Benthic Nitrogen Dynamics in Response to Deltaic Succession, Anthropogenic Fertilization and Hurricane Events in an Emerging Coastal Deltaic Floodplain within the Mississippi River Delta Plain

Song Li
Louisiana State University and Agricultural and Mechanical College

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BENTHIC NITROGEN DYNAMICS IN RESPONSE TO DELTAIC SUCCESSION, ANTHROPOGENIC FERTILIZATION AND HURRICANE EVENTS IN AN EMERGING COASTAL DELTAIC FLOODPLAIN WITHIN THE MISSISSIPPI RIVER DELTA PLAIN

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

In

The Department of Oceanography and Coastal Sciences

by

Song Li
B.S., Liaocheng University, 2012
M.S., Ocean University of China, 2016
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ABSTRACT

Increasing concentrations of nitrogenous fertilizer (NO$_3^-$) in rivers have potentially altered benthic nitrogen (N) cycling in coastal deltaic floodplains at the continental margins of major rivers. Here I evaluated the N removal capacity of a newly emergent coastal deltaic floodplain [Wax Lake Delta (WLD)] within the Mississippi River Delta Plain using continuous flow-through incubations. I investigated the response of benthic N dynamics in WLD to deltaic succession, anthropogenic fertilization and hurricane events. The estimated annual NO$_3^-$ removal of 896 Mg N yr$^{-1}$ in WLD accounts for 10 to 27% of total NO$_3^-$ load to WLD, most of which is converted to N$_2$ through denitrification. WLD is a continuously emerging ecosystem where the capacity of N removal increases by 0.2 to 2% per year prior to riverine NO$_3^-$ export to coastal ocean. Organic matter content increased from younger to older deltaic sediments from subtidal to supratidal hydrogeomorphic zones, which were coupled with increasing benthic NO$_3^-$ uptake and N$_2$ production. Direct denitrification, as the dominant N pathway in WLD, was one to two magnitudes larger than coupled nitrification-denitrification, dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonium oxidation (anammox). Increased NO$_3^-$ loading due to anthropogenic fertilization enhanced denitrification rates but inhibited heterotrophic N$_2$ fixation rates. N$_2$ fixation was comparable to denitrification under historically lower NO$_3^-$ concentrations (10 µM) and less than 32% of N removed via denitrification was from riverine NO$_3^-$ loading. In contrast, currently higher overlying NO$_3^-$ concentration (100 µM) makes denitrification the dominated benthic N pathway compared to N$_2$ fixation and over 61% of the removed N via denitrification was from riverine NO$_3^-$ loading. Hurricane Barry generated a decrease of
denitrification, enhanced proportion of DNRA to total NO$_3^-$ loss and increased NH$_4^+$ effluxes in most experimental sites in WLD. These hurricane effects lead to a decreased capacity of coastal deltaic floodplain to process eutrophic riverine water.

This research highlights the increasing capacity of an emerging coastal deltaic floodplain in processing elevated riverine NO$_3^-$ and clarifies the contribution of denitrification, N$_2$ fixation, DNRA and anammox to benthic N dynamics at different stages of deltaic development and anthropogenic NO$_3^-$ loading.
CHAPTER 1. INTRODUCTION

Nitrogen (N), as a necessary building block for organic molecules in primary productivity, can cause harmful ecological effects at higher concentrations (Damashek and Francis 2018). The vast majority of N on Earth exists as non-reactive dinitrogen gas (N$_2$) whereas fewer N$_2$ (0.002%) could be converted to bio-reactive N (nitrate, nitrite, and ammonium) through natural biochemical process in pre-industrial periods (Howarth 2008; Reddy and DeLaune 2008; Schlesinger and Bernhardt 2013; Damashek and Francis 2018). However, advanced industrial methods anthropogenically converted dinitrogen gas (N$_2$) to bio-reactive N for agricultural fertilization in the early 20$^{th}$ century, causing a two to four-fold increase in bio-reactive N concentrations since 1960 in estuarine and coastal ecosystems in North America (Goolsby et al. 2000b; Rabalais et al. 2002; Howarth et al. 2002). Elevated bio-reactive N in coastal waters stimulates phytoplankton and algal blooms, depletes water dissolved oxygen concentrations, decreases water quality and reduces biodiversity in coastal ecosystems (Paerl et al. 2002; Rabalais et al. 2002; Diaz 2008).

Increasing concentrations of nitrogenous fertilizer (mainly NO$_3^-$) in rivers over the last several decades have potentially altered benthic N cycles in coastal deltas (Broussard and Turner 2009; Henry and Twilley 2014). Before industrial production of bio-reactive N for agricultural use, coastal deltas were important sinks of atmospheric N$_2$ gas and source of bio-reactive N (Delwiche 1970; Galloway et al. 1995; Vitousek et al. 1997; Galloway 1998). Today deltas are considered an important sink of riverine bio-reactive N as a function of connectivity between river channels, benthic zone, and adjacent wetlands (Lane et al. 2002; Henry and Twilley 2014; Krause et al. 2017). The shift of N fluxes from a source to a sink in
coastal deltas is a result of the balance among several important N processes like N₂ fixation, denitrification, coupled nitrification denitrification and anaerobic ammonium oxidation (anammox; Fig. 1.1).

N₂ fixation is performed by diazotrophic bacteria that break down the triple bond in N₂ and fix N into ammonia using the nitrogenase (nifH) enzyme in concert with other cofactors and enzymes (Postgate 1970; Klotz and Stein 2008). Denitrification is categorized as ‘direct denitrification’ that reduces external nitrate to N₂ gas and ‘coupled nitrification-denitrification’ that uses NO₃⁻ generated by in situ nitrification as electron acceptor. Dissimilatory nitrate reduction to ammonium (DNRA), as a process that converts NO₃⁻ to more bio-reactive NH₄⁺, is reported to be a major N pathway in saline coastal and estuarine ecosystems (Gardner et al. 2006; Koop-Jakobsen and Giblin 2010; Bernard et al. 2015). Anammox that converts NH₄⁺ and NO₂⁻/NO₃⁻ to N₂ was firstly discovered in 1990s in wastewater treatments and, thereafter, reported to contribute substantially to N removal in some marine, coastal and estuarine sediments (Thamdrup and Dalsgaard 2002; Kuypers et al. 2003; Trimmer et al. 2003; Damashek and Francis 2018).
Benthic N cycles are complex in coastal wetlands as different processes are influenced by different environmental factors and interact with each other under different conditions. For example, N₂ fixation rates are related to the availability of dissolved inorganic N (DIN = NH₄⁺ + NO₃⁻ + NO₂⁻) and organic carbon (Howarth 1988; Fulweiler et al. 2007; Scott et al. 2008; Dias et al. 2012). N₂ fixation can be significantly repressed when NH₄⁺ concentration is higher than 1 mM in the rhizosphere of seagrasses (Welsh et al. 1997; Welsh 2000) or when NO₃⁻ concentration is higher than 10 µM in the ocean (Mulholland et al. 2001). However, the repression is not universal as many heterotrophic diazotrophic bacteria are not sensitive to increasing concentrations of DIN (McCarthy et al. 2016). Long-term denitrification studies indicate that denitrification is positively related to NO₃⁻ loading, and denitrification can be closely linked to sediment organic matter (SOM) as higher SOM concentration results in depleted oxygen which favors denitrification while inhibits nitrification (Cornwell et al. 1999; Piña-Ochoa and Álvarez-Cobelas 2006; Eyre and...
Ferguson 2009; Scaroni et al. 2011; Henry and Twilley 2014; Li et al. 2020). Recent studies report that DNRA is more competitive than denitrification under highly labile organic carbon and low overlying NO$_3^-$ availability in estuarine and coastal ecosystems (Megonigal et al. 2004; Algar and Vallino 2014; Hardison et al. 2015; Peng et al. 2016). The response of anammox to SOM concentration differs in different ecosystems, as some research found that anammox was positively correlated with SOM in estuaries (Brin et al. 2014; Lisa et al. 2014), some reported that anammox was more favorable at low SOM in some estuarine and coastal sediments (Engström et al. 2005a; Algar and Vallino 2014; Plummer et al. 2015), while several studies indicated that anammox was independent of SOM in some coastal marine sediments (Trimmer and Nicholls 2009).

Coastal deltaic floodplains, as the deposition zone where major rivers reach the ocean, demonstrate significant capacity to remove bio-reactive N from river waters prior to transport to nearshore waters (Henry and Twilley 2014; Twilley et al. 2019). N removal capacity in an emerging coastal deltaic floodplain may be associated with delta development and vegetation succession (Baldwin and Mitchell 2002; Henry and Twilley 2014). Active coastal deltaic floodplains that continuously receive riverine sediments and freshwater illustrate unique landscape feature of annual progradation. During early delta development, mineral sedimentation from riverine input increases soil surface elevation, transforming subaqueous delta to emergent ecosystems (Cahoon et al. 2011; Ma et al. 2018; Twilley et al. 2019). Once subaerial emergence, deltaic floodplains are colonized by vegetation during growing season, which stimulate SOM accumulation (Bevington and Twilley 2018). Both mineral sedimentation and organic production in deltaic wetlands increase soil surface elevation and
slowly shift subtidal hydrogeomorphic zones to supratidal hydrogeomorphic zones. Delta vegetation enhances depositional processes as vegetation roots decrease soil erosion, vegetation production contributes SOM to soil volume and dense vegetation traps suspended sediments (Gyssels et al. 2005; Baustian et al. 2012; Bevington and Twilley 2018; Ma et al. 2018; Larsen 2019; Twilley et al. 2019). The increased elevation of emerging delta islands drives ecological succession from simple submersed aquatics to emergent herbaceous vegetation to woody vegetation communities (Cahoon et al. 2011; Bevington and Twilley 2018). The ecological succession associated with SOM accumulation changes N removal capacity in freshwater deltaic floodplains (Henry and Twilley 2014). Nevertheless, the variation of each individual N pathway in response to morphological development and ecological succession during the early stages of delta development has not been well defined.

In addition to morphological development and vegetation succession with delta age, coastal deltaic floodplains in the Mississippi River Delta Plain experience frequent disturbance from natural climatic events like tropical storms and hurricanes. The episodic and energetic events of hurricane alter wetland hydrodynamics, vegetation coverage and sedimentation, which may further impact the capacity of coastal deltaic floodplains in processing riverine pollutions and nutrients (Michener et al. 1997; Davis et al. 2004; Turner et al. 2006; Deng et al. 2010; Liu et al. 2014; Wang et al. 2016). The responses of coastal wetlands to hurricane effects are diverse and complex, which are related to not only the trace and magnitude of a hurricane event, but also the morphological and ecological characteristics of disturbed wetlands (Davis et al. 2004; Wang et al. 2016). Firstly, the disturbed delta may decrease SOM concentrations at the sediment-water interfaces due to allochthonous mineral
sedimentation from hurricanes (Walker 2001; Turner et al. 2006; Castañeda-Moya et al. 2010; Bevington et al. 2017). Also, saltwater intrusion and salt spray during a hurricane event build higher salt stress to vegetation and causes substantial plant mortality (Chabreck and Palmisano 1973; Blood et al. 1991; Michener et al. 1997; Lacoul and Freedman 2006; Wang et al. 2016). Increasing fluxes of labile organic carbon and nutrients leaching from dead vegetation and litter to overlying water columns, which may alter benthic N cycling and impact N removal ability (Michener et al. 1997; Davis et al. 2004). However, the response of coastal deltaic floodplains to hurricane events in terms of benthic N cycles is unclear as there is a lack of research on hurricane effects on benthic N dynamics in coastal deltas.

