Nitrate reduction pathways in the presence of excess nitrogen in a shallow eutrophic estuary

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ABSTRACT

The eutrophication of estuaries results from increasing anthropogenic nutrient inputs to coastal waters. Ecosystem recovery from eutrophication is partly dependent on the ability of a system to assimilate or remove nutrients, and denitrification and dissimilatory nitrate reduction to ammonium (DNRA) are important pathways for nitrogen (N) retention or removal. We measured rates of denitrification and DNRA over an annual cycle at two stations in Weeks Bay, AL, a shallow microtidal estuary receiving freshwater from two rivers with agricultural watersheds and high N inputs. We hypothesized that rates of DNRA would exceed denitrification in the sulfidogenic sediments in this estuary. Consistent with our hypothesis, we found that DNRA (44.4 ± 5.5 μmol N m^{-2} hr^{-1}) exceeded *in situ* denitrification (0.9 ± 2.3 μmol N m^{-2} hr^{-1}) and that even in the presence of abundant water column nitrate DNRA was favored over denitrification by a factor of two. DNRA is estimated to provide N to the water column at a rate equivalent to 15% of the N input that is retained within the estuary and is a significant component of the N budget in this highly impacted estuary. DNRA by retaining N in the system contributes to the nitrogen demand by primary producers can impact this estuary through enhanced rates of primary production. Weeks Bay, like many coastal estuaries, experiences periods of hypoxia, blooms of harmful algae and fish kills. Future management efforts should focus on reducing nutrient input to this estuary without which the significant retention of N in this system through DRNA will contribute to the undesirable ecosystem attributes associated with eutrophication.

Capsule: DNRA is a significant process even in the presence of elevated nitrate concentrations in the sulfidogenic sediments of Weeks Bay, Alabama, and provides a significant fraction of the nitrogen demand by primary producers. It is conceivable that higher inputs of nutrients will contribute to the initiation and retention of algal blooms and subsequent deposition of organic matter to the sediments, degradation of which will lead to more hypoxic events and fish kills in this and similarly impacted ecosystems if management decisions do not lead to nutrient input reductions.

Key Words Nitrogen cycling, DNRA, denitrification, hydrogen sulfide, National Estuarine Research Reserve

INTRODUCTION

Nearshore marine ecosystems are especially sensitive to anthropogenic nutrient inputs (Smith et al., 1999) with ecosystem structure and function markedly altered as a consequence (Cloern, 2001; Halpern et al., 2007; Harley et al., 2006). Anthropogenically-driven increases in N loads (primarily as nitrate, NO_3^-) to aquatic systems and associated water quality problems have focused attention on understanding the variables that affect processes within the N cycle, and more specifically the pathways of NO_3^- reduction within estuarine sediments. These processes include canonical denitrification, anaerobic ammonium (NH_4^+) oxidation (anammox), and dissimilatory nitrate reduction to NH_4^+ (DNRA). Denitrification is carried out by bacteria that reduce NO_3^- at low (0.2 mg/L) oxygen (O_2) concentrations and produce nitrous oxide (N_2O) and dinitrogen gas (N_2) (Knowles, 1982; Seitzinger et al., 2006). Anammox oxidizes NH_4^+ with NO_2^- as the electron acceptor to produce N_2, however, it generally accounts for only a minor fraction of the N_2 produced (Dalsgaard et al., 2005). As a result of DNRA, NO_3^- is reduced to NH_4^+ (Gardner et al., 2006; Kaspar et al., 1981). In contrast to denitrification and anammox that lead to the removal of N from the system, DNRA retains N as NH_4^+ (An and Gardner, 2002). In addition to N and phosphorus (P) regenerated through mineralization of sediment organic matter (Twilley et al., 1999) N retained through DNRA contributes to primary production in estuaries.

Understanding the factors that control how NO_3^- is cycled has implications for predicting the impact of excess nutrient inputs to nearshore marine systems (Christensen et al., 2003; Seitzinger et al., 2006). Indeed, anthropogenic N loading in the watershed and the fate of nutrients once they enter the estuary are primary management concerns (Paerl et al., 2014). Denitrification has empirically been shown to vary as a function water column NO_3^- concentration, the water column residence time, (Nixon et al., 1996; Seitzinger et al., 2006), as well the overall rate of sediment organic matter mineralization (Fennel et al., 2009). With higher water residence time and elevated NO_3^- concentrations, primary production is enhanced which leads to higher inputs of organic matter to the sediment and leads to higher denitrification rates (Middelburg et al., 1996). However, the same factors, namely NO_3^- availability and organic matter content of the sediments (Tiedje, 1988), have also been shown to influence DNRA (Christensen et al., 2000; Dong et al., 2011). The ratio of NO_3^- to organic matter content is a primary factor that determines if NO_3^- is lost through denitrification or retained in the system through DNRA (Burgin and Hamilton, 2007). Other variables such as the presence of reduced sulfur in the sediments also influence

denitrification and DNRA. The presence of sulfides in sediments lead to reduced denitrification (Tobias et al., 2001) and coupled nitrification-denitrification (Christensen et al., 2003), though autotrophic denitrification coupled to reduced sulfur compounds is noted (Batchelor and Lawrence, 1978). But DNRA can proceed chemolithoautotrophically through oxidation of reduced sulfur species (Brunet and Garcia-Gil, 1996; Dalsgaard and Bak, 1994), and in the presence of sulfides a larger fraction of the available NO_3^- can be retained in the system as opposed to lost from the systems through denitrification (Christensen et al., 2003; Christensen et al., 2000). These complexities make it challenging to predict how excess NO_3^- delivered to the coast will be processed.

