A shift in the archaeal nitrifier community in response to natural and anthropogenic disturbances in the northern Gulf of Mexico

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Summary

The Gulf of Mexico is affected by hurricanes and suffers seasonal hypoxia. The Deepwater Horizon oil spill impacted every trophic level in the coastal region. Despite their importance in bioremediation and biogeochemical cycles, it is difficult to predict the responses of microbial communities to physical and anthropogenic disturbances. Here, we quantify sediment ammonia-oxidizing archaeal (AOA) community diversity, resistance and resilience, and important geochemical factors after major hurricanes and the oil spill. Dominant AOA archetypes correlated with different geochemical factors, suggesting that different AOA are constrained by distinct parameters. Diversity was lowest after the hurricanes, showing weak resistance to physical disturbances. However, diversity was highest during the oil spill and coincided with a community shift, suggesting a new alternative stable state sustained for at least 1 year. The new AOA community was not significantly different from that at the spill site 1 year after the spill. This sustained shift in nitrifier community structure may be a result of oil exposure.

Introduction

The northern Gulf of Mexico (GOM) receives a heavy nutrient load from the Mississippi River, which is associated with seasonal hypoxia [<2 mg l⁻¹ dissolved oxygen (DO)] (Rabalais et al., 2007). This study initially intended to determine the response of sediment ammonia-oxidizing archaea (AOA) to seasonal hypoxia, but we also evaluated the community response to three major events during the study (July 2008–May 2011). Two Category 2 hurricanes (Ike and Gustav) passed directly over the study sites in September 2008. The Deepwater Horizon (DWH) oil spill began on 20 April 2010, but was not capped until July 2010. The extent and fate of the oil plume is still unknown, but oil mousse was observed on the water surface at our sampling sites during this study (Liu et al., 2012).

Coupled nitrification-denitrification is the major removal process for nitrogen (N) in the GOM (Childs et al., 2002). Nitrification is sensitive to low oxygen, and water column nitrification can decouple from sediment denitrification in hypoxic environments (Ogilvie et al., 1997). Ammonia oxidation to nitrite is the first step of nitrification, mediated by ammonia-oxidizing bacteria (AOB) and AOA. Studies have linked nitrifier community structure to salinity (Francis et al., 2003; Bernhard et al., 2007; 2010), but consistent relationships with other geochemical parameters (e.g. oxygen, ammonium and nitrite) have not been observed (Erguder et al., 2009; Bouskill et al., 2011). Previous work in the GOM indicated high nitrification rates (Carini et al., 2010) but low diversity and abundance of bacterial amoA (encoding ammonia monooxygenase) (Mills et al., 2008); thus, the focus of this investigation was restricted to AOA.

The goals of this project were to: (i) characterize the AOA community using a functional gene microarray to identify the most abundant archetypes at stations in the northern GOM (Fig. 1) outside [Sta. Control (CT)] and within (Sta. C6) the seasonal hypoxic zone; (ii) determine how AOA community structure varied with time and, in retrospect, was linked to the hurricanes and DWH oil spill; and (iii) quantify community resistance and resilience to the disturbances.

Results and discussion

AOA community composition

Sediment AOA community composition was investigated with an amoA functional gene microarray (Supplementary Experimental Procedures), such that the signal intensity of 30 probes (A1–A31, hybridizing to any amoA sequence
≥ 85% similar) was used to evaluate relative abundance. AOA communities at CT and C6 are shown as stacked bars (Fig. 2), where archetype signals (normalized fluorescence ratio) are proportional to the total community signal (sum of all normalized fluorescence ratios). Two archetypes, A4 (representing sequences detected in hot springs and the South China Sea) and A26 (in soil), were highest before May 2010. After May 2010, A4 remained the highest-signal archetype while A26 decreased. The community shifted in May 2010, with some new groups appearing only in May and August 2010, and others appearing in May 2010 and remaining through May 2011. A9, representing sequences from various (mainly low oxygen) marine environments, and A12, from soil, had the second and third highest signals (after A4) at CT from May 2010 onward. Both were also present at the spill site (DWH) in May 2011. A1, representing *Nitrosopumilus maritimus* (the only marine AOA in culture), was only detected once at each station (included in ‘all others’ in Fig. 2). Post-oil spill communities were significantly (P ≤ 0.03) different from pre-oil spill communities (Fig. 2, Fig. S1, and Table S1).