The overall objective of this research is to clarify the role of coastal deltaic floodplains in processing riverine nutrients in response to deltaic succession, anthropogenic fertilization and hurricane effects. To achieve this goal, I firstly evaluated the seasonal variation of benthic fluxes and special patterns of nutrients fluxes as a function of hydrogeomorphology and delta age. Then I investigated each individual N process and the interaction of these N processes responding to increasing NO$_3^-$ loading and elevated SOM matter concentrations associated with anthropogenic fertilization and deltaic succession. In addition, hurricane effects on benthic N cycling were explored to understand the short-term response of coastal deltas to extreme climatic events. The detailed dissertation outline is listed below:

Chapter 2 explores spatial and seasonal variations of benthic fluxes in response to developing hydrogeomorphic zones along a chronosequence of the emerging Wax Lake Delta (WLD) within the Mississippi River Delta Plain. A continuous flow-through system with
intact sediment core incubation was applied to test the hypothesis that benthic fluxes will increase spatially in a coastal deltaic floodplain as sediment organic matter increases from subtidal to supratidal hydrogeomorphic zones from younger to older deltaic soils. Annual rates of N removal across WLD were estimated based on observed seasonal NO$_3^-$ fluxes, inundation time and seasonal temperature. I compared the estimates of denitrification using stoichiometry of benthic fluxes with direct measures of net denitrification to test assumptions of denitrification rates using different methodologies in deltaic environments.

Chapter 3 evaluates the relative importance of denitrification, DNRA and anammox at hydrogeomorphic zones with three distinct concentrations of SOM in WLD. Coupled nitrification-denitrification rates were measured to determine its significance to gross denitrification and total N$_2$ loss under different conditions of SOM concentration. Also, I manipulated two NO$_3^-$ concentrations (present ambient and a reduced concentration) to test how decadal changes in river fertilization may change benthic N cycling in coastal deltaic floodplains.

Chapter 4 focuses on heterotrophic N$_2$ fixation and its correlation with denitrification as functions of elevated SOM due to ecological succession as well as increased NO$_3^-$ loading due to agricultural fertilization in WLD. Heterotrophic N$_2$ fixation rates were measured directly using continuous flow-through incubations with $^{30}$N$_2$ addition. In addition, $nifH$ functional gene was quantified and $\delta^{15}$N of total N was measured in sediment cores after incubation to support the occurrence of N$_2$ fixation.

In chapter 5, I investigate short-term hurricane effects on benthic N cycling in the WLD using intact sediment core incubations with $^{15}$NO$_3^-$ enrichment before versus post
Hurricane Barry in 2019. Direct denitrification, coupled nitrification-denitrification and DNRA rates were measured 25 days post Hurricane Barry and compared to the corresponding rates from Chapter 3. The changes of benthic biogeochemical characteristics (salinity, bulk density, chlorophyll $a$ and organic and inorganic nutrients) one month prior compared to post Hurricane Barry were explored to test hurricane effects on soil properties, biotic communities and benthic nutrient fluxes.

Chapter 6 summarizes the response of different benthic N processes to deltaic succession, anthropogenic fertilization and hurricane events in the young tidal freshwater ecosystem of WLD. I highlighted the significance of these research findings and analyze the implications of these findings to delta restoration. In addition, I discuss unsolved questions and future research directions at the end.
CHAPTER 2. BENTHIC FLUXES OF DISSOLVED OXYGEN AND NUTRIENTS ACROSS HYDROGEOMORPHIC ZONES IN A COASTAL DELTAIC FLOODPLAIN WITHIN THE MISSISSIPPI RIVER DELTA PLAIN

INTRODUCTION

The application of nitrogen (N) fertilizers to agricultural fields has increased by about 800% over the last several decades, but agricultural N use efficiency has remained below 40% (Fixen and West 2002; Canfield et al. 2010). As a result, significant amounts of inorganic N leach into rivers, causing a two to four-fold increase in nitrate (NO$_3^-$) concentrations since 1960 in estuarine and coastal ecosystems in North America (Goolsby et al. 2000b; Rabalais et al. 2002; Howarth et al. 2002). Meanwhile, coastal phosphorus (P) concentrations have increased due to anthropogenic fertilization and industrial wastewater (Conley et al. 2009). The elevated loads of inorganic N and P enhance coastal net primary productivity, stimulate harmful algal blooms, decrease water quality, and exacerbate hypoxia (oxygen depletion < 2 mg L$^{-1}$ in bottom water) (Rabalais et al. 2002; Paerl et al. 2002; Diaz 2008).

Elevated inorganic nutrient concentrations in river basins have stimulated research on how patterns of denitrification in alluvial floodplains may reduce the potential for eutrophication of downstream ecosystems (Goolsby et al. 2000; Noe and Hupp 2009; Jordan et al. 2011). Less information is available for the significance of coastal deltaic floodplains in reducing eutrophication at continental margins with major rivers. Coastal deltaic floodplains

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form in the deposition zone where major rivers reach the ocean, representing wetlands at the interface of land and oceans (Henry and Twilley 2014; Hiatt and Passalacqua 2015; Bevington and Twilley 2018). Even though coastal deltaic floodplains are also depositional environments connected to rivers as alluvial floodplains, multiple factors like tides, waves, tropical storms and meteorological fronts make the geomorphology and connectivity (daily and seasonal inundation) between channels and coastal deltaic floodplains more complex in comparison to alluvial floodplains (Twilley et al. 2019). The morphological development of coastal deltaic floodplains results in spatial and temporal differences in elevation, vegetation, and sediment organic matter content (Lorenzo-Trueba et al. 2012; Carle 2013; Bevington and Twilley 2018). Soil elevations of different hydrogeomorphic zones favor specific vegetation communities and generate different rates of organic matter accumulation (Bevington and Twilley 2018).

Recent research reported that patterns of hydrogeomorphology along longitudinal zones of major river systems control the processing of NO$_3^-$ in alluvial floodplains (Clawson et al. 2001; Noe and Hupp 2005; Welti et al. 2012; Noe et al. 2013). However, the influence of hydrogeomorphic zones in processing nutrients in a coastal deltaic floodplain has not been resolved compared with alluvial floodplain ecosystems. As hydrogeomorphic zones age during active deltaic development, benthic ecological and biogeochemical processes may change, representing a chronosequence of ecosystems (Twilley et al. 2019). Previous work in our research group indicated that benthic nutrient fluxes did vary along the deltaic chronosequence, but these findings did not clearly differentiate the influence of hydrogeomorphic zones (Henry and Twilley 2014). More information on landscape patterns
of benthic fluxes is required to further understand the role of active coastal deltaic floodplains in permanent NO$_3^-$ removal in response to both the chronosequence and hydrogeomorphic variations.

Denitrification is an ecologically important pathway to permanently remove bioreactive N under anaerobic condition (Cornwell et al. 1999; Piña-Ochoa and Álvarez-Cobelas 2006; Eyre and Ferguson 2009; Scaroni et al. 2011; Henry and Twilley 2014). Denitrification is categorized as ‘direct denitrification’ that reduces external NO$_3^-$ to N$_2$ gas and ‘coupled nitrification-denitrification’ that uses NO$_3^-$ generated by in situ nitrification. Determination of denitrification is impeded by methodological difficulties from high background N$_2$ concentrations and large spatial and temporal heterogeneity of N$_2$ production in coastal ecosystems (Cornwell et al. 1999; Davidson and Seitzinger 2006). Previous research on benthic nutrient fluxes estimated denitrification indirectly based on a stoichiometric assumption that the molar organic carbon (C):oxygen (O) and N:P ratios of sediment fluxes should follow Redfield composition (C:O:N:P=106:138:16:1). A discrepancy between measured O:N:P ratio of these elements exchanged at the sediment-water interface compared to the expected Redfield ratio may reflect the occurrence of benthic denitrification (Nielsen 1992; Kana et al. 1994; Gilbert et al. 1997; Cornwell et al. 1999). Recent improvements in direct techniques such as N$_2$:Ar method and isotope pairing technique have demonstrated the significance of benthic fluxes to N$_2$ production (Seitzinger et al. 1984; Gardner and McCarthy 2009; Henry and Twilley 2014). The direct measurements of benthic denitrification are reported to be correlated with indirect estimates of denitrification using stoichiometric assumption in Massachusetts Bay (Giblin et al. 1995, 1997). However, the correlation of
estimated benthic denitrification rates with directly measured rates is not well clarified in most coastal regions. To get a general idea of the relationship between benthic dissolved inorganic N (DIN = NH$_4^+$ + NO$_3^-$ + NO$_2^-$) fluxes and denitrification rates, it is important to investigate if the estimated denitrification rates from stoichiometric assumptions give comparable rates as directly measured denitrification rates in coastal ecosystems.

Here, we compared the spatial and seasonal patterns of nutrient processing in coastal deltaic floodplains as a function of hydrogeomorphology and delta age since emergence. We hypothesized that benthic fluxes of dissolved oxygen (O$_2$), N$_2$ and inorganic nutrients will increase in a newly emergent coastal deltaic floodplain from lower to higher hydrogeomorphic zones and from younger to older chronosequence within each hydrogeomorphic zone. We used seasonal measures of benthic fluxes and denitrification rates across hydrogeomorphic zones with different hydroperiods (duration of inundation per year) to estimate the annual NO$_3^-$ removal and N$_2$ release in a coastal deltaic floodplain at the mouth of the Atchafalaya River. We compared the estimates of denitrification using stoichiometry of benthic fluxes with direct measures of N$_2$ production to test assumptions of denitrification rates using different methodologies in deltaic environments (Rivera et al. 2010). This analysis will describe the role of newly emergent deltaic floodplains in NO$_3^-$ removal from riverine loading compared to other ecosystems of the Mississippi River Delta Plain.
METHODS

Experimental Design

Wax Lake Delta (WLD) is an emergent coastal deltaic floodplain in the Atchafalaya Coastal Basin within the Mississippi River Delta (Fig. 2.1). The U.S. Army Corps of Engineers constructed the Wax Lake Outlet (WLO) in 1941 to divert flow from the Atchafalaya River and provide flood relief to Morgan City, Louisiana. Riverine sediments carried by WLO began to form a subaqueous delta at the outlet mouth upon completed construction. In 1973, an unusually high spring flood resulted in a large sediment deposition and WLD became subaerial (Roberts et al. 2003). Deltaic islands emerged rapidly throughout the next several decades at a rate of about 1 to 5 km² yr⁻¹ with a minor anthropogenic influence from navigation (Roberts et al. 1997; Wellner et al. 2005; Allen et al. 2012; Carle et al. 2015). WLD provides a natural laboratory to study the ecological succession and biogeochemistry of a coastal deltaic floodplain and to test the hypotheses presented concerning chronosequence, elevation and sediment organic matter content (Henry and Twilley 2014; Bevington and Twilley 2018).
Figure 2.1. Map of Mike Island in Wax Lake Delta (WLD), Louisiana, with the location of study sites. Elevation records are from USGS Atchafalaya 2 project LiDAR Survey 2012 digital elevation model (4m resolution). The black lines across Mike island delineate four chronosequence transects (from younger to older: T4 to T1) mainly defined by the distance to apex of Mike Island and the characterization of cross-sectional morphology (Bevington and Twilley 2018). Three hydrogeomorphic zones (subtidal, intertidal, and supratidal) are distinguished by sediment surface elevation relative to mean high water (MHW) and mean low water (MLW). White square in WLD indicates the location of CRMS 0479 station established by the Coastal Resources Monitoring System (CRMS) to measure water temperature.

Experimental sites were selected on Mike Island (Fig. 2.1), a well-developed island of WLD, to test the hypotheses of benthic fluxes with hydrogeomorphology. Three
hydrogeomorphic zones were defined on the basis of sediment surface elevations from USGS Atchafalaya 2 Project LiDAR Survey 2012 (4m resolution, Bevington and Twilley 2018). Area with sediment surface elevation lower than mean low water (MLW, -0.04 m NAVD 88) is a subtidal zone while area with elevation higher than mean high water (MHW, 0.30 m NAVD88) is a supratidal zone. Area with sediment surface between MLW and MHW is an intertidal zone. The area of each hydrogeomorphic zone was calculated based on a digital elevation model (DEM) of Mike Island (Bevington and Twilley 2018). The subtidal zone is the dominant hydrogeomorphic zone on Mike Island followed by the intertidal zone and the supratidal zone. Subaqueous areas below -0.75 m NAVD were excluded. The subtidal zone has a molar organic carbon to total nitrogen (C:N) ratio of 9.7 in the top 4 cm of soil, whereas the supratidal and intertidal zones show larger C:N ratios of 11.6.