We determined rates of denitrification and DNRA in Weeks Bay, AL, USA, a shallow (1.4 m depth) microtidal (0.4 m) estuary in the northern Gulf of Mexico that is part of the National Estuarine Research Reserve System. Weeks Bay is fringed with a variety of wetland habitats receiving freshwater from the Fish and Magnolia Rivers that both have highly agricultural watersheds with dissolved inorganic nitrogen (DIN) concentrations in the rivers exceeding at times 140 μM (Lehrter, 2008). Caffrey et al. (2013) reported total N inputs into Weeks Bay of 10 mol N m^{-2} yr^{-1}, which is one of the highest rates of N loading to an estuary in the northern Gulf of Mexico estuaries. Previous studies in Weeks Bay found high porewater sulfide concentrations (Caffrey et al., 2007), significant sediment uptake of NO_3^- and high NH_4^+ fluxes, but concurrent low net denitrification rates (Mortazavi et al., 2012; Riggs, 2010). Therefore, we hypothesized that DNRA is the significant reduction pathway for NO_3^- in Weeks Bay and because of the sulfidogenic sediments, DNRA would also be a significant NO_3^- reduction pathway in the presence of excess NO_3^-. Periods of anoxia are common occurrences in Weeks Bay (http://cdmo.baruch.sc.edu/), as are blooms of harmful algae (Canion et al., 2013) and fish kills and understanding the fate of nutrients in this system has management implications.

METHODS

Field Collections

Intact sediment cores and water column samples for experiments were collected quarterly from bare sediments by hand near the mouth and in the mid bay area of the Weeks Bay National Estuarine Research Reserve (hereafter referred to as MidBay and Mouth stations) between December 2011 and October 2013 **(Fig. 1)**. At both sites, we measured temperature, salinity, pH, and dissolved oxygen (DO) with a YSI Model 556 Multiparameter Meter. Water column samples for nutrient analysis were collected by hand, filtered in the field (GF/F, 0.7 micron) and frozen until DIN and phosphate (PO_4^{3-}) analyses. All nutrient concentrations from the field and experimental

samples described below were measured with a Skalar SAN$^+$ Autoanalyzer. Total nitrogen and carbon content were measured in triplicate from the top 1 cm of sediment. Samples were acidified to remove carbonates (Harris et al., 2001) and total C and N were analyzed with an elemental combustion analyzer (Costech Instruments, model ECS 4010). Based on the ASTM C136-06 standard, grain size distribution was determined by sieve analysis using sieves #10, #60, and #230 from a haphazard sediment grab of approximately 2 kg at each site (ASTM C136-06, 2006).

Denitrification and DNRA from intact sediment cores with N enrichment

In a darkened environmental chamber set to site temperature, denitrification, and DNRA at the sediment-water interface were measured on sediment cores with N enrichment (9.5 cm inner diameter; 19 cm sediment with 5 cm overlying water; 3 per station in 2011 and 2012; 6 per station in 2013) set up in a flow-through system. Site water was filtered (0.7 micron) and amended to ~100 µM $Na^{15}NO_3^-$ (99 atom %) representing similar N concentrations reported by Lehrter (2008), and used as the inflow water at a continuous flow rate (1.2 mL min^{-1}) into each core. The outflow from each core was collected in a reservoir. Inflow and outflow samples for dissolved gas and nutrient analysis were collected at 36 hours to allow the systems to approach steady-state conditions (Eyre et al., 2002). Benthic flux calculations were calculated according to: $(C_o - C_i) * f/a$, where C_o and, C_i are the outflow and inflow concentration in µmole L^{-1}, f is the flow rate (0.072 L hr^{-1}), and a is the sediment surface area (0.00708 m^{-2}) (Lavrentyev et al., 2000).

Samples for dissolved gas analysis were collected in 12 mL Exetainers and preserved with 250 µL of 50% (w/v) $ZnCl_2$ before analysis on a membrane inlet mass spectrometer (MIMS) (Kana et al., 1998) fitted with a copper column heated to 600^{o}C to remove oxygen (O_2) (Eyre et al., 2002). Following the Isotope Pairing Technique (IPT) (Nielsen, 1992), denitrification rates were calculated under ambient environmental conditions (D_{14}) (which can be further portioned as ambient $^{14}NO_3^-$ from the water column (D_w) and coupled nitrification-denitrification (D_n)) and amended denitrification rates ($D_{14} + D_{15}$), calculated as the sum of denitrification rates of ambient NO_3^- (D_{14}) and denitrification stimulated by the added labeled $^{15}NO_3^-$ (D_{15}), and hereafter will be referred to as the denitrification capacity. Denitrification was explicitly calculated from the $^{29}N_2$ and $^{30}N_2$ fluxes calculated directly from dissolved $^{29}N_2$:$^{28}N_2$ and $^{30}N_2$:$^{28}N_2$ measured with a MIMS. Sediment-water interface gas flux (µmol m^{-2} hr^{-1}) greater than zero indicates a release from the sediments to the water column. All rates and fluxes pertaining to N species are expressed on N atom basis.

After sample collection for denitrification, approximately 1 L of inflow reservoir water and outflow water from each core were collected for DNRA analysis. Samples and standards for $^{15}NH_4^+$ were prepared according to Holmes et al. (1998) and as described in Bernard et al. (2015). ^{15}N analysis was performed at Utah State University's Stable Isotope Lab. DNRA was determined from the production rate of $^{15}NH_4^+$ ($p^{15}NH_4^+$) according to Christensen et. al (2000), assuming that (i) DNRA takes place in the same sediment layers as denitrification and (ii) that the $^{15}NO_3^-$ that was reduced to NH_4^+ is similar to that of the $^{15}NO_3^-$ that was reduced to N_2 (Christensen et al., 2000).

