., Paisie, T., and Roberts, B.J. (2016) Mississippi River plume enriches microbial diversity in the northern Gulf of Mexico. *Front Microbiol* **7**: 1048. <https://doi.org/10.3389/fmicb.2016.01048>.
- McCann, M.J., Able, K.W., Christian, R.R., Fodrie, F.J., Jensen, O.P., Johnson, J.J., et al. (2017) Key taxa in food web responses to stressors: the Deepwater Horizon oil spill. *Front Ecol Environ* **15**: 142–149.
- Moin, N.S., Nelson, K.A., Bush, A., and Bernhard, A.E. (2009) Distribution and diversity of archaeal and bacterial ammonia-oxidizers in salt marsh sediment. *Appl Environ Microbiol* **75**: 7461–7468.
- Mosier, A.C., and Francis, C.A. (2008) Relative abundance and diversity of ammonia-oxidizing archaea and bacteria in the San Francisco Bay Estuary. *Environ Microbiol* **10**: 3002–3016.
- Mußmann, M., Brito, I., Pitcher, A., Sinnighe Damsté, J.S., Hatzenpichler, R., Richter, A., et al. (2011) Thaumarchaeotes abundant in refinery nitrifying sludges express *amoA* but are not obligate autotrophic ammonia oxidizers. *Proc Natl Acad Sci U S A* **108**: 16771–16776.
- Newell, S.E., Babbín, A.R., Jayakumar, A., and Ward, B.B. (2011) Ammonia oxidation rates and nitrification in the Arabian Sea. *Global Biogeochem Cycles* **25**: GB4016.
- Newell, S.E., Eveillard, D., McCarthy, M.J., Gardner, W.S., Liu, Z., and Ward, B.B. (2014) A shift in the archaeal nitrifier community in response to natural and anthropogenic disturbances in the Northern Gulf of Mexico. *Environ Microbiol Rep* **6**: 106–112.
- Peng, X.F., Yando, E., Hildebrand, E., Dwyer, C., Kearney, A., Waciega, A., et al. (2013) Differential responses of ammonia-oxidizing archaea and bacteria to long-term fertilization in a New England salt marsh. *Front Microbiol* **3**: 445.
- Qin, W., Amin, S.A., Martens-Habbena, W., Walker, C.B., Urakawa, H., Devol, A.H., et al. (2014) Marine ammonia-oxidizing archaeal isolates display obligate mixotrophy and wide ecotypic variation. *Proc Natl Acad Sci U S A* **111**: 12504–12509.
- Rabalais, N.N., Turner, R.E., and Wiseman, W.J., Jr. (2002) Gulf of Mexico hypoxia, a.k.a. "The Dead Zone.". *Annu Rev Ecol Syst* **33**: 235–263.
- Sahan, E., and Muyzer, G. (2008) Diversity and spatio-temporal distribution of ammonia-oxidizing archaea and bacteria in sediments of the westerschelde estuary. *FEMS Microbiol Ecol* **64**: 175–186.
- Santoro, A.E., Francis, C.A., De Sieyes, N.R., and Boehm, A.B. (2008) Shifts in the relative abundance of ammonia-oxidizing bacteria and archaea across physico-chemical gradients in a subterranean estuary. *Environ Microbiol* **10**: 1068–1079.
- Seitzinger, S.P. (1988) Denitrification in fresh-water and coastal marine ecosystems - ecological and geochemical significance. *Limnol Oceanogr* **33**: 702–724.
- Tartariw, C., Floumoy, N., Kleinhuisen, A.A., Tollette, D., Overton, E.B., Sobocky, P.A., et al. (2018) Salt marsh denitrification is impacted by oiling intensity six years after the Deepwater Horizon oil spill. *Environ Pollut* **243**: 1606–1614.
- Turner, R.E., Overton, E.B., Meyer, B.M., Miles, M.S., McClenachan, G., Hooper-Bui, L., et al. (2014) Distribution and recovery trajectory of Macondo (Mississippi Canyon 252) oil in Louisiana coastal wetlands. *Mar Pollut Bull* **87**: 57–67.

- Urakawa, H., Kurata, S., Fujiwara, T., Kuroiwa, D., Maki, H., Kawabata, S., *et al.* (2006) Characterization and quantification of ammonia-oxidizing bacteria in eutrophic coastal marine sediments using polyphasic molecular approaches and immunofluorescence staining. *Environ Microbiol* **8**: 787–803.
- Vipindas, P.V., Anas, A., Jayalakshmy, K.V., Lallu, K.R., Benny, P.Y., and Shanta, N. (2018) Impact of seasonal changes in nutrient loading on distribution and activity of nitrifiers in a tropical estuary. *Cont Shelf Res* **154**: 37–45.
- Zhang, Y., Chen, L., Sun, R., Dai, T., Tian, J., and Wen, D. (2015) Ammonia-oxidizing bacteria and archaea in wastewater treatment plant sludge and nearby coastal sediment in an industrial area in China. *Appl Microbiol Biotechnol* **99**: 4495–4507.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Changes in relative elevation at TB, WB, and EB as distance from the marsh edge increases.

Fig. S2. Relative abundance of AOA (panel A) and AOB (panel B) TRFs in July 2012–2016 in TB, WB, and EB. Different letters above the bars indicate significantly different communities in each region. Phylogenetic affiliations based on analysis of *amoA* sequences, determined by *in silico* analysis of *amoA* sequences from Bernhard *et al.* (2016), of some of the dominant TRFs are indicated for each panel.

Fig. S3. Phylogenetic relationships among archaeal *amoA* deduced amino acid sequences from OTU1 (Bernhard

et al., 2016). The tree was inferred with the neighbour-joining algorithm and is based on 133 amino acids. Numbers above the nodes represent bootstrap values based on 100 replicates. Numbers inside the polygons indicate number of sequences.

Fig. S4. Simpson's Diversity Index (based TRFLP analysis of *amoA* genes) for AOA (top panels) and AOB (bottom panels) in July 2012–2016 in the three regions. Different letters indicate significantly different values within each region.

Fig. S5. Average annual water level in the three regions (TB, WB, and EB) over the 5 years of the study. Data were downloaded from the Coastwide Reference Monitoring System (<https://www.lacoast.gov/crms/Home.aspx#>) using sites 0355, 0178, and 4529 to represent TB, WB, and EB respectively.

Table S1. 5-year mean \pm SE values for sediment chemistry measurements for the three regions. Different letters after the values indicate significantly different means between regions.

Table S2. Differences in sediment chemistry within each region using distance from marsh edge or year as the grouping variable, analysed by ANOVA. Only P values ≤ 0.05 are shown. No analysis for salinity in TB by year was possible due to missing values.

Table S3. Pearson's correlation coefficients and P values for comparisons of gene abundance and diversity with precipitation. P-values ≤ 0.05 are indicated with bold numbers and asterisks. Precipitation data for the entire area were retrieved from the Weather Collector (<http://www.theweathercollector.com/>) using the Galliano, LA station.