The chronosequence zones along Mike Island were defined based on a conceptual model from Bevington and Twilley (2018). Briefly, island morphology and elevation development are controlled primarily by island age. Four chronosequence zones were thus determined depending on the distance to delta apex and the characterization of cross-sectional morphology (Bevington and Twilley 2018). Transect I (T1) is ≥ 35 years since emergence and is dominated by the supratidal zone with higher elevations (≥ 0.3 m), while transect II (T2) is estimated at 20-35 years since emergence and dominated by the intertidal zone. Transects III and IV (T3 and T4) are younger zones (≤ 20 yr since emergence) of Mike island dominated by the subtidal zone with a lower slope from levee ridge to the interdistributary bay.
Nine experimental sites were selected in three hydrogeomorphic zones (subtidal, intertidal and supratidal) along four chronosequence zones (Fig. 2.1 and Table 2.1). There was an uneven distribution of experimental treatments of hydrogeomorphic zones within the four chronosequence zones. For example, the intertidal zone was the only hydrogeomorphic zone that occurred in all chronosequence zones (T1-T4). The subtidal zone did not occur in the older region of Mike Island (T1) while the supratidal zone was mostly absent in the younger zones of Mike Island (T3 and T4). The assignment of experimental sites to a hydrogeomorphic zone were confirmed with elevations (NAVD 88) based on real-time kinematic (RTK) positioning using a Trimble R8 GNSS. Supratidal-T2 did not have available elevation measurement nearby, so its elevation was roughly estimated from a digital elevation model (m NAVD 88; LiDAR Survey 2012; Bevington and Twilley 2018).

Table 2.1. Site distributions and sampling dates in different hydrogeomorphic and chronosequence zones in Wax Lake Delta (WLD), Louisiana.

<table>
<thead>
<tr>
<th>Hydrogeomorphic</th>
<th>Chronosequence</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>T4</td>
</tr>
<tr>
<td></td>
<td>Younger</td>
</tr>
<tr>
<td>Supratidal</td>
<td>NA*</td>
</tr>
<tr>
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</table>

*No data available for these hydrogeomorphic and chronosequence combinations.

**Ambient Conditions**

Replicate porewater (4 cm depth) and surface water (within top 5 cm of the air-water interface) samples were collected at each experimental field site to capture sediment and...
overlying water characteristics of hydrogeomorphic and chronosequence zones. Water samples were stored on ice and filtered through GF/F glass microfiber filters (25 mm diameter, 0.7 µm particle retention) immediately upon arrival at the laboratory. Samples were stored in a freezer (-20 °C) until dissolved inorganic nutrients [ammonium (NH$_4^+$), nitrite (NO$_2^-$), nitrate (NO$_3^-$) and phosphate (PO$_4^{3-}$)] were determined on a flow solution IV autoanalyzer (OI analytical, College Station, Texas). Temperature and salinity of surface water and porewater were measured immediately after collection in the field with a YSI salinity-conductivity-temperature meter.

**Benthic incubations**

Triplicate sediment cores (10 cm internal diameter by 20 cm depth) were collected randomly in each of the nine experimental sites to measure nutrient fluxes at the sediment-water interface. Cores were collected during two different seasons representing cooler temperatures in spring (February and March 2018) and warmer temperatures in summer (May to August 2017). In summer 2017, supratidal-T1 was skipped for technical reasons, but it was collected in summer 2018. Aboveground vegetation was excluded when collecting intact sediment cores, but rhizomes, roots, infauna and benthic algae were included. Headspace over the sediment in each core was carefully adjusted to 10 ± 1 cm in height. The cores were sealed using silicone-greased bottoms, then gently filled with ambient water in the field. Cores were stored in a cooler under in situ temperature and transported back to the laboratory. Ambient water was collected from WLO at the Calumet boat launch, LA, representing river water flowing to WLD. Ambient water was then filtered using a five-stage filtration system (30, 20, 5, 1, and 0.2 µm) and used to gently replace overlying water inside
sediment cores with minimal disturbance to the sediment-water interfaces in the laboratory. Using filtered water during incubations excludes most microbial processes in the overlying water column and attributes changes in nutrient concentrations, dissolved $O_2$ and $N_2$ gases to benthic processes (Miller-Way and Twilley 1996). The top of each core was sealed with a lid containing inlet and outlet tubing and affixed with a magnetic stir bar to gently mix the overlying water. The sealed cores were incubated in a water chamber at controlled temperatures of either 12 °C for spring months or 22 °C for summer months (Table 2.3).

All the incubations were conducted in the dark with a continuous flow-through system (Miller-Way and Twilley 1996). Sediment cores were connected to water reservoirs through inlet tubing with a peristaltic pump controlling the flow rate at 2.4 ± 0.2 ml min$^{-1}$ in spring (residence time = 6h) and 4.6 ± 0.5 ml min$^{-1}$ in summer (residence time = 3h). The flow rate in each season was determined through previous experiments performed at the corresponding temperature in each season. We chose an overlying water residence time that was long enough to produce a measurable difference between influent and effluent concentrations, but short enough to support an aerobic condition with dissolved $O_2$ concentration $\geq$ 3 mg L$^{-1}$ in each season (Miller-Way and Twilley 1996). At the optimal and constant flow rate in each season, the continuous flow-through system achieved steady state conditions inside sediment cores (non-varying flux with time), which indicated representative benthic fluxes in each site (Miller-Way and Twilley 1996). Cores were pre-incubated for at least three turnover times of the overlying water in the cores (about 18 h in spring and 9 h in summer) to allow fluxes at the sediment-water interface to reach an equilibrium.
After the pre-incubation period, influent and effluent water was collected to measure inorganic nutrients, dissolved O$_2$ and N$_2$ gas concentrations at the completion of a single water residence time for a total of three turnovers per experiment. Two blank cores with only filtered ambient water were incubated for each experiment in each season to correct all possible interferences not related to changes in nutrient concentrations due specifically to benthic activities. Water samples were filtered immediately through 25 mm GF/F glass microfiber filters into triplicate vials (20 ml) and stored in a freezer (-20 °C) until analyzed for NH$_4^+$, NO$_2^-$, NO$_3^-$ and PO$_4^{3-}$ on the OI autoanalyzer. Replicate samples for dissolved gas analysis were collected in 12ml gas-tight exeters (Labco Limited, Lampeter, Wales, UK). After each exeter was filled, 200 µL of ZnCl$_2$ solution (50% of saturation concentration) was injected (Nielsen and Glud 1996), and the exeter was tightly capped immediately. The gas samples were then stored under water in a water bath at 4°C. Dissolved N$_2$ was measured in a membrane inlet mass spectrometer (MIMS) within one month (precision < 0.03%; Kana and others 1994). Benthic fluxes of inorganic nutrients and dissolved gas (N$_2$ and O$_2$) were determined by the equation:

$$\text{Flux} = \frac{[\text{Co} - \text{Ci}] - [\text{Cbo} - \text{Cbi}]}{\text{flow rate} \times \text{Core surface area}}$$

(EQ 1)

where Co and Ci (µM) refer to outflow and inflow concentrations of a sediment core while Cbo and Cbi (µM) are the average outflow and inflow concentrations of two blank cores in corresponding incubation events.

The top 4 cm of sediment in each core was sampled with a piston core (2.4 cm internal diameter) and segmented at 2 cm depth intervals. Each slice of sediment was oven-
dried at 60 °C to a constant mass and bulk density was measured by dividing dry sediment mass by sediment volume (8.75 cm³). Each dried sediment sample was ground to less than 250 µm in a Wiley Mill, and a 1 ± 0.01 g subsample was ignited at 550 °C for 2 h to estimate organic matter content.

**Scaling benthic fluxes to annual rates**

Incubations for cores sampled in early spring were controlled at 12 °C, compared to 22 °C for cores sampled in summer. The median of these two temperatures (17 °C) was used to define ambient water temperatures into either summer or spring seasons. The study area is in the warm temperate region with annual surface water temperatures ranging from 5.6 to 31.3 °C. Here we assumed the study area has only two seasons per year, spring and summer, when water temperatures are less than or no less than 17 °C, respectively. Annual spring days and summer days were calculated from daily water temperature measurements at station CRMS 0479 (29° 31.4' N, 91° 27.0' W) located in the intertidal zone of WLD, from May 2017 to April 2018 (recognized as an experimental year). There are several days showing undefined water temperature during this period. We used water temperature readings in the same dates, but one or two years ahead, to fill in these missing values. We assumed the supratidal and subtidal zones had the same water temperature as the intertidal zone when submerged. The hydroperiod for each experimental site (hours inundated per day) from May 2017 to April 2018 was based on continuous water level records from a tidal gauge near WLD (29° 27.0' N, 91° 20.3' W, Amerada Pass, LA). The difference between the sediment surface elevation of the tide gauge and the sediment surface elevation of an experimental site was compared to continuous water level records to determine daily inundation time in each
experimental site. Summer NO$_3^-$ and N$_2$-N fluxes were applied only if the site was inundated and, meanwhile, the overlying water was at or above 17 °C. Spring NO$_3^-$ and N$_2$-N fluxes were applied if the site was inundated and the overlying water was below 17 °C.

**Statistical analyses**

We used repeated measures analysis of variances (ANOVAs) to test the difference in nutrients, dissolved O$_2$ and N$_2$ fluxes among experimental sites in each of the sampling season. We used repeated measures for the three repeated sampling events of an individual core over time to confirm that each core achieved a steady state (no significant difference over time in each core) during the sampling period (Miller-Way and Twilley 1996). Seasonal differences were tested using one-way ANOVA by treating the results from the nine experimental sites in each season as a whole and ignoring site-to-site variations. Significant differences were also tested on the interaction between seasons and hydrogeomorphic zones with chronosequence results nested in each hydrogeomorphic zone using ANOVA. When differences were significant at a 95% confidence level, Tukey’s HSD post hoc test was used to do all pairwise comparisons and letters designated significant differences (p < 0.05). Data analyses were performed using SAS and JMP software. Benthic fluxes were presented as means with error bars of standard error (SE).

**RESULTS**

**Sediment properties and benthic fluxes**

WLD was a tidal freshwater system with surface water salinity ranging from 0.1 to 0.2 and porewater salinity ranging from 0.2 to 0.4 (Table 2.2). Ambient NO$_3^-$ concentrations of *in-situ* surface water varied from 0.1 to 50.8 µM in spring and from 0.1 to 84.1 µM in
summer. Site-to-site variations in overlying NO$_3^-$ concentrations demonstrated different NO$_3^-$ removal capacities from different experimental sites to process riverine nutrients from the same water source (WLO). Ambient NH$_4^+$ concentrations varied from 0.7 to 6.5 $\mu$M in surface water, which were lower than porewater NH$_4^+$ concentrations from 5.0 to 470.9$\mu$M in respective experimental site in each season. Ambient NO$_2^-$ and PO$_4^{3-}$ concentrations in surface water and porewater were generally low in all the experimental sites in the field.