Anammox from slurry assays

Following intact sediment core collection, sediments (n=3) at each site were collected by hand with a sediment core (9.5 cm ID) and the top 5 cm were combined and homogenized. At each sampling event, anammox rates were determined with ^{15}N (99 atom %, 100 μmol NO_3^- L^{-1}) tracer slurry incubations at each station in triplicate according to Thamdrup and Dalsgaard (2002). Anammox on average contributed to 2% of the overall N_2 production and is not discussed further.

Oxygen and hydrogen sulfide sediment profiles

We also collected duplicate sediment cores (17 cm x 9.5 cm ID) at each site to determine sediment O_2 and hydrogen sulfide (measured as HS^-) concentrations. Concentrations just above the sediment-water interface and in the sediments to a depth of 1 cm at 1 mm intervals were determined with a microelectrode system (Unisense Ox-500, H_2S-50) with sensors calibrated as recommended by the manufacturer.

Statistical Analysis

To test the seasonal flux variability between sites in Weeks Bay, two-way ANOVAs with site and date as independent variables were performed. If data could not be transformed to meet ANOVA assumptions, we carried out Wilcoxon/Kruskal-Wallis nonparametric tests with all parameters with site and date as independent factors. When differences were significant, Tukey HSD or Steel-Dwass post hoc tests were used to test for interactions. A Principal component analysis (PCA) was conducted on all biogeochemical parameters to identify underlying multivariate components that may be influencing DNRA and denitrification. Statistical significance was set at α=0.05 and error is reported as standard error. We used SAS JMP 10 (SAS Institute Inc.) to carry out all statistical analysis.

RESULTS

Site Characteristics

Temperature exhibited significant seasonal variability (p=0.042) and a moderate 10 °C seasonal range (**Fig. 2a**). Salinity fluctuated substantially and was lowest in March 2013 (1.6) **(Fig. 2a)** coinciding with a spike in Magnolia River discharge (USGS daily discharge data not shown) even though it was only marginally correlated with seasons (p=0.0539). Water column nutrient concentrations did not differ between site nor season (**Fig. 2b**). Water column NO_3^- ranged from 0.6 ± 0.4 μM in June 2012 to 16.8 ± 3.1 μM in March 2013. Water column NH_4^+ ranged from 0.3 ± 0.3 μM in March 2012 to 3.4 ± 1.5 μM in June 2013. Water column PO_4^{3-} generally was less than 0.2 μM throughout the study and resulted in elevated N:P ratios (average 118:1).

The sediments at the Mouth consisted of 85% medium sand, 13% very fine sand and <1% silt and only differed marginally in composition from sediments at MidBay (76% medium sand, 15% very fine sand, and 4% silt). The sediment C:N averaged 15.0 ± 1.3 and ranged from 12.0 to 21.0 but did not differ between sites (p=0.753) or seasons (p=0.110). Sediments in Weeks Bay were often anoxic by 3 mm in the winter and by 1 mm in the summer (**Fig. 3 top panel**). The only months with oxygen present past 1mm were December 2011 and June 2013 at the Mouth and March 2012 and March and October 2013 at MidBay. Hydrogen sulfide was nearly always present in the top 1 cm of the sediment at both sites and maximum values were found in March 2012 and ranged from 37.7 ± 0.9 μM at MidBay to 57.2 ± 1.2 μM at the Mouth (**Fig. 3 bottom panel**). A second event of high surficial HS^- concentrations was observed at both locations in March 2013. The only months without HS^- in the top 1 cm of sediment were June 2012 and October 2013 at the Mouth and June 2012, June and October 2013 at MidBay.

Denitrification and DNRA

In situ denitrification, D_{14}, (**Fig. 4a)** was low and averaged 0.8 ± 0.5 μmol N m^{-2} hr^{-1} at the Mouth and 1.6 ± 0.4 μmol N m^{-2} hr^{-1} at MidBay with an overall average of 0.9 ± 2.3 μmol N m^{-2} hr^{-1}. D_{14} denitrification partitioned into D_w and D_n averaged 0.4 ± 0.2 and 0.4 ± 0.3 μmol N m^{-2} hr^{-1} respectively, at the Mouth and 0.5 ± 0.2 and 1.1 ± 0.3 μmol N m^{-2} hr^{-1} respectively, at MidBay. Overall, D_n contributed 55% and 69% to D_{14} at the Mouth and Midbay stations, respectively. The denitrification capacity averaged 22.9 ± 15.0 μmol N m^{-2} hr^{-1} and was similar (p=0.365) between MidBay (33.6 ± 10.8 μmol N m^{-2} hr^{-1}) and the Mouth (21.6 ± 12.9 μmol N m^{-2} hr^{-1}). Only denitrification capacity in June 2012 was significantly higher than the rest of the study period.

DNRA ranged from a low of 8.8 ± 3.1 at the Mouth to a high of 89.7 ± 18.4 μmol NH_4^+ m^{-2} hr^{-1} at MidBay **(Fig. 4b)** and the rates were significantly higher at MidBay than at the Mouth (p=0.001). DNRA and water column

NO_3^- concentrations were positively correlated (r^2=0.41, p=0.025) over the study duration. DNRA at MidBay (average 56.1 ± 7.7 μmol N m^{-2} hr^{-1}) was also generally greater than denitrification capacity (average 33.6 ± 10.8 μmol N m^{-2} hr^{-1}) at this location, while at the Mouth (DNRA average 34.5 ± 7.0 μmol N m^{-2} hr^{-1}), DNRA only exceeded denitrification capacity in March 2012 and October 2013. DNRA in March 2013 was significantly lower than the rest of the study period. Average DNRA for Weeks Bay (44.4 ± 5.5 μmol N m^{-2} hr^{-1}) exceeded *in situ* denitrification by an order of magnitude and the average denitrification capacity twofold.