Archetypes exhibiting similar temporal patterns are clustered (Patterns 1–6), and behavioral similarity is depicted as a dendrogram (Fig. 3). In Pattern 1, the archetypes peaked in May 2010, but were either absent or had minimal signals at other times. Pattern 2 consisted only of A26 and was highest through January 2009, but this archetype decreased during and after the spill. Archetypes A4 and A22 (Pattern 3) were highest in July 2008, decreased post-hurricanes and returned as the dominant signal by May 2010. Archetypes in Patterns 4 and 5 appeared in May 2010, and remained high through May 2011. Archetypes in Pattern 4 peaked in May 2010 while those in Pattern 5 peaked in August 2010. Archetypes in Pattern 6 were low through May 2010 and peaked in August 2010.

Similar AOA amoA microarrays, using the same method and archetypes, have been applied elsewhere: the Bermuda Atlantic Time-Series Study (Bouskill *et al.*, 2011; Newell *et al.*, 2013), Chesapeake Bay and the North Atlantic (Bouskill *et al.*, 2011), and the Eastern Tropical South Pacific and the Arabian Sea (Bouskill *et al.*, 2011; Peng *et al.*, 2013). A subset of dominant sequences (A4, A9, A12, A15, A17, A23 and A26) recurred across all of these environments. These studies suggest that some AOA are ubiquitous and consistently important across marine (and potentially terrestrial) systems.

**AOA community diversity**

Diversity was lowest after the hurricanes and highest after the oil spill. CT and C6 were less diverse (Shannon Weiner Diversity Index; Table 1) in September 2008 post-hurricanes than in July 2008. Post-oil spill AOA communities were more diverse than pre-oil spill communities. The DWH community was also diverse in May 2011 (oil was still visible in the sediment) and similar to those at C6 and CT (linear regression slope = 1.15; R^2 = 0.84; P < 0.0001). Overall, the oil spill coincided with the highest diversity at both sites, with the new communities very similar to those at DWH.

The diversity estimated here is likely an underestimate for coastal sediments. During probe design, we used 1400 sequences available at the time, and our algorithm [Bulow *et al.*, 2008; comparable with MOTHUR or DOTUR (Schloss and Handelsman, 2005)] defined 30 probes at an 85% cut-off. Pester and colleagues (2012) also defined AOA species at 85–87% divergence for *amoA*. Using > 12 000 *amoA* sequences from soils at 85%, Pester and colleagues (2012) calculated 83–108 OTUs (operational taxonomic units, functionally equivalent with archetypes in
In a similar study, Biller and colleagues (2012) distilled 8000 sequences into OTUs. At 90%, they calculated 315 OTUs, 27 from the marine environment. At 85%, they calculated 138 OTUs, 15 from the marine environment. Thus, both studies predict more OTUs than represented on our array, although few of those occur in the marine environment. The data reported here most likely underestimated diversity, but the most abundant groups are probably well represented.

**Geochemical constraints on diversity**

The initial goal of the study was to determine if hypoxia impacts the AOA community. The bottom water (17 m) at C6 was hypoxic (< 2 mg l⁻¹ DO) at all times except September 2008 (post-hurricanes) and January 2009 (Table 2). Bottom-water (27 m) DO at CT was > 2 mg l⁻¹ except for May 2010 (during the oil spill). However, community composition was not significantly different between C6 (hypoxic) and CT (oxic). In the principal component analysis (Fig. 4), A26 correlated with bottom-water DO (within 8°) and nitrite concentrations (within 14°). A26 was the only archetype positively correlated with nitrite and DO (Fig. 4) and did not cluster temporally with other archetypes (Fig. 3). A26 also correlated with DO in other marine environments (Bouskill *et al*., 2011; Peng *et al*., 2013). This evidence suggests that changes in DO may have affected A26 primarily and cannot explain the community shifts observed here.