Bulk density and organic matter content in the top 4 cm of sediment exhibited significant differences (F = 130.6, P < 0.001 and F = 120.9, P < 0.001, respectively) along the chronosequence of each hydrogeomorphic zone (Fig. 2.2), but no significant difference between seasons (Table 2.3). Chronosequence-averaged bulk density decreased while organic matter content increased as elevation increased from subtidal to supratidal zones in each season (Table 2.3). Bulk density decreased from 1.5 g cm$^{-3}$ to 0.3 g cm$^{-3}$ while organic matter content increased from 2.2% to 10.7% from younger (T4) to older (T2) chronosequence sites in the subtidal zone (Fig 2.2). Similar patterns were observed in the intertidal zone with bulk density decreasing from 1.56 to 0.40 g cm$^{-3}$ and organic matter increasing from 2.4 to 10.0% from the younger (T4) to the older (T1) sites. Trends in bulk density and organic matter in the two supratidal sites were different from those in the subtidal and intertidal zones. The supratidal-T1 (site near island apex) was assumed to be the older site but exhibited higher bulk density (0.9 g cm$^{-3}$) and lower organic matter content (8.5%) than the younger supratidal-T2 site (0.3 g cm$^{-3}$ and 18.2%, respectively).
Table 2.2 Ambient surface water and porewater conditions in spring and summer in experimental sites in Wax Lake Delta (WLD), Louisiana.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subtidal</th>
<th>Intertidal</th>
<th>Supratidal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>T3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>T2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>19.1</td>
<td>19.3</td>
<td>22.8</td>
</tr>
<tr>
<td>Surface Water</td>
<td>15</td>
<td>16.3</td>
<td>24.2</td>
</tr>
<tr>
<td>Salinity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Surface Water</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;⁻</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>25.7</td>
<td>6.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Surface Water</td>
<td>37.9</td>
<td>38</td>
<td>10.2</td>
</tr>
<tr>
<td>NO&lt;sub&gt;2&lt;/sub&gt;⁻</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>1.3</td>
<td>0.3</td>
<td>0</td>
</tr>
<tr>
<td>Surface Water</td>
<td>1.4</td>
<td>1.4</td>
<td>0.8</td>
</tr>
<tr>
<td>NH&lt;sub&gt;4&lt;/sub&gt;⁺</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>131.5</td>
<td>122.1</td>
<td>150.6</td>
</tr>
<tr>
<td>Surface Water</td>
<td>4.9</td>
<td>5.3</td>
<td>3.8</td>
</tr>
<tr>
<td>PO&lt;sub&gt;4&lt;/sub&gt;³⁻</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>0.5</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Surface Water</td>
<td>1.3</td>
<td>1.3</td>
<td>2.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>Supra-T1 and Supra-T2 had no surface water records because the water level was zero;
<sup>b</sup>Superscripts over the chronosequence refer to sampling dates: <sup>c</sup>=Feb 16, 2018; <sup>d</sup>=Mar 2, 2018; <sup>e</sup>=Mar 21, 2018; <sup>f</sup>=May 23, 2017; <sup>g</sup>=July 26, 2017; <sup>h</sup>=Aug 14, 2017; <sup>i</sup>=Jul 20, 2018.
### Summer

<table>
<thead>
<tr>
<th>Variable</th>
<th>Subtidal</th>
<th>Intertidal</th>
<th>Supratidal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T4\textsuperscript{f}</td>
<td>T3\textsuperscript{f}</td>
<td>T2\textsuperscript{e}</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>27</td>
<td>28.5</td>
<td>30.8</td>
</tr>
<tr>
<td>Surface Water</td>
<td>27.2</td>
<td>27.5</td>
<td>29.5</td>
</tr>
<tr>
<td>Salinity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Porewater</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Surface Water</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>NO\textsubscript{3}\textsuperscript{−} (µM)</td>
<td>38.7</td>
<td>4.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Surface Water</td>
<td>64.5</td>
<td>63</td>
<td>0.1</td>
</tr>
<tr>
<td>NO\textsubscript{2}\textsuperscript{−} (µM)</td>
<td>0.7</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Surface Water</td>
<td>0.3</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>NH\textsubscript{4}\textsuperscript{+} (µM)</td>
<td>23.9</td>
<td>112.7</td>
<td>92.5</td>
</tr>
<tr>
<td>Surface Water</td>
<td>1.6</td>
<td>0.7</td>
<td>1.6</td>
</tr>
<tr>
<td>PO\textsubscript{4}\textsuperscript{3−} (µM)</td>
<td>1.3</td>
<td>0.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Surface Water</td>
<td>2.6</td>
<td>2</td>
<td>6.3</td>
</tr>
</tbody>
</table>

*Supra-T1 and Supra-T2 had no surface water records because the water level was zero;

*Superscripts over the chronosequence refer to sampling dates: \textsuperscript{a}=Feb 16, 2018; \textsuperscript{b}=Mar 2, 2018;
\textsuperscript{c}=Mar 21, 2018; \textsuperscript{d}=May 23, 2017; \textsuperscript{e}=July 26, 2017; \textsuperscript{f}=Aug 14, 2017; \textsuperscript{g}=Jul 20, 2018.
Table 2.3. Characteristics of the three hydrogeomorphic zones (subtidal, intertidal and supratidal) in spring and summer based on chronosequence-averaged results in each hydrogeomorphic zone. ANOVA results are shown with superscript letters representing significant difference (p < 0.05) for a specific analyte among the two-way interactions of hydrogeomorphic zones and seasons using Tukey’s HSD test.

<table>
<thead>
<tr>
<th></th>
<th>Subtidal</th>
<th>Intertidal</th>
<th>Supratidal</th>
<th>Subtidal</th>
<th>Intertidal</th>
<th>Supratidal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spring</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bulk density (g cm(^{-3}))</td>
<td>0.9(^a)</td>
<td>0.9(^a)</td>
<td>0.6(^a)</td>
<td>1.0(^b)</td>
<td>0.8(^b)</td>
<td>0.6(^a)</td>
</tr>
<tr>
<td>Sediment organic matter (%)</td>
<td>5.9(^b)</td>
<td>6.6(^b)</td>
<td>12.4(^a)</td>
<td>5.3(^b)</td>
<td>6.3(^b)</td>
<td>14.4(^a)</td>
</tr>
<tr>
<td>O(_2) (g O(_2) m(^{-2}) d(^{-1}))</td>
<td>-0.8(^a)</td>
<td>-0.7(^a)</td>
<td>-1.2(^bc)</td>
<td>-1.4(^c)</td>
<td>-1.3(^c)</td>
<td>-2.6(^d)</td>
</tr>
<tr>
<td>N(_2)-N (µmol N m(^{-2}) h(^{-1}))</td>
<td>34.8(^c)</td>
<td>50.0(^c)</td>
<td>296.2(^b)</td>
<td>315.2(^b)</td>
<td>322.3(^b)</td>
<td>734.4(^a)</td>
</tr>
<tr>
<td>NO(_3)- (µmol m(^{-2}) h(^{-1}))</td>
<td>-15.0(^a)</td>
<td>-74.9(^ab)</td>
<td>-84.3(^ab)</td>
<td>-204.0(^ab)</td>
<td>-235.3(^b)</td>
<td>-916.4(^c)</td>
</tr>
<tr>
<td>NO(_2)- (µmol m(^{-2}) h(^{-1}))</td>
<td>0.8(^cd)</td>
<td>5.0(^cd)</td>
<td>-2.9(^d)</td>
<td>13.0(^bc)</td>
<td>18.3(^ab)</td>
<td>32.6(^a)</td>
</tr>
<tr>
<td>NH(_4)+ (µmol m(^{-2}) h(^{-1}))</td>
<td>42.2(^b)</td>
<td>52.5(^b)</td>
<td>-31.5(^c)</td>
<td>64.0(^ab)</td>
<td>96.3(^a)</td>
<td>33.7(^b)</td>
</tr>
<tr>
<td>PO(_4)(^3-) (µmol m(^{-2}) h(^{-1}))</td>
<td>2.0(^a)</td>
<td>-0.3(^a)</td>
<td>-0.8(^ab)</td>
<td>-7.5(^b)</td>
<td>-0.1(^a)</td>
<td>4.2(^a)</td>
</tr>
</tbody>
</table>

Benthic dissolved O\(_2\) fluxes ranged from -0.2 to -3.5 g O\(_2\) m\(^{-2}\) d\(^{-1}\) and fluxes in spring were lower than fluxes in summer (Fig. 2.3a). In spring, dissolved O\(_2\) consumption increased from the younger (T4) to older (T2) chronosequences in the subtidal zone. The chronosequence-averaged fluxes of dissolved O\(_2\) in the subtidal and intertidal zones were significantly lower than the chronosequence-averaged flux in the supratidal zone in spring (Table 2.3, P < 0.001). In summer, there was no obvious hydrogeomorphic or chronosequence variation except for the supratidal-T2 site with significantly higher dissolved O\(_2\) consumption of -3.5 g O\(_2\) m\(^{-2}\) d\(^{-1}\) (F = 15.7, P < 0.001).
Figure 2.2. Bulk density (a) and sediment organic matter content (b) in the top 4 cm of sediment among hydrogeomorphic zones (subtidal, intertidal, and supratidal) along a chronosequence of younger (T4) to older (T1) sites within each hydrogeomorphic zone (mean ±1 SE, n = 3). One-way ANOVA was used to test the difference among experimental sites and letters designate significant (p < 0.05) differences using Tukey’s HSD test.
Figure 2.3. Benthic fluxes of (a) dissolved oxygen and (b) N$_2$-N among hydrogeomorphic zones (subtidal, intertidal, and supratidal) along a chronosequence of younger (T4) to older (T1) sites within each hydrogeomorphic zone in the spring and summer (mean ± 1 SE, n = 9). ANOVA with repeated measures was used to test the difference among experimental sites in each season and letters designate significant (p < 0.05) differences using Tukey’s HSD test.

Benthic fluxes of N$_2$ gas ranged from -22 to 911 µmol N$_2$-N m$^{-2}$ h$^{-1}$ with a dominance of positive rates resulting from net denitrification (Fig. 2.3b). N$_2$ gas fluxes in spring were significantly lower than fluxes in summer (F = 82.1, P < 0.001). In spring, N$_2$ fluxes increased along the chronosequence as deltaic sediments increased in organic matter content in the subtidal and intertidal zones. Chronosequence-averaged N$_2$ fluxes from the subtidal
and intertidal zones were significantly lower than chronosequence-averaged flux from the supratidal zones in spring (Table 2.3, P < 0.001). In summer, no obvious chronological or hydrogeomorphic variations were observed, but N₂ fluxes in the supratidal-T2 site were much higher than fluxes in the other sites.

Table 2.4 Laboratory incubation conditions in continuous flow-through experiments in WLD. Note that the pre-filtered incubation water was collected from Wax Lake Outlet (WLO).

<table>
<thead>
<tr>
<th></th>
<th>Spring</th>
<th></th>
<th>Summer</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subtidal</td>
<td>Intertidal</td>
<td>Supratidal</td>
<td></td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>T4ᵇ</td>
<td>T3ᵇ</td>
<td>T2ᵇ</td>
<td>T4ᶜ</td>
</tr>
<tr>
<td></td>
<td>13.1</td>
<td>13.1</td>
<td>13.1</td>
<td>11.0</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>NO₃⁻ (µM)</td>
<td>39.5</td>
<td>39.5</td>
<td>39.5</td>
<td>48.7</td>
</tr>
<tr>
<td>NO₂⁻ (µM)</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>0.6</td>
</tr>
<tr>
<td>NH₄⁺ (µM)</td>
<td>2.8</td>
<td>2.8</td>
<td>2.8</td>
<td>1.2</td>
</tr>
<tr>
<td>PO₄³⁻ (µM)</td>
<td>1.1</td>
<td>1.1</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>T4ᶠ</td>
<td>T3ᶠ</td>
<td>T2ˣ</td>
<td>T4ᵉ</td>
</tr>
<tr>
<td></td>
<td>22.0</td>
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<td>22.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>NO₃⁻ (µM)</td>
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<td>67.5</td>
<td>90.4</td>
<td>90.4</td>
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<tr>
<td>NO₂⁻ (µM)</td>
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<td>0.2</td>
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<tr>
<td>NH₄⁺ (µM)</td>
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<td>0.3</td>
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<tr>
<td>PO₄³⁻ (µM)</td>
<td>2.4</td>
<td>2.4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

NO₃⁻ concentrations in overlying water during the incubations ranged from 39.5 to 71.9 µM in spring and from 67.5 to 105.5 µM in summer (Table 2.4). Benthic NO₃⁻ fluxes were mostly negative (sediment uptake) in both seasons with a range of -1439 to 40 µmol m⁻² h⁻¹ (Fig. 2.4a). In spring, benthic NO₃⁻ fluxes increased from the younger to older chronosequences in the subtidal and intertidal zones. Chronosequence-averaged NO₃⁻ flux in the subtidal zone was lower than chronosequence-averaged fluxes in the intertidal and
supratidal zones in spring (Table 2.3). In summer, NO$_3^-$ fluxes were significantly higher than fluxes in spring (F = 38.0, P < 0.001) and there was no significant chronosequence variation in each hydrogeomorphic zone. However, the supratidal-T2 site indicated much higher benthic NO$_3^-$ removal than the other sites. NO$_2^-$ fluxes varied from -3 to 11 µmol m$^{-2}$ h$^{-1}$ in spring and from 2 to 67 µmol m$^{-2}$ h$^{-1}$ in summer (Fig. 2.4b). There was no significant chronosequence variation in each hydrogeomorphic zone, but the supratidal-T2 site in summer showed significantly higher NO$_2^-$ release than other sites (F = 10.7, P < 0.001).