Principal Component Analysis

The PCA analysis resulted in a two-component model that explained a cumulative 58% of the total variance in the abiotic variables (**Table 1)**. Water column inorganic N and salinity were correlated with PC1 which explained 35% of the total variance and indicated higher water column nutrient availability during times of greater freshwater delivery. Temperature and HS^- and DO were correlated with PC2 and explained 23% of the total variance and indicated higher HS^- concentrations during the lower DO and warmer months. Denitrification capacity was negatively correlated with PC1 (rho=-0.577, p=0.019) driven by the water column inorganic N. DNRA did not correlate with either PC1 or PC2 but in the presence of excess NO_3^-, DNRA accounted for 66% of the total NO_3^- reduction (**Table 2**).

DISCUSSION

DNRA exceeds denitrification in Weeks Bay

DNRA, consistent with our hypothesis, by far exceeded *in situ* denitrification in Weeks Bay. At the Mouth, denitrification capacity was slightly lower or comparable to DNRA rates and at MidBay denitrification was consistently lower than DNRA. Denitrification capacity in Weeks Bay varied seasonally, a pattern that is similar to previously studies in other coastal ecosystems (Piehler and Smyth, 2011; Seitzinger, 1994). In Weeks Bay, D_n is responsible for between 55 to 69% of *in situ* denitrification at the Mouth and MidBay respectfully, but the magnitude of these fluxes are low because of the presence of HS^- and suggest a minimal role for nitrification and coupled nitrification-denitrification in this system. In Weeks Bay denitrification increased in the presence of higher NO_3^- concentrations consistent with predictions from Seitzinger (1988); Seitzinger and Giblin (1996). DNRA dominated *in situ* denitrification in these carbon rich (C:N =15:1) and sulfidogenic sediments (Caffrey et al., 2013) and it remained the dominant NO_3^- reduction pathway despite increases in denitrification at elevated NO_3^-

concentrations. In the presence of excess NO_3^-, DNRA accounted for 66% of the total NO_3^- reduction, and remained a significant pathway for N reduction in this system, consistent with other studies that have found DNRA contribution to NO_3^- reduction to range from <3% to >60-99% (Giblin et al., 2013). The significant relationship between water column NO_3^- concentrations and DNRA implies that allochthonous NO_3^- inputs can potentially support DNRA and lead to retention of bioavailable N in the systems. DNRA, by retaining N in the system, exacerbates eutrophication in estuaries and may have major implications for how coastal ecosystems respond to elevated N loading.

While the prevalence of DNRA over denitrification has been observed in other estuaries (An and Gardner, 2002; Gardner and McCarthy, 2009; Koop-Jakobsen and Giblin, 2010), in some systems, DNRA rates are lower than or comparable to denitrification rates (Lansdown et al., 2012; McCarthy et al., 2007; Tobias et al., 2001). The average DNRA rate in Weeks Bay was on the lower range of reported rates for Gulf of Mexico estuaries (1 to 241 μmol N m^{-2} hr^{-1}) (An and Gardner, 2002; Gardner and McCarthy, 2009) and other sub-tropical estuaries (up to 1137 μmol N m^{-2} hr^{-1}) (Dong et al., 2011; Dunn et al., 2013; Dunn et al., 2012; Porubsky et al., 2009). DNRA is energetically favored over denitrification (597 versus 559 kJ mol^{-1} NO_3^- at 30 °C) under NO_3^- limiting conditions (Algar and Vallino, 2014; Dong et al., 2011), and low NO_3^- availability has been regarded as a mechanism favoring DNRA over denitrification. But other factors such as temperature, salinity, the presence of porewater sulfides (Burgin and Hamilton, 2007; Howarth et al., 2011; Yoon et al., 2015), the abundance of labile organic carbon relative to NO_3^- (Algar and Vallino, 2014; Babbin and Ward, 2013; Burgin and Hamilton, 2007; Hardison et al., 2015; Tiedje, 1988), and the overall rates of benthic metabolism (Burgin and Hamilton, 2007; Dong et al., 2011; Giblin et al., 2010; Nizzoli et al., 2006) can work independently or in concert to determine if NO_3^- is used by DNRA or denitrification. Many of these factors often covary and it is difficult to attribute the influence of a single factor as a driver on these two processes. Multiple influential factors may be at work in Weeks Bay, given the lack of a relationship between the DNRA flux and either of the PCA principal components, as well as a lack of a strong individual influence from abiotic variables, save water column NO_3^-.

Mesocosm and modeling studies found anammox to dominate in C limited systems, while heterotrophic denitrification and DNRA dominate in N limited environments as the C:N input increases (Algar and Vallino, 2014) and at higher ratios of C:N input, there is a switch to denitrification and finally DNRA as the environment switches from being C limited to N limited (Hardison et al., 2015). Porubsky et al. (2009) found C:N ratios of 50-200

favored DNRA over denitrification, while Algar and Vallino (2014) found DNRA to exceed denitrification at CH_20: NO_3^- ratios around 3. In Weeks Bay, the C:N ratio ranges from 12 to 21 (average 15), lending support that the system favors DNRA before we simulated estuarine N loading. Moreover, the presence of sulfides in Weeks Bay sediments most probably limits nitrification (Joye and Hollibaugh, 1995), the supply of nitrate to the denitrifiers (Brunet and Garcia-Gil, 1996), and can be used chemolithoautotrophically to support DNRA. Though DNRA and denitrification can coexist in environments with high C:N ratios (van den Berg et al., 2016), our data is consistent with the interpretation that HS^- appears to be a contributing driver for the dominance of DNRA over denitrification. These findings mirror those found for Little Lagoon, a nearby anthropogenically impacted coastal lagoon (Bernard et al., 2015). In Little Lagoon, DNRA averaged 52.1 μmol N m^{-2} hr^{-1} and exceeded denitrification capacity by an order of magnitude (Bernard et al., 2015). Sediments in Little Lagoon were also sulfidic, with concentrations that at times exceeded 4 mM and Bernard et al. (2015) attributed the high DNRA and low denitrification rates to high porewater sulfide concentrations.