Ammonia oxidation rates often correlate with nitrite concentrations (Santoro *et al*., 2010; Newell *et al*., 2011), while diversity and distribution of AOA vs. AOB sometimes correlate with salinity (Santoro *et al*., 2008; Bernhard *et al*., 2010; Bouskill *et al*., 2011). However, no single geochemical factor consistently constrains AOA diversity and composition. Here, bottom water ammonium concentration at C6 was 2.65 μM or less, except in July 2008 and May 2010, but was < 0.50 μM at CT. Bottom-water nitrate

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**Fig. 2.** AOA amoA functional gene microarray results at (A) CT and (B) C6. The community is represented by a stacked bar plot, showing the percentage of each probe’s normalized Cy3/C5 fluorescence ratio (FRn) to the sum of the community FRn. The AOA amoA community is shown from July 2008 through May 2011; the last column represents the Deepwater Horizon community of surface sediment in May 2011. Significant but minor archetypes are grouped together in the black bars; the number of archetypes represented is written on the bar. Significant (P < 0.05) differences between sampling dates within each site are indicated above the bars.
and nitrite concentrations varied at both sites, ranging from 0.01 μM to 9.11 μM for nitrite and from 0.54 μM to 10.52 μM for nitrate. A4 correlated with ammonium and soluble reactive phosphorus (SRP) concentrations (within 5° of both). A9 and A12 were also closely correlated (within 3°) with each other, as well as with total dissolved inorganic nitrogen (DIN) concentration (within 10°). No archetypes were strongly correlated with nitrate concentrations or with nitrate or nitrite sediment fluxes. Similarly, no archetypes plotted near salinity or temperature; both factors were relatively constant and not discriminative in our analysis. Changes in other nutrient concentrations, however, correlated with the major archetypes. The two dominant archetypes here, A4 and A26,

![Diagram](image_url)

**Fig. 3.** The left panel is a dendrogram of all the probes at C6 and CT showing the similarity in their temporal patterns based on pairwise comparisons. The centre panel shows the patterns of clustered archetypes over the entire time series, beginning with July 2008 and ending in May 2011. The right panel shows the centroid, the overall temporal pattern of all of the clustered archetypes combined. To create the dendrogram and clusters, the temporal signal of each probe was extracted from fluorescence ratio (FRn) data and transposed into a similarity matrix using pairwise Spearman correlation scores when significant (< 10^-5, 0 otherwise). Joint classification and discrimination analyses of temporal behaviors were applied to group the archetypes by their qualitative temporal behavior.

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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.668</td>
<td>0.316</td>
<td>1.665</td>
<td>1.989</td>
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<td>2</td>
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<td>11</td>
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<td>20</td>
</tr>
<tr>
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<td></td>
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<td></td>
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<tr>
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<td>10</td>
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H' is diversity, E is evenness and S is the number of species (probes) detected.

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correlated with different nutrients (ammonium and SRP, and nitrite, respectively), and the major cluster of archetypes (A9, A10 and A12) correlated with total DIN concentration. These patterns indicate that AOA are not constrained by a single geochemical parameter; rather, different AOA groups are driven by distinct parameters.

**Physical constraints on diversity**

Lowest diversity occurred immediately after the hurricanes, suggesting physical disturbance may have reduced the nitrifier biomass such that only the highest signal archetypes, A4 and A26, were detected. Alternatively, the physical disturbance may have allowed only

<table>
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<tr>
<th>Site ID</th>
<th>Date</th>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
<th>DO (mg l⁻¹)</th>
<th>SRP (μM)</th>
<th>NO₂⁻ (μM)</th>
<th>NO₃⁻ (μM)</th>
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<td>25.79</td>
<td>34.1</td>
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<td>3.62</td>
<td>0.78</td>
<td>0.75</td>
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<td>33.4</td>
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<td>0.57</td>
<td>4.07</td>
<td>0.70</td>
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<td>35.2</td>
<td>4.32</td>
<td>0.24</td>
<td>2.86</td>
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<td>1.06</td>
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<td>0.25</td>
<td>3.10</td>
<td>0.59</td>
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<td>22.15</td>
<td>36.4</td>
<td>4.52</td>
<td>0.05</td>
<td>3.14</td>
<td>4.10</td>
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<td>May 2010</td>
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<td>2.15</td>
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<td></td>
<td>May 2011</td>
<td>19.50</td>
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<td>1.30</td>
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‘DO’ indicates dissolved oxygen and SRP indicates soluble reactive phosphorus concentrations.