NH$_4^+$ concentrations in the overlying water during incubations varied from 1.2 to 4.0 µM in spring and 0.3 to 1.0 µM in summer (Table 2.4). Benthic NH$_4^+$ fluxes were mostly positive in both seasons (-34 to 216 µmol m$^{-2}$ h$^{-1}$), representing a net release of NH$_4^+$ from sediments to the water column (Fig. 2.5a). In spring, NH$_4^+$ fluxes significantly increased from the younger (T4) to the older (T2) chronosequences in the subtidal zone (P < 0.05). A similarly increasing pattern was observed in the intertidal zone along the chronosequence except for the intertidal-T1 site in spring. NH$_4^+$ fluxes in the subtidal and intertidal zones were positive, whereas fluxes in the supratidal zone were negative in spring. In summer, NH$_4^+$ fluxes increased along the chronosequence from the younger sites (T4) to the older sites (T2 or T1) in the subtidal and intertidal zones. Chronosequence-averaged NH$_4^+$ flux in the supratidal zone was lower than fluxes in the other two hydrogeomorphic zones in both seasons (Table 2.3). PO$_4^{3-}$ fluxes were variable with no notable trend in either season (Fig. 2.5b).
Figure 2.4. Benthic fluxes of (a) NO$_3^-$ and (b) NO$_2^-$ among hydrogeomorphic zones (subtidal, intertidal, and supratidal) along a chronosequence of younger (T4) to older (T1) sites within each hydrogeomorphic zone in the spring and summer (mean ±1 SE, n = 9). ANOVA with repeated measures was used to test the difference among experimental sites in each season and letters designate significant (p < 0.05) differences using Tukey’s HSD test.
Figure 2.5. Benthic fluxes of (a) NH$_4^+$ and (b) PO$_4^{3-}$ among hydrogeomorphic zones (subtidal, intertidal, and supratidal) along a chronosequence of younger (T4) to older (T1) sites within each hydrogeomorphic zone in the spring and summer (mean ± 1 SE, n = 9). ANOVA with repeated measures was used to test the difference and letters designate significant (p < 0.05) differences among nine study sites using Tukey's HSD test.

Significant positive relationships were observed between sediment organic matter content and dissolved O$_2$, N$_2$-N and NO$_3^-$ fluxes, respectively, in both seasons (P < 0.001; Fig. 2.6). Note that the slope in the fitted equation between organic matter content and N$_2$-N flux in summer was twice the slope in the fitted equation in spring (Fig. 2.6b). Such seasonal patterns were even obvious in the correlation of organic matter content and NO$_3^-$ flux, as the slope in summer (-66.8) was about 5-times larger than the slope in spring (-12.9, Fig. 2.6c).
In summary, benthic fluxes of N$_2$-N and NO$_3^-$ were more sensitive to increases in sediment organic matter content in summer than in spring.

Figure 2.6. Benthic fluxes of (a) dissolved oxygen, (b) N$_2$-N and (c) NO$_3^-$ as functions of sediment organic matter content in Wax Lake Delta (WLD). Note each data point represents a sediment core and values of O$_2$ and NO$_3^-$ fluxes were inversed on the x-axis. Henry and Twilley (2014) data in spring and summer were added as filled diamonds in corresponding colors; however, these values were not included in correlation models.
Scaling N fluxes to annual rates

We used 17 °C as a critical value of water temperature to categorize each day in a year in WLD as either spring or summer seasons. Overall, there were 225 days defined as summer and 140 days defined as spring during the experimental year (May 2017 to April 2018). Sites from different hydrogeomorphic zones had distinct seasonal patterns of inundation depending on topography (Fig. 2.7). Generally, subtidal sites were submerged for over 97% of the year, while intertidal sites were inundated more than 80% of the year, compared to < 50% for supratidal sites. Over 67% of the inundation period was distinguished as summer season with warmer (≥ 17°C) overlying water in the nine experimental sites (Table 2.5)

Table 2.5 Inundation days that categorized as spring (cold) or summer (warm) seasons based on the overlying water level and water temperature in each experimental site during the experimental year (May 2017 to April 2018).

<table>
<thead>
<tr>
<th>Site</th>
<th>Cold</th>
<th>Warm</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-T4</td>
<td>140</td>
<td>225</td>
<td>365</td>
</tr>
<tr>
<td>Sub-T3</td>
<td>139</td>
<td>225</td>
<td>364</td>
</tr>
<tr>
<td>Sub-T2</td>
<td>137</td>
<td>225</td>
<td>362</td>
</tr>
<tr>
<td>Int-T4</td>
<td>102</td>
<td>224</td>
<td>326</td>
</tr>
<tr>
<td>Int-T3</td>
<td>98</td>
<td>222</td>
<td>320</td>
</tr>
<tr>
<td>Int-T2</td>
<td>134</td>
<td>225</td>
<td>359</td>
</tr>
<tr>
<td>Int-T1</td>
<td>137</td>
<td>225</td>
<td>362</td>
</tr>
<tr>
<td>Supra-T2</td>
<td>13</td>
<td>68</td>
<td>81</td>
</tr>
<tr>
<td>Supra-T1</td>
<td>31</td>
<td>118</td>
<td>149</td>
</tr>
</tbody>
</table>

Annual rates of NO$_3^-$ removal and N$_2$-N production were estimated by converting hourly NO$_3^-$ and N$_2$-N fluxes into daily rates based on how long the experimental sites were inundated and whether the inundation time was recognized as spring or summer. The sum of daily rates in the experimental year provided a rough estimate of annual N fluxes. The intertidal-T1, rather than the supratidal-T2, was the most significant site in removing NO$_3^-$ (-3.4 mol m$^{-2}$ yr$^{-1}$) and releasing N$_2$-N (3.2 mol m$^{-2}$ yr$^{-1}$) on the annual basis when considering
seasonal temperature and annual inundation (Fig. 2.8). The lowest annual \( \text{NO}_3^- \) removal (-0.5 mol m\(^{-2}\) yr\(^{-1}\)) occurred at the younger intertidal zone, and the lowest \( \text{N}_2\text{-N} \) production (1.0 mol m\(^{-2}\) yr\(^{-1}\)) was at the younger intertidal and subtidal zones of WLD. Over 90% of annual \( \text{NO}_3^- \) loss and \( \text{N}_2\text{-N} \) production occurred during the summer season when water temperatures were at or above 17 °C while less than 10% of these fluxes occurred during the spring season.

Figure 2.7. Monthly flooded times for (a) subtidal, (b) intertidal and (c) supratidal hydrogeomorphic zones in Wax Lake Delta (WLD) from May 2017 to April 2018 (recognized as a sampling year). Error bars are standard errors among different chronosequence zones within each hydrogeomorphic zone. Flooded time was estimated based on the elevations of nine sites and continuous water level records at 6 min time intervals from a tidal gauge (29° 27.0’ N, 91° 20.3’ W) in Amerada Pass near WLD.
To calculate total N removal in WLD, the annual NO$_3^-$ and N$_2$-N fluxes from different chronosequences within a certain hydrogeomorphic zone were averaged and multiplied by the total area of each hydrogeomorphic zone in WLD. The three hydrogeomorphic zones are distributed in the 50.0 km$^2$ WLD as 66.3% subtidal zone, 23.9% intertidal zone and 9.8% supratidal zone (Fig. 2.9a). We estimated the subtidal, intertidal and supratidal zones could remove 520, 254 and 122 Mg N yr$^{-1}$ of NO$_3^-$, respectively, for a total of 896 Mg N yr$^{-1}$ (Fig. 2.9b). WLD released N$_2$-N to the atmosphere at the rates of 712 Mg N yr$^{-1}$ from the subtidal zone, 324 Mg N yr$^{-1}$ from the intertidal zone, and 116 Mg N yr$^{-1}$ from the supratidal zone (Fig. 2.9c). This budget of N fluxes for this coastal deltaic floodplain suggests that there was an additional source of N, other than NO$_3^-$ uptake, that contributed to N$_2$ production to the atmosphere.

Figure 2.8. Annual rates for (a) NO$_3^-$ removal and (b) N$_2$-N production based on hourly NO$_3^-$ and N$_2$-N fluxes at the sediment-water interface applying spring vs summer rates based on ambient water temperature together with the amount of time that a specific hydrogeomorphic zone is inundated. The critical water temperature we used to separate months into spring/summer seasons is 17 °C.
Figure 2.9. Using (a) the area of each hydrogeomorphic zone at Wax Lake Delta and per unit area rates of nitrogen fluxes to estimate (b) annual NO$_3^-$ removal and (c) N$_2$-N production in each hydrogeomorphic zone.
DISCUSSION

The role of organic matter content in benthic N fluxes

Combining results across hydrogeomorphic and chronosequence zones demonstrated a pattern where sediment organic matter content increased with a decrease in bulk density (Fig. 2.10). Wetland soil formation in a coastal deltaic floodplain is a process of mineral sedimentation promoting increased elevation with time that transforms subtidal to intertidal hydrogeomorphic zones (Bevington and Twilley 2018; Twilley et al. 2019). The increase in elevation as wetland soils age during delta formation causes a shift in wetland vegetation that increases the organic matter production (both aboveground and belowground), which further increases elevation (White 1993; Callaway et al. 1997; Cahoon et al. 2011; Bevington and Twilley 2018). Sediment organic matter content alters the sediment compressibility and decreases sediment bulk density (Ruehlmann and Körschens 2009). As such, bulk density decreased along the chronosequence within a hydrogeomorphic zone and from the subtidal to the supratidal zones of this coastal deltaic floodplain.

Figure 2.10. Bulk density as a function of sediment organic matter content in Wax Lake Delta (WLD). Note each data point represents a sediment core.
Surface sediments in the supratidal-T1 site with much lower sediment organic matter content and higher bulk density than the supratidal-T2 site indicated an alternative rather than chronosequence controlling sediment development. The supratidal-T1 site was located near the fringe along a primary channel of WLD with tree vegetation. This site was exposed to high sedimentation associated with flood pulses of inorganic sediments during river flood events (Bevington 2016; Bevington and Twilley 2018). The supratidal-T2 site, unlike the supratidal-T1, was located within the interior of the island dominated by the herbaceous community of *Colocasia esculenta*. Thus, while the supratidal-T1 and T2 sites may represent older chronosequence with higher elevation, the differences in organic matter content and bulk density between these two sites may be related to levee vs interior location and exposure to sedimentation during a flood event. As such, chronosequence is an important factor to explain sediment organic matter content during the earlier development stages of an active deltaic floodplain, but sediment organic matter content in the supratidal zone may also depend on locations along the fringe (exposed to high mineral sedimentation) compared to interior locations (less mineral sedimentation).