Ecosystem Implications

The primary management goals for many nearshore marine ecosystems focuses on restoring the hydrology, establishing the natural shorelines and marshes, as well as reducing delivery of nutrients (Pinckney et al., 2001). The increased urban and agricultural developments in the Weeks Bay watershed have lead to higher inputs of N resulting in high chlorophyll, blooms of harmful algae, and fish kills. This study empirically confirms that DNRA as opposed to denitrification is the dominant NO_3^- reduction pathway in Weeks Bay and the dominance of DNRA over denitrification has important ecosystem implications.

Caffrey et al. (2013) reported total N input to Weeks Bay of 10 mol N m^{-2} yr^{-1}, which is one of the highest rates of N loading to an estuary in the northern Gulf of Mexico estuaries. However, because the residence time of the estuary is short (mean 13 days, Schreiber and Pennock, 1995) using Nixon et al. (1996) relation between N retention and residence time, we estimate that 75% of the N input is exported from the estuary. The flux of bioavailable N to the water column through DNRA is equivalent to 15% of the TN input retained in the estuary, and therefore, is a significant component of the N budget. Caffrey et al. (2013) also determined primary production in Weeks Bay to be 599 g C m^{-2} yr^{-1}, which after applying the Redfield ratio (Redfield, 1958) amounts to a phytoplankton N demand of 7.5 moles N m^{-2} yr^{-1}. We estimate that DNRA provides 5% of the N demand by primary producers.

Inputs of nutrients to the estuary stimulate phytoplankton growth leading to bloom events that will result in the delivery (deposition) of phytoplankton C to the benthos that once mineralized leads to further nutrient release to the water column promoting water column primary production. The balance between N and P supply to the water column is a dominant factor shaping the phytoplankton community and has been implicated in blooms of harmful algae (Glibert et al., 2005). The role of DNRA in supplying bioavailable N to the water column and as a factor shaping the phytoplankton community composition remains to be determined. Because DNRA remains a significant process even in the presence of elevated NO_3^- concentrations in these sulfidogenic sediments, if management decisions do not lead to a reduction of nutrient inputs to this estuary, it is conceivable that higher inputs of nutrients to Weeks Bay will contribute to the initiation and retention of algal blooms (An and Gardner, 2002) and subsequent deposition of organic matter to the sediments, degradation of which will lead to more hypoxic events (Pinckney et al., 2001) and fish kills in this and similarly impacted ecosystems.

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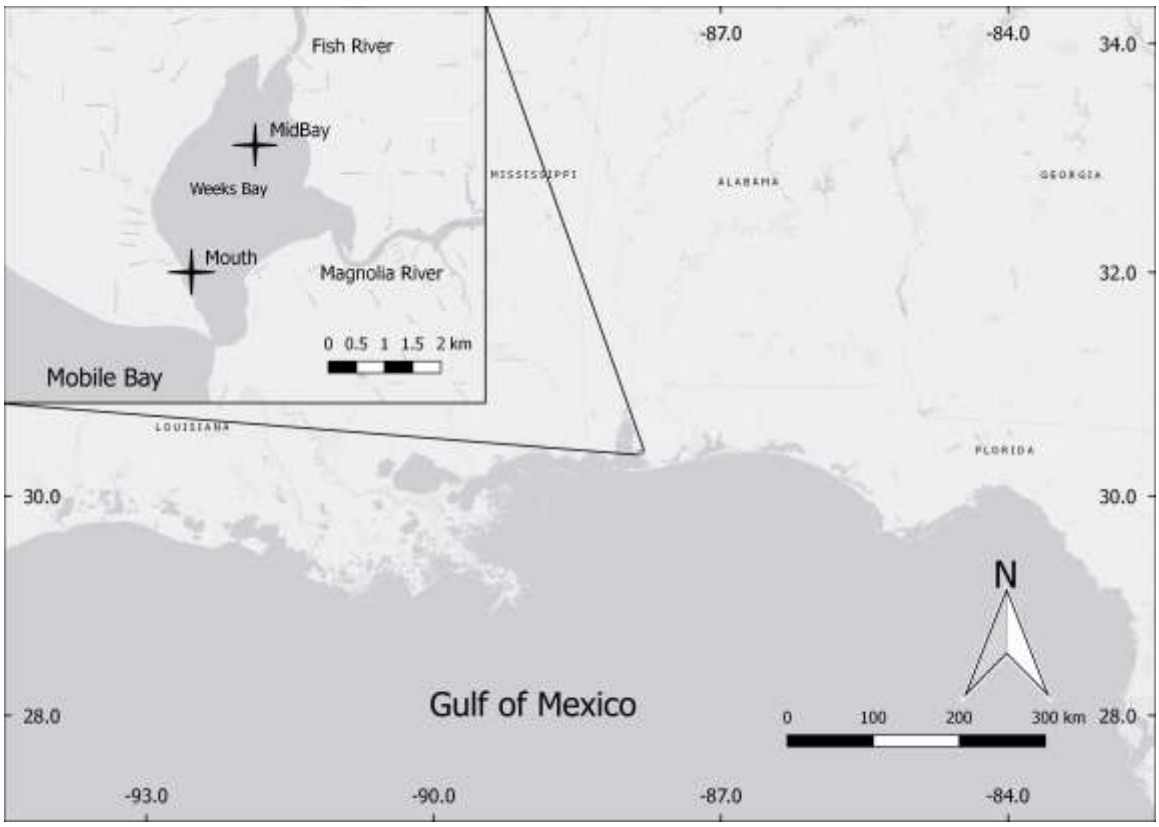