Table 2. Geochemistry data for ambient bottom water (∼1 m above sediment) at sites C6 and control (CT).

![Fig. 4. Principal component analysis (PCA) diagram of all archetypes and geochemical data over all time points.

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Table 3. Resistance (RS) values for AOA amoA communities at CT and C6 post-hurricanes and post-oil spill based on changes in diversity (values range from 0–1, with 1 being most resistant).

<table>
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<th>Site</th>
<th>Event</th>
<th>Timeframe (days)</th>
<th>RS</th>
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<td>Hurricanes</td>
<td>7</td>
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<td>Oil spill</td>
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<td>0.67</td>
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<tr>
<td></td>
<td></td>
<td>390</td>
<td>0.52</td>
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<tr>
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<td>Hurricanes</td>
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<td></td>
<td></td>
<td>390</td>
<td>0.27</td>
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dominant archetypes to persist, or phylotypes not represented on the array were more abundant after the hurricanes. Biomass reduction from hurricane-induced physical disturbance seems most likely because lowest sediment oxygen consumption was observed after the hurricanes (McCarthy et al., 2013), suggesting depletion of total microbial biomass.

Highest diversity occurred after the oil spill in August 2010, and diversity remained high through May 2011, when the communities were similar to the DWH community and significantly different from the pre-hurricane community. This altered community may result from long-term oil impact or the persistence of oil in sediments (Liu et al., 2012). The sustained community structure in May 2011 implies that nutrient influx from record Mississippi River flooding in May 2011 either did not significantly impact the AOA community or that the impact was not yet detectable.

Community resistance and resilience were quantified as described by Shade and colleagues (2012) (Table 3). The GOM AOA community experienced pulse disturbances (sequential hurricanes) and a press disturbance (the oil spill). AOA communities at both sites showed weak resistance to the hurricanes in September 2008 [resistance (RS) = 0.6 and 0.2 for CT and C6, respectively, Table 3]. The C6 community was more resilient than the CT community, almost completely recovering from the hurricanes by January 2009, while CT recovered between the January and August 2009 sampling dates. AOA communities changed significantly (P < 0.0001) during and after the oil spill, also indicating weak resistance (RS = 0.2 and 0.6 for CT and C6, respectively). Resilience could not be quantified in this case, as instead of recovering to the pre-oil spill state, both communities stabilized to a new alternative stable state.

Possible impacts of oil on diversity

Urakawa and colleagues (2012) suggested that oil may shift the nitrifying community from AOA to AOB. They reported that *N. maritimus* (A1) was only detected once at each site, with a low signal, before the oil spill, but a diverse AOA community was found during and after, when polycyclic aromatic hydrocarbon concentration ranged from 96 ppb to 234 ppb (Z. Liu, unpubl. data). *Nitrosopumilus maritimus* does not therefore appear to represent the natural GOM AOA assemblage. AOA community diversity was highest during and after the spill, and high at the DWH site. Thus, AOA diversity was not adversely affected by the oil. Conversely, oil exposure appears to promote AOA diversity via an unknown mechanism.

Other studies have shown a temporary shift in the microbial community in response to oil (e.g., Bordenave et al., 2007; Chronopoulou et al., 2013) and deepwater plumes from the DWH spill (Bik et al., 2012; Lu et al., 2012). The shift in the nitrifier community in sediments reported here may be a sustained response to oil. The similarity between the AOA communities at CT and C6 to the DWH site in May 2011 suggests that linking AOA amoA genes to the rest of the genome is critical to determining additional functions for AOA and what, if any, role they play in remediating hydrocarbon releases.

Acknowledgements

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References


Supporting information

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

**Supplementary Experimental Procedures.**

Fig. S1. A cluster-gram (bootstrapped 20 000 times) showing correspondence between sampling dates (CT and C6 were combined, and August 2009 was excluded as there was no data for C6). Correspondence values and P-values are shown in Table S1.

Table S1. Correspondence values (R) and P-values for Fig. S1. R-values (shown above the diagonal) closest to 1 are most similar. P-values are shown below the diagonal.