The significantly increasing trend in organic matter content from younger to older chronosequences explained general patterns in benthic nutrient fluxes. There is evidence in estuarine systems that elevated sediment organic matter content enhances denitrification in wetland sediments along with increased rates of sediment oxygen demand and N mineralization (Eyre and Ferguson 2009; Hardison et al. 2015). Elevated benthic metabolism in sediments with greater organic matter content decreases dissolved oxygen concentrations in wetland sediments, providing a suitable condition for denitrification (Caffrey et al. 1993a; Cai and Sayles 1996; Cornwell et al. 1999; Eyre and Ferguson 2009). Both benthic N₂ and NO₃⁻ fluxes increased with sediment organic matter content in our study, which are consistent with previous research results of the correlation between N₂ (NO₃⁻) fluxes and sediment
organic matter in WLD (Henry and Twilley 2014; their data were added in Fig. 2.6 as filled diamonds). The positive linear correlation between N₂ fluxes and sediment organic matter content indicates that denitrification potential is stimulated by increased sediment organic matter content as a function of biotic feedback associated with active deltaic succession. NO₃⁻ is an important electron acceptor for organic matter respiration under anaerobic conditions, and thus NO₃⁻ removal is positively associated with organic matter content in mostly inundated wetland soils that are anaerobic.

Sediment oxygen demand and benthic fluxes of N₂-N and NO₃⁻ were significantly higher in summer than in spring demonstrating the effect of temperature in this warm temperate delta in stimulating benthic biogeochemical processes (Eyre and Ferguson 2005; Giblin et al. 2010). In our experiments NO₃⁻ removal and N₂-N production were more sensitive to sediment organic matter content in summer than in spring, which may relate to a combination of higher NO₃⁻ concentrations and temperature in influent riverine waters in the summer than in the spring. Denitrification rates were enhanced at warmer temperature as increasing temperature stimulates benthic microbial activity including denitrifying bacteria (Dawson and Murphy 1972). Also, increased microbial metabolism at higher temperature reduced benthic dissolved O₂ concentrations and created a favorable condition for the anaerobic process of denitrification (Dawson and Murphy 1972; Cornwell et al. 1999). On the other hand, a pulse of riverine discharge from snowmelt and rainfall usually occurs in late winter and early spring in WLD, followed by higher NO₃⁻ concentrations in river waters from March to July resulting from agricultural fertilization (Xu 2006; Shaw et al. 2013; Carle et al. 2015). Influent NO₃⁻ concentrations were positively correlated with benthic NO₃⁻ fluxes (Fig. 2.11), which supports previous research results that sediment NO₃⁻ fluxes and denitrification rates are proportional to NO₃⁻ concentrations in overlying waters (Ogilvie et al. 1997; Kana et al. 1998; Piña-Ochoa and Álvarez-Cobelas 2006; Scaroni et al. 2011).
Figure 2.11. Correlation between NO$_3^-$ fluxes versus influent NO$_3^-$ concentrations during incubations. Note that each data point represents an averaged value in each site.

**N reactions in response to benthic fluxes**

N$_2$ production, measured here using the N$_2$:Ar method, represents a combination of processes that produce N$_2$ [anaerobic ammonium oxidation (anammox) and denitrification] and a process that consumes N$_2$ (N$_2$ fixation). Anammox is an anaerobic reaction dependent on a supply of both NH$_4^+$ and NO$_x^-$ (NO$_2^-$ + NO$_3^-$) whereas denitrification requires only NO$_x^-$ as a N substrate (Damashek and Francis 2018). In most freshwater and estuarine ecosystems, high NO$_3^-$ loading stimulates denitrification directly. The dominance of denitrification suppresses anammox by competition for NO$_x^-$, which makes anammox insignificant compared to denitrification (Wang et al. 2012; Yin et al. 2014; Damashek and Francis 2018). With relatively higher NO$_3^-$ concentrations and lower NH$_4^+$ concentrations in the overlying water, WLD is expected to have much lower rates of anammox compared to denitrification as found in other similar tidal freshwater ecosystems (Trimmer et al. 2003; Koop-Jakobsen and Giblin 2009; Brin et al. 2014). However, N$_2$ fixation is becoming more evident as a significant process in estuarine and coastal ecosystems (Gardner et al. 2006; Yin et al. 2014;
Bentzon-Tilia et al. 2015; Damashek and Francis 2018). Previous estimates of N₂ fixation in WLD sediments using the N₂:Ar method with a batch core incubation system observed that N₂ fixation exceeded denitrification when overlying NO₃⁻ concentration was low (about 2.0 µM; Henry and Twilley 2014). Nevertheless, our experiments were under eutrophic NO₃⁻ concentrations (39.5 to 105.5 µM) and showed mainly positive N₂-N production without obvious evidence of net N₂ fixation. Several studies have also reported that bio-reactive N at high concentrations (≥ 10 µM) could significantly inhibit N₂ fixation (Howarth et al. 1988; Mulholland et al. 2001; Capone et al. 2008; Scott et al. 2008). Assuming this threshold of NO₃⁻ concentration for WLD, the N₂-N production rates measured in our study represent denitrification rates in WLD with little impact of N₂ fixation.

Incomplete denitrification to N₂O is frequently reported to be small compared to complete denitrification in riverine and coastal ecosystems (Seitzinger et al. 1984; Yu et al. 2006; Beaulieu et al. 2011). Assuming incomplete denitrification is negligible in our research, N₂-N production rates should be balanced with NO₃⁻ removal rates if all N₂-N is from direct denitrification and there is little impact of N₂ fixation. However, if coupled nitrification-denitrification occurs, some atoms of N₂-N are produced from NO₃⁻ formed within sediments from nitrification, leading to higher N₂-N production rates compared to NO₃⁻ removal rates. On the other hand, when N₂-N production rates are significantly less than NO₃⁻ removal rates, there is evidence that some NO₃⁻ is not used in denitrification, but instead contributes to dissimilatory nitrate reduction to ammonium (DNRA) assuming incomplete denitrification is negligible. N₂-N production was higher than NO₃⁻ removal in the sub-T3 site and most sites in the intertidal zone, indicating coupled nitrification-denitrification was present in these sediments and outcompeted DNRA (assuming anammox and N₂ fixation are negligible; Fig. 2.8a and b). In contrast, N₂-N production was lower than NO₃⁻ removal rates in the supratidal-T2 site, indicating DNRA was more favorable than coupled nitrification-
denitrification in this site. DNRA was reported to be a prominent NO$_3^-$ reduction pathway in estuarine and coastal ecosystems, especially under high organic carbon content (An and Gardner 2002; Koop-Jakobsen and Giblin 2010; Giblin et al. 2013; Hardison et al. 2015). It is reasonable that the supratidal-T2 site had higher DNRA rates because of the significantly higher organic matter content (18.2%) in this site compared to other sites in WLD. However, these estimates should be treated with some caution since N cycles are more complex than the methodologies used in this study to discern specific transformations of N in delta sediments.

**Stoichiometry of benthic fluxes**

Denitrification can be estimated based on stoichiometric ratios of dissolved oxygen and inorganic N assuming constant ratios of elements related to the dominant terminal electron acceptors in eutrophic coastal sediments (Boynton and Kemp 1985; Cowan et al. 1996; Cornwell et al. 1999; Boynton et al. 2018):

$$(\text{CH}_2\text{O})_{106}(\text{NH}_3)_{16}\text{H}_3\text{PO}_4 + 138 \text{O}_2 \rightarrow 106 \text{CO}_2 + 16 \text{HNO}_3 + \text{H}_3\text{PO}_4 + 122 \text{H}_2\text{O}$$  \hspace{1cm} (EQ 2)

Every 17.25 atoms of dissolved oxygen that are consumed in sediments should result in 1 atom of DIN released to porewater and then diffused across the sediment-water interface to the overlying water to reach an equilibrium (Redfield 1934; Boynton and Kemp 1985). Dissolved oxygen consumption we measured in the flow-through system was presumed to represent sediment oxygen demand (the benthic consumption of O$_2$ due to aerobic respiration as shown in EQ 2) since our incubations occurred in the dark and the overlying water was filtered before use. We assumed that the C:O:N ratio of sediment organic matter in WLD followed the Redfield composition. Multiplying the measured rates of sediment oxygen demand by the Redfield ratio of 17.25 provided expected DIN fluxes across the sediment-water interface. The expected DIN fluxes were different from measured DIN fluxes, which is reasonable since denitrification occurred and converted DIN to N$_2$ gas during the incubations.
As such, the measured DIN fluxes are combinations of the expected DIN production from benthic aerobic respiration following Redfield ratio and the DIN uptake due to denitrification. Denitrification rates can be indirectly estimated by subtracting the measured DIN fluxes with the expected DIN fluxes (Cowan and Boynton 1996). Estimated denitrification rates calculated indirectly based on the stochiometric ratio were significantly correlated to measured net denitrification rates from the N₂:Ar method (Fig. 2.12; $R^2 = 0.73$ and $p < 0.0001$). However, the slope of estimated denitrification to measured denitrification rates was significantly higher than one (slope = 1.27, $p < 0.01$), indicating that the estimated denitrification rates using the stoichiometric ratio of 17.25 were higher than the measured denitrification rates. An implicit assumption with using this stoichiometric ratio to estimate denitrification is that the sediment organic matter has the Redfield composition of C:O:N ratio (Cornwell et al. 1999). Nevertheless, the Redfield ratio (C:O:N:P=106:138:16:1) is an average ratio of phytoplankton biomass composition (Redfield 1934; Redfield 1958; Hillebrand and Sommer 1999). Previous research showed that this ratio had significant local variations, which could depart broadly from the average due to different nutrient availabilities and planktonic species (Geider and La Roche 2002; Ptacnik et al. 2010). WLD is mainly N limited with low C:N (9.7 to 11.6) and N:P ratios (Henry and Twilley 2014), which may result in a lower stochiometric ratio than 17.25 and an overestimate of denitrification using the ratio of 17.25. Stoichiometry of benthic fluxes is a complex balance between several biological processes rather than a fixed ratio, and thus rates based on the Redfield ratio should be treated with caution (Geider and La Roche 2002).

Another explanation for higher estimated denitrification than measured denitrification is that the N₂:Ar method may underestimate denitrification because of the occurrence of N₂ fixation, incomplete denitrification and/or gas bubbles during the incubation (Eyre and Ferguson 2002; Ferguson and Eyre 2007; Henry and Twilley 2014). As discussed, N₂ fixation
and incomplete denitrification are reported to be small compared to total denitrification in eutrophicated coastal wetlands, rivers and coastal bays. Also, our incubations were in the dark with pre-filtered overlying water and closely monitored for the occurrence of bubbles. The influence of gas bubbles to the measured net denitrification should be small.

Figure 2.12. Correlation between direct measurements of benthic denitrification using the N\textsubscript{2}: Ar method versus estimated denitrification rates based on the stoichiometric assumption that the molar organic carbon (C):oxygen (O):nitrogen (N): phosphorus (P) ratios of sediment fluxes should follow Redfield composition (C:O:N:P=106:138:16:1). Note that each data point represents a sediment core in each site.

**Are coastal deltas hotspots of N removal?**

The sediment organic matter content and hydroperiod of each hydrogeomorphic zone are important factors to determine annual benthic fluxes of a coastal deltaic floodplain. Organic matter determines benthic flux per unit time of inundation, and hydroperiod defines the duration that an area of sediment is inundated with overlying water (and NO\textsubscript{3}\textsuperscript{-}). The intertidal and supratidal zones are submerged seasonally, with less hours per month during late fall and early winter seasons when river stage and frequency of cold fronts are the lowest (Bevington 2016; Twilley et al. 2019). Annual estimates of N removal for intertidal and
supratidal sites account for both seasonal water temperature and flood duration. For subtidal sediments, water temperature is a key factor since these sites are inundated nearly all year. Considering these seasonal and hydroperiod factors among sites representing subtidal, intertidal and supratidal hydrogeomorphic zones, our annual benthic NO\textsubscript{3}\textsuperscript{-} fluxes varied from -0.5 to -3.4 mol N m\textsuperscript{-2} yr\textsuperscript{-1}. The upper range of NO\textsubscript{3}\textsuperscript{-} removal at WLD are comparable to a recent estimate in the Mississippi River Basin based on a mass balance calculation (average = -3.3 mol N m\textsuperscript{-2} yr\textsuperscript{-1}; Mitsch et al. 2005). Annual N\textsubscript{2}-N production rates varied from 1.0 to 3.2 mol N m\textsuperscript{-2} yr\textsuperscript{-1}, which is comparable to annual NO\textsubscript{3}\textsuperscript{-} removal.