Fig.1
Map of study area at Weeks Bay, AL showing study sites (Mouth and MidBay)

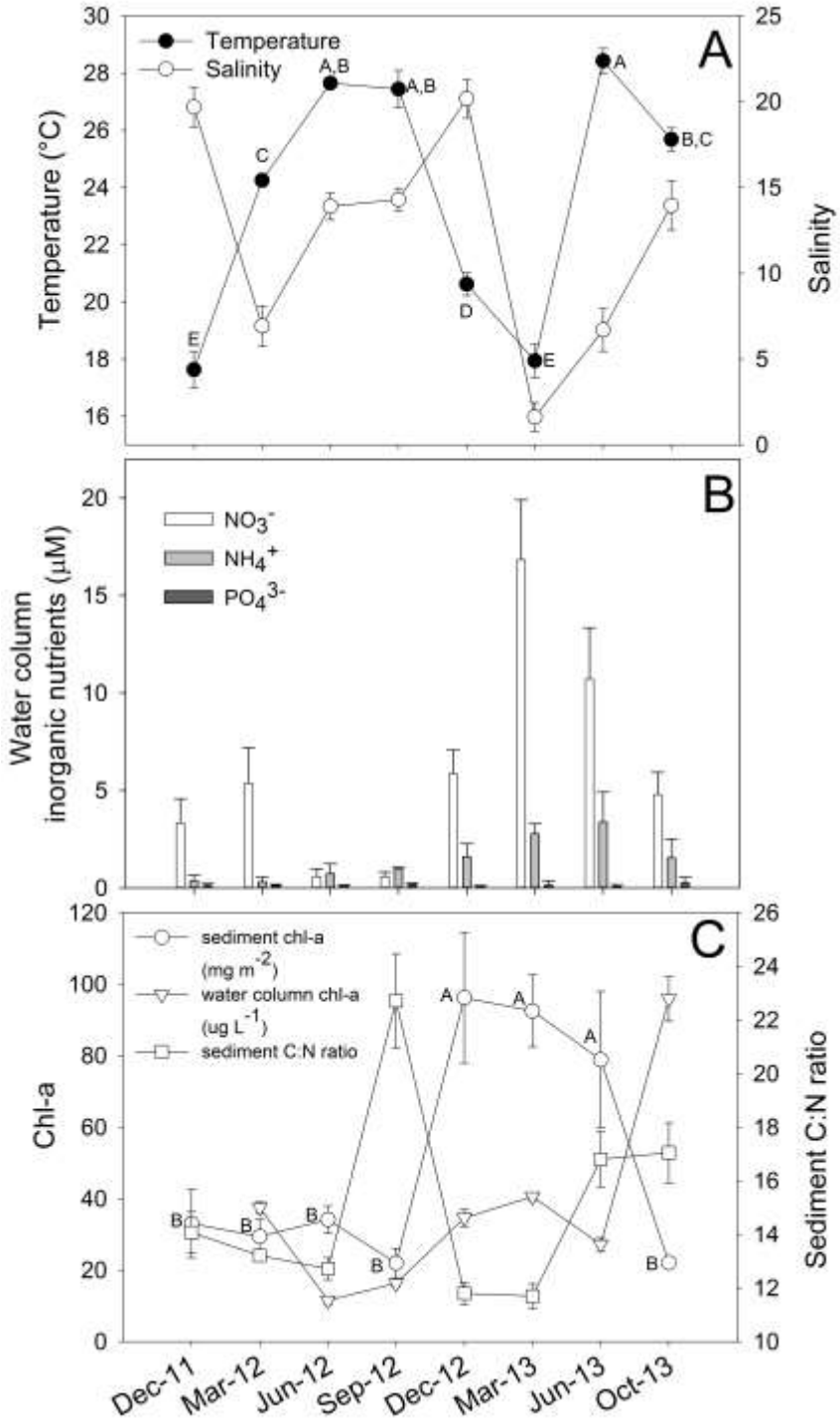


Fig.2 (A) Average values of the two sites for water temperature and salinity from the sites in Weeks Bay. Letters indicate significant seasonal differences for temperature; salinity was not statistically seasonally different. (B) Average values of the two sites for water column inorganic nutrients (n=3 each site). Water column nutrient concentrations did not differ between site nor season during the study. (C) Average values of sediment chlorophyll-a (mg m^{-2}) and water column chlorophyll-a (ug L^{-1}) and sediment C:N ratio. Letters indicate significant seasonal differences for sediment chlorophyll-a, while water column chlorophyll-a and sediment C:N were not statistically seasonally different. Error is reported as ± 1 SE

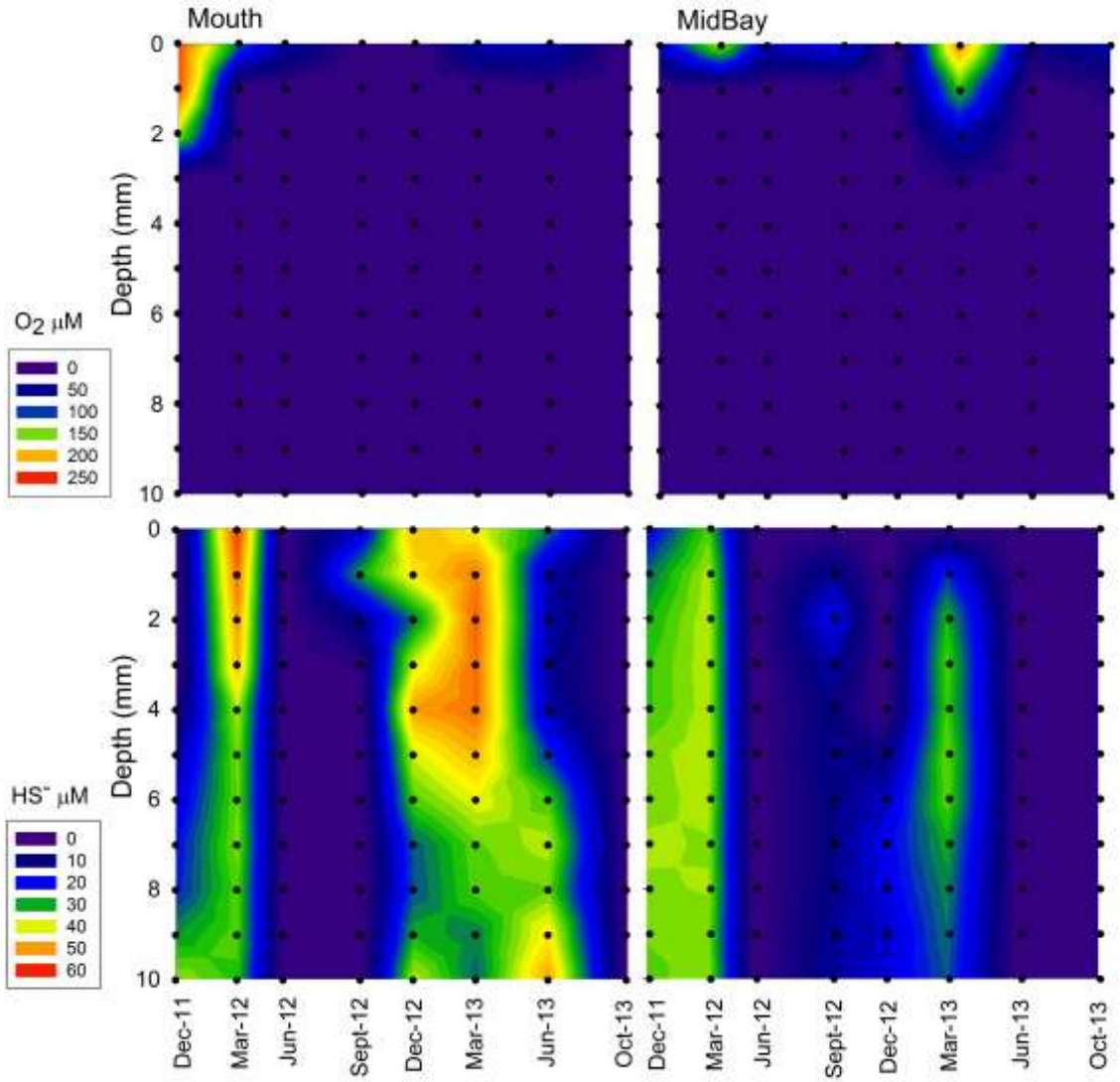


Fig.3 Oxygen (top panel) and hydrogen sulfide (bottom panel) concentrations (µM) in the top 1 cm of sediment at Mouth and MidBay. Note the differences in scale