Total NO\textsubscript{3}\textsuperscript{-} load to WLD can be calculated by multiplying the estimated NO\textsubscript{3}\textsuperscript{-} loading rate of 66 to 172 g N m\textsuperscript{-2} yr\textsuperscript{-1} in the Atchafalaya River estuarine complex (Lane et al. 2002) with the area of WLD (50 km\textsuperscript{2}). Based on this calculation, WLD could receive 3300 to 8600 Mg N yr\textsuperscript{-1}. The estimated annual NO\textsubscript{3}\textsuperscript{-} removal of 896 Mg N yr\textsuperscript{-1} in WLD accounts for 10 to 27\% of total NO\textsubscript{3}\textsuperscript{-} load to WLD, most of which is converted to N\textsubscript{2} gas since NO\textsubscript{3}\textsuperscript{-} removal rate is comparable to N\textsubscript{2}-N production in our study. Over 90\% of the annual NO\textsubscript{3}\textsuperscript{-} removal occurred during warmer temperatures (≥17 °C) when higher NO\textsubscript{3}\textsuperscript{-} concentrations due to agricultural fertilization were present in the overlying water. As such, coastal deltaic floodplains like WLD play an important role in decreasing nutrient concentrations in warm seasons before nutrient enriched water enters the Gulf of Mexico (Henry and Twilley 2014).

Active deltas, such as WLD, have the unique landscape feature of annual progradation as young subtidal hydrogeomorphic zones, which increases the N removal capacity. For example, WLD with the land growth rate of 1.0 to 5.0 km\textsuperscript{2} yr\textsuperscript{-1} (Allen et al. 2012; Shaw et al. 2013; Bevington 2016) increases the capacity of NO\textsubscript{3}\textsuperscript{-} removal by 14 to 70 Mg N yr\textsuperscript{-1} (0.2 to 2\% of total NO\textsubscript{3}\textsuperscript{-} load) as a result of continuous delta progradation. In addition to annual delta growth, there is a shift in proportion of hydrogeomorphic zones from subtidal to intertidal and supratidal zones in response to organic matter accumulation and infilling as emergent
vegetation expands with increased sediment elevation (Bevington and Twilley 2018; Ma et al. 2018; Twilley et al. 2019). Increasing areas of intertidal and supratidal zones favor N removal in WLD as denitrification rates are higher with higher sediment organic matter. The expansion of the intertidal zone may contribute more to benthic N removal after years of organic matter accumulation as this area is inundated more frequently compared to the supratidal zone. Active delta formation at the mouth of rivers that have waters enriched with NO$_3^-$ is a dynamic process in reducing eutrophication of the coastal ocean. Mineral sedimentation stimulates delta formation during winter and spring flood-pulse of the river (Bevington and Twilley 2018) whereas the warmer growing season of wetlands contribute organic production in summer and fall (Aarons 2019). The combination of both processes contributes to the formation of depositional landscapes that increases N removal capacity in active deltaic basins with connectivity between channels and hydrogeomorphic zones (Hiatt et al. 2018).

Our estimates of NO$_3^-$ removal and N$_2$ production are conservative for several reasons. Firstly, subtidal zones with elevation lower than -0.75 m and all creeks and channels are excluded as part of the total subtidal area estimate for WLD. These regions account for about 40% of total WLD, and their benthic biogeochemical processes may be different from our experimental sites due to distinct environmental conditions (sediment organic matter content, water residence time, water temperature). Denitrification that occurs in these regions is not included in our analysis of NO$_3^-$ removal. Secondly, our estimates focus on the hydroperiod of wetland submergence that determines NO$_3^-$ removal when river waters are in contact with deltaic sediments. The emergence of deltaic sediments in intertidal and supratidal hydrogeomorphic zones may prompt nitrification that is later denitrified when sediments are submerged (Cornwell et al. 1999; Baldwin and Mitchell 2002; Pinay et al. 2002). Partial drying of previously submerged sediments will produce a zone for coupled
nitrification-denitrification (Patrick and Reddy 1976; Baldwin and Mitchell 2002). Coupled nitrification-denitrification rates may be enhanced by seasonal hydroporperiod from emergence to submergence in more elevated hydrogeomorphic zones. Since our focus is on estimates of riverine NO$_3^-$ removal in coastal deltaic floodplains, this coupled process of nitrification as source of NO$_3^-$ may not be relevant to our analysis, but impact the total N$_2$ flux from active deltaic floodplains to the atmosphere.

Our estimate of N removal at the sediment-water interface may not account for field boundary conditions at the sediment-water interface as result of flow currents that can impact benthic fluxes. This may be particularly important at the subtidal zone in the younger chronosequences (T4 and T3), where more open water with strong hydrological connectivity may present different conditions than found in our incubation system (Hiatt and Passalacqua 2015). Additionally, dark incubations reflect limited diurnal variability of benthic fluxes compared to field conditions. Benthic fluxes in WLD are reported to have diurnal variability in the field because of the photosynthesis of benthic microalgae in light condition (Henry 2012). In the light, experimental sites with benthic microalgae could have higher benthic NO$_3^-$ and NH$_4^+$ uptakes, lower dissolved O$_2$ consumption and lower denitrification rates compared to the dark incubations (Sundback et al. 1991; Henry 2012). Besides, two temperature settings simplified the seasonal variability of benthic fluxes, but our incubations at 12 °C and 22 °C did mirror environmental conditions as these two temperatures are representative temperatures for cold (winter and spring) and warm (summer and fall) seasons that provide a first approximation of seasonal temperature variation. Surface water temperatures in WLD fell within 12 °C to 22 °C for about half time of the year. Also, the supratidal and intertidal zones tend to have higher water temperature than the subtidal zone because longer water residence time in supratidal and intertidal zones allows water temperatures to increase from solar radiation (Christensen 2017; Noe et al. 2013). Applying
water temperature records from the intertidal zone to subtidal and supratidal hydrogeomorphic zones may weaken hydrogeomorphic variations in annual N removal. Model simulation of benthic N processes and field experiments that include physical conditions like hydrological connectivity and currents may expand our understanding of these additional factors that control NO$_3^-$ removal in coastal deltaic floodplains (Christensen 2017).

Our analysis demonstrates the importance of active deltaic floodplains in N removal as these landscapes become subaerially emergent before significant N enters the coastal ocean. The potential capacity of N removal can be further demonstrated by applying the N removal capacity in WLD to the Atchafalaya River Coastal Basin (ARB). ARB is about 4678 km$^2$ in area with over 150 km$^2$ of newly emergent delta including WLD in Atchafalaya Bay (Coleman et al. 1998; Xu 2006). Annual NO$_3^-$ loading to ARB is about 170,000 Mg (Xu 2006). Assuming the N removal capacity in WLD is similar to the capacity in wetland landscapes across ARB, about 50% of riverine NO$_3^-$ could be removed before entering the Gulf of Mexico. Though the N removal capacity of 50% in ARB may be underestimated as describe above, it is consistent with the conclusion that ARB removes 41 to 47% of NO$_3^-$ from the Atchafalaya River (Lane et al. 2002). With increasing coastal water temperature due to global climate change, the role of coastal deltaic floodplain in benthic N removal may be enhanced since warmer temperature increases benthic denitrification rates (Dawson and Murphy 1972; Michener et al. 1997). Our study provides new insights into how active deltaic floodplains may remove riverine N as part of delta restoration efforts. We propose that the extension of older intertidal zone as the most efficient N-removal area, annual progradation of younger subtidal areas, vegetative production that elevates SOM concentrations and frequent riverine water inundation all lead to higher N removal capacity of a newly restored delta.
The combined effects of anthropogenic changes to sediment supply and river flow, along with subsidence and global sea level rise, are causing river deltas all over the world to decrease in emergent area along deltaic coasts (Syvitski et al. 2009; Vörösmarty et al. 2009; Twilley et al. 2016). The Mississippi River Delta Plain covers an area of 30,000 km² (Coleman et al. 1998), which represents coastal deltaic floodplains with potential to remove N before riverine nutrients reach the ocean. However, anthropogenic modifications, such as the construction of levees and flood-control structures along the Mississippi River, convert this once active delta into a wetland area abandoned from riverine waters (Twilley et al. 2016). These inactive deltaic floodplains no longer contribute to riverine N removal that could potentially reduce coastal eutrophication on the Louisiana Bight (Diaz 2009; Bargu et al. 2019; Twilley et al. 2019; White et al. 2019). Reconnecting the previously isolated coastal deltaic floodplains to river flood pulse is proposed as a feasible way to not only restore coastal wetland habitats, but also re-build ecosystems in processing polluted riverine water (Mitsch et al. 2005; Bargu et al. 2019; Twilley et al. 2019; White et al. 2019). Scaling the capacity of an active deltaic floodplain such as WLD, accounting for both chronosequence and hydrogeomorphic zones as influential factors in N removal, can serve as the model of how riverine nutrients will be processed under the ecological succession of a young emergent delta created from major river diversions.

CONCLUSIONS

We investigated spatial and seasonal patterns in benthic fluxes and estimated annual N removal in the newly emergent WLD using continuous flow-through incubations of intact sediment cores. To our knowledge, it is the first assessment on benthic fluxes as a function of hydrogeomorphology and delta age since emergence in coastal deltaic floodplains. This study suggests that benthic fluxes have obvious variations within different hydrogeomorphic zones and chronosequence zones in response to the change in sediment organic matter content.
Denitrification rates estimated from the stochiometric ratio of C:O:N (106:138:16) explained 73% of the measured net denitrification rates using the N₂:Ar method. But the use of stoichiometry in benthic denitrification calculation should be used with caution as the Redfield ratio is a complex balance between several biological processes rather than a fixed ratio. The older intertidal hydrogeomorphic zones with relatively higher sediment organic matter was the most efficient site in annual N removal. Subtidal zone had the lowest denitrification rates associated with lower organic matter content, but was the largest hydrogeomorphic zone with the longest flood duration, and therefore contributed over half of the N removal in WLD. The estimated annual NO₃⁻ removal of 896 Mg N yr⁻¹ in WLD accounted for 10 to 27% of total NO₃⁻ load to WLD, most of which was converted to N₂ gas. Over 90% of the annual N removal occurred during warmer temperatures (≥17 °C) when higher NO₃⁻ concentrations were observed in the overlying water. WLD is a continuously emerging ecosystem where the capacity for N removal increases by 0.2-2% per year prior to riverine NO₃⁻ is export to coastal ocean.

We conclude that coastal deltaic floodplains play an important role in decreasing nutrient concentrations before riverine water entering the Gulf of Mexico. The capacity for N removal in active coastal deltaic floodplains like WLD increases with continuously emerging ecosystems at continental margins. This research of nutrient cycling in WLD concerning chronosequence and hydrogeomorphic zones as influential factors provides a basic understanding of how riverine nutrients will be processed under the ecological succession of a young emergent delta created from major river diversions. Further analysis of model simulation incorporating actual hydrological connectivity, water residence time and the growth of an active delta with newly emergent subtidal hydrogeomorphic zones and continued development of intertidal to supratidal zones will more clearly define the role of coastal deltaic floodplains in processing elevated riverine NO₃⁻ in major river basin.
CHAPTER 3. BENTHIC NITROGEN DYNAMICS IN AN EMERGING COASTAL DELTAIC FLOODPLAIN IN MISSISSIPPI RIVER DELTA USING ISOTOPE PAIRING TECHNIQUE TO TEST RESPONSE TO NITRATE LOADING AND SEDIMENT ORGANIC MATTER

INTRODUCTION

Synthetic production of nitrogenous fertilizers have significantly increased bio-reactive nitrogen (N) inputs to river and coastal waters, which causes coastal eutrophication, stimulates harmful algal blooms and aggravates hypoxia (Rabalais et al. 2002; Paerl et al. 2002; Erisman et al. 2008; Diaz 2009; Canfield et al. 2010; Steffen et al. 2015; Damashek and Francis 2018). Alluvial floodplains are ecohydrological interfaces that are considered hotspots that remove N as a function of connectivity between river channels and wetlands as rivers transport N to the coastal ocean (Krause et al. 2017). Coastal deltaic floodplains, which form in the deposition zone at the continental margins of major river watersheds, also play an important role in N removal prior to riverine nutrient is export to the coastal ocean (Fagherazzi et al. 2015; Bevington and Twilley 2018; Twilley et al. 2019). The capacity of coastal deltaic floodplains to remove bio-reactive N depends on morphological development of these newly emergent ecosystems at the mouths of major river systems (Li et al. 2020). The composition of delta islands with different hydrogeomorphic zones under different age defines how N is processed in deltaic floodplains prior to riverine NO$_3^-$ is export to coastal ocean.

Biotic feedback associated with ecological succession on soil development in coastal deltaic floodplains may be key factor in how riverine N is processed along river channels as has been observed in alluvial floodplains (Ma et al. 2018; Bevington and Twilley 2018; Twilley et al. 2019). During early delta development, mineral sedimentation increases soil surface elevation, transforming subaqueous delta to emergent ecosystems (Cahoon et al. 2011; Twilley et al. 2019). Once subaerial emergence, deltaic floodplains are colonized by
vegetation during growing season, which increases sediment organic matter (SOM) accumulation (Bevington and Twilley 2018). Both mineral sedimentation and organic production in deltaic wetlands increase soil surface elevation and slowly shift subtidal hydrogeomorphic zones to supratidal hydrogeomorphic zones as biotic feedback enhances depositional processes including: a) vegetation production contributes SOM to soil volume; b) vegetation roots decrease soil erosion and c) dense vegetation decreases water velocity increasing deposition of suspended sediments (Gyssels et al. 2005; Baustian et al. 2012; Bevington and Twilley 2018; Ma et al. 2018; Larsen 2019; Twilley et al. 2019). The elevation capital of emerging delta islands drives ecological succession from simple submersed aquatics to emergent herbaceous vegetation to woody vegetation communities in supratidal hydrogeomorphic zones (Cahoon et al. 2011; Bevington and Twilley 2018). The shift from mineral to organic sedimentation in the development of a coastal deltaic floodplain results in changes in benthic N cycling (Li et al. 2020).

Previous studies indicate that nitrate (NO$_3^-$) removal and nitrogen gas (N$_2$) production are significantly correlated with increased SOM as delta islands age from younger to older chronosequence and subtidal to supratidal hydrogeomorphic zones in coastal deltaic floodplains (Henry and Twilley 2014; Li et al. 2020). Patterns of NO$_3^-$ removal and N$_2$ production are a result of interaction between several important N pathways like denitrification, anaerobic ammonium oxidation (anammox), and dissimilatory nitrate reduction to ammonium (DNRA; Fig. 1.1). Denitrification and anammox, as pathways to permanently remove NO$_3^-$ to N$_2$ gas, are crucial means of NO$_3^-$ removal in coastal deltaic floodplains that can reduce coastal eutrophication. In contrast, DNRA converts NO$_3^-$ to NH$_4^+$ and retains bioavailable N in the floodplain, which could be exported to coastal waters or buried during soil development (Gardner et al. 2006; Koop-Jakobsen and Giblin 2010; Bernard et al. 2015). Whether or not coastal deltaic floodplains can serve as hot spots to
reduce coastal eutrophication primarily depends on the relative importance of denitrification, anammox and DNRA to total NO\textsubscript{3}\textsuperscript{-} loss before river waters are exported to coastal ocean. However, the relative importance of denitrification, anammox and DNRA and the interaction between these pathways have not been directly measured in active coastal deltaic floodplains (Henry and Twilley 2014; Twilley et al. 2019; Li et al. 2020). Insight of how SOM enrichment in emerging coastal deltaic floodplains may shift the relative significance of these N pathways is essential to understand how ecological succession of floodplains at continental margins may mitigate coastal eutrophication.

Increasing concentrations of nitrogenous fertilizer (NO\textsubscript{3}\textsuperscript{-}) in rivers over the last four decades (Broussard and Turner 2009) have potentially shifted how coastal deltaic sediments process N, as evidence from net N\textsubscript{2} gas uptake to net N\textsubscript{2} gas release when oligotrophic overlying waters were enriched with NO\textsubscript{3}\textsuperscript{-} in experimental studies of benthic processes (Henry and Twilley 2014). Net N\textsubscript{2} gas release is related to several N processes like direct denitrification, coupled nitrification-denitrification and/or anammox. It is important to separate benthic N pathways from total N\textsubscript{2} release and NO\textsubscript{3}\textsuperscript{-} removal to examine the relative important of each process since each N pathway, as discussed above, plays different roles in coastal N cycling. For example, DNRA, which reduces NO\textsubscript{3}\textsuperscript{-} to more bio-reactive forms of NH\textsubscript{4}\textsuperscript{+}, is reported to be more competitive than denitrification under low NO\textsubscript{3}\textsuperscript{-} loading when labile organic carbon is high in some estuarine and coastal ecosystems (Megonigal et al. 2004; Algar and Vallino 2014; Hardison et al. 2015; Peng et al. 2016). Further study of the interaction between denitrification and DNRA in response to increased NO\textsubscript{3}\textsuperscript{-} loading is necessary to better understand how the role of coastal floodplains to process riverine NO\textsubscript{3}\textsuperscript{-} may have changed during decades of nutrient enrichment.
We evaluated the relative importance of denitrification, DNRA and anammox as a function of biotic feedback to soil development (three ranges in SOM), and to decadal changes in river fertilization (comparing reduced to enriched NO$_3^-$ concentrations). Coupled nitrification-denitrification rates were measured to determine its significance to gross denitrification and total N$_2$ loss under different conditions of SOM concentration and NO$_3^-$ loading. We propose that evaluating shifts in N dynamics in newly emergent coastal deltaic floodplains under different NO$_3^-$ concentrations helps define the role of coastal deltaic floodplain ecosystems in reducing eutrophication to coastal oceans (Twilley and Rivera-Monroy 2009; Paola et al. 2011). These patterns serve as an analog of benthic N dynamics during the early stages of deltaic development in other continental margins of major rivers around the world.

METHODS

Wax Lake Delta (WLD) is a newly emergent coastal deltaic floodplain within the Atchafalaya Basin in the Mississippi River Delta in coastal Louisiana, United States (Fig. 3.1). It is a river-dominated delta with small tidal range (mean = 0.4 m) and wave height (monthly maximum = 0.5 m; Syvitski and Saito 2007; Edmonds et al. 2011; Shaw and Mohrig 2014). The deltaic floodplain has been developing for 46 yrs, and sediment stoichiometry suggests the productivity is limited by N based upon low molar N:phosphorus (P) ratio (0 to 15.6; Henry and Twilley 2014; Bevington and Twilley 2018; Aarons 2019; Li et al. 2020; Twilley et al. 2019). Annual NO$_3^-$ loading to the Atchafalaya Basin is around 140,000 Mg N, with about half transported to Wax Lake Outlet (WLO) and 25% enters the interdistributary islands of WLD (Allison et al. 2012; Hiatt and Passalacqua 2015). NO$_3^-$ removal in WLD accounted for 10 to 27% of total riverine NO$_3^-$ load to WLD, and the benthic NO$_3^-$ fluxes were reported to be linked to deltaic development with increased SOM (Henry and Twilley 2014; Li et al. 2020). The strong increasing pattern of SOM from
younger subtidal zones to older supratidal zones with increased biotic feedback in WLD makes it an ideal ecosystem to investigate the influence of SOM enrichment as depositional environments with ecological succession controlling benthic N dynamics (Li et al. 2020).

Figure 3.1. Map of Mike Island in the Wax Lake Delta (WLD), Louisiana, with the location of study sites. Experimental sites include lower sediment organic matter (Lower-SOM), intermediate sediment organic matter (Int-SOM) and higher sediment organic matter (Higher-SOM). Elevation records are from USGS Atchafalaya 2 project LiDAR Survey 2012 digital elevation model (4m resolution).

Benthic fluxes of inorganic nutrients and dissolved gases ($O_2$, $N_2$) were examined at three sites representing lower, intermediate, and higher SOM concentrations in WLD in the summer 2018 (Fig. 3.1). Lower-SOM site (29°28’48.4”N, 91°26’53.4”W) is a young subtidal hydrogeomorphic site ($\leq$ 20 yr since emergence, about -0.6 m NAVD 88 in elevation) with
mainly mineral sedimentation. Submersed aquatics in this site represents the very early
development stage of a coastal deltaic floodplain (Bevington and Twilley 2018). Sites with
intermediate SOM concentrations (int-SOM) and higher SOM concentrations (higher-SOM)
were supratidal sites (≥ 30 yr) with increased biotic feedback on soil development. The int-
SOM site (29°30’47.9”N, 91°26’33.4”W, about 0.2 m NAVD 88 in elevation) is located near
a levee along a primary channel of WLD, occupied by trees (Salix nigra) and exposed to
flood pulses of inorganic sediments (Bevington and Twilley 2018; Li et al. 2020). The
higher-SOM site (29°30’21.2’’N, 91°26’18.5”; about 0.3 m NAVD 88 in elevation), in
contrast, is located more interior from the int-SOM site with less flood disturbance, and
greater biotic feedback from Colocasia esculenta as the dominate plant.

In situ surface and porewater samples were collected at each of the three sites and
stored on ice. In situ temperature and salinity of surface water and porewater were measured
with a YSI salinity-conductivity-temperature meter. Water samples were filtered immediately
after arrival at the laboratory using GF/F glass microfiber filters (25mm diameter, 0.7 µm
particle retention) and stored frozen for inorganic nutrient analyses. Concentrations of
ammonium (NH₄⁺), nitrite (NO₂⁻), NO₃⁻ and phosphate (PO₄³⁻) were measured with a flow
solution IV autoanalyzer (OI analytical, College Station, Texas).

Twelve intact sediment cores (10cm internal diameter by 20cm depth) with 10 ± 1cm
sediments in height and 10 ± 1cm ambient overlying water in height were collected at each
study site. Aboveground vegetation was excluded but rhizomes, roots, infauna and benthic
algae were collected in sediment cores. Cores were sealed with silicone-greased bottoms and
capped with detachable lids, then stored in a cooler under in situ temperature and transported
to lab within 4 h. Ambient water was collected from Wax Lake Outlet (WLO) and filtered
using a five-stage filtration system (30, 20, 5, 1, and 0.2 µm) in the laboratory. Isotopes were
added to the filtered water and four treatments were conducted in each site: TRT 1 = ~5 µM
$^{15}$NO$_3^-$ addition after nitrate-specific resin (ResinTech SIR-100-HP, West Berlin, New Jersey) removed all background NO$_3^-$ (K$^{15}$NO$_3^-$, 99%, Cambridge stable isotope laboratories) (Henry and Twilley 2014); TRT 2 = ~100 µM $^{15}$NO$_3^-$ addition after resin removed all background NO$_3^-$; TRT 3 = ~50 µM $^{15}$NH$_4^+$ addition ($^{15}$NH$_4$Cl, 99%, Cambridge stable isotope laboratories) in aerobic condition; and TRT 4 = ~50 µM $^{15}$NH$_4^+$ addition in anaerobic condition (helium was bubbled in inflow water reservoir). Triplicate cores were assigned randomly to each of the four treatments in each site. Overlying water in each sediment core was gently replaced with treated filtered water with minimal disturbance to the sediment-water interfaces. Each core was then sealed with a lid containing inlet and outlet tubing and affixed with a magnetic stir bar to gently mix the overlying water (Henry and Twilley 2014).

Sediment cores were incubated in a dark water chamber at room temperature (around 20.8 ± 0.4°C) and a continuous flow through system was used in core incubations (Miller-Way and Twilley 1996). Briefly, sediment cores were connected to water reservoirs with different isotope treatments and a peristaltic pump was used to adjust the flow rate to 4 to 5 ml min$^{-1}$ (overlying water residence time ~3 h). Cores were pre-incubated for 10 h to allow fluxes at