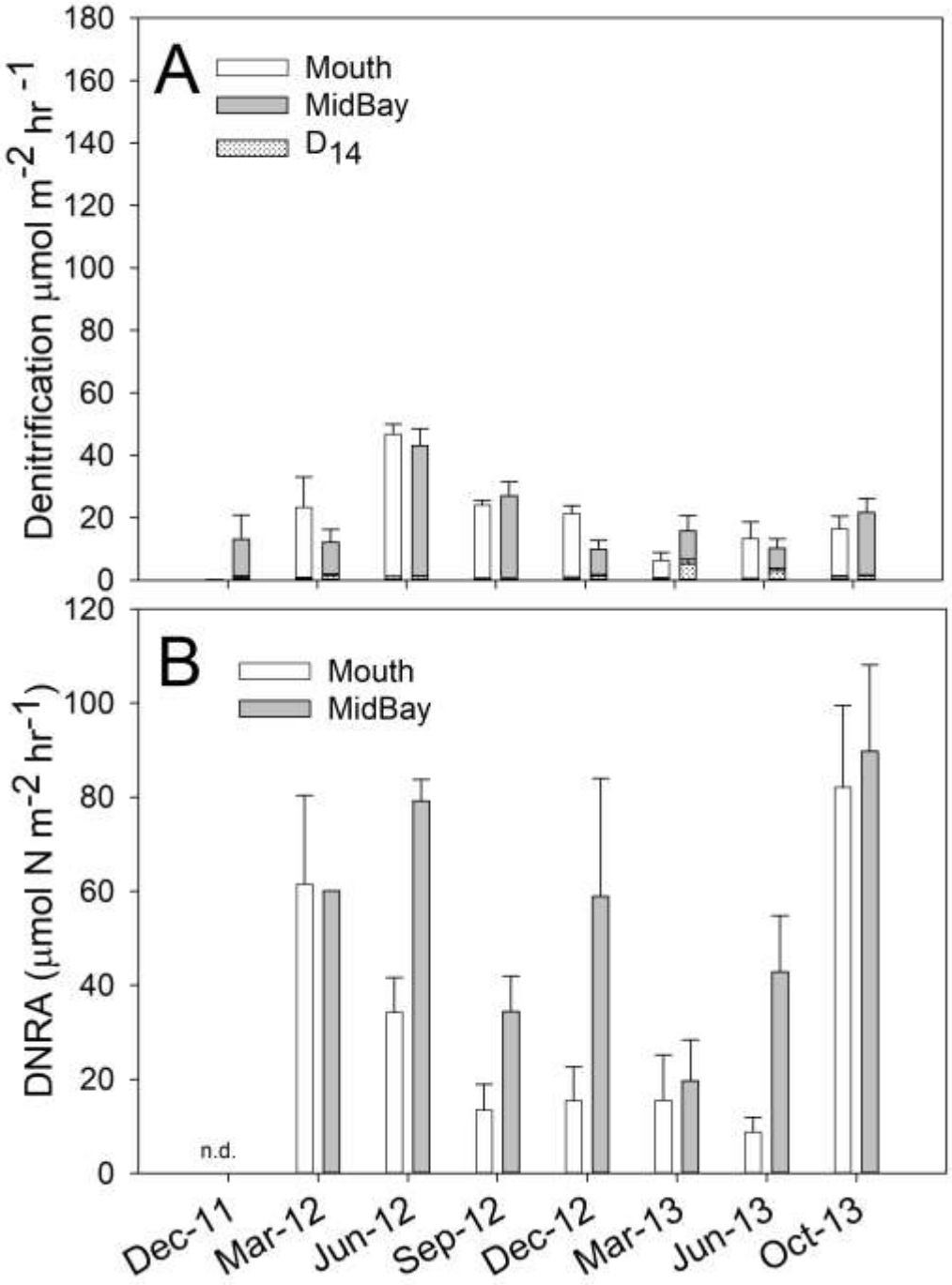


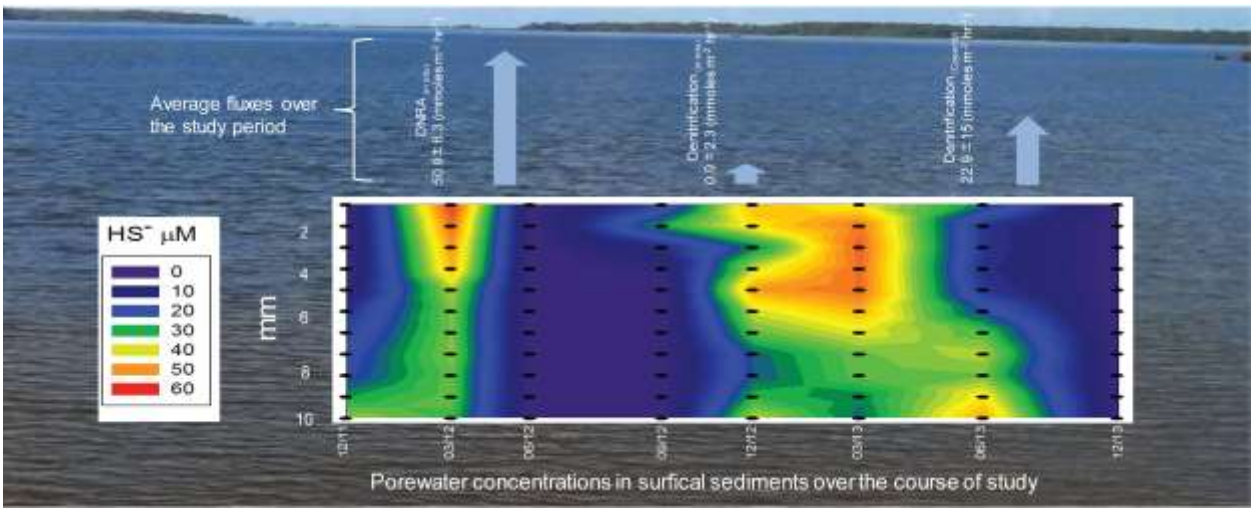
Fig.4 (A) The system capacity for denitrification at the Mouth (white bar) and MidBay (gray bar) with D_{14}, in situ denitrification (dotted bars), (n=5). (B) DNRA at the Mouth (white bar) and MidBay (gray bar). DNRA rates were significantly higher at MidBay than at the Mouth. Error bars are ± 1 SE

Table 1 Eigenvector values from the principal components analysis. Bolded values had strongest relationships.

Eigenvector	**Principal 1 (35.4%)**	**Principal 2 (22.9%)**
Temperature (°C)	-0.1993	**0.4344**
Salinity	**-0.3524**	-0.2192
DO (mg L^{-1})	0.2262	**-0.5064**
pH	-0.3424	0.1394
Water column NO_2^- (µM)	**0.4494**	0.0902
Water column NO_3^- (µM)	**0.4372**	0.1758
Water column NH_4^+ (µM)	**0.3728**	0.2653
Water column PO_4^{3-} (µM)	-0.0902	0.2752
Water column chl-a (mg m^{-2})	0.3461	-0.1879
Highest sediment HS^- (µM)	0.0875	**0.5167**

Table 2. Average site % DNRA contribution to N reduction with ± 1 SE in parentheses.

Date	Average % DNRA to N reduction
Dec. 2011	N/A
March 2012	77% (0.1)
June 2012	56% (0.2)
Sept. 2012	48% (0.2)
Dec. 2012	70% (0.3)
March 2013	62% (0.2)
June 2013	69% (0.3)
Oct. 2013	82% (0.1)
Study Average	66%


Average fluxes over the study period
DNRA
Denitrification
Denitrification
HS⁻ µM
0
10
20
30
40
50
60
mm
2
4
6
8
10
12/11
03/12
06/12
09/12
12/12
03/13
06/13
12/13
Porewater concentrations in surfical sediments over the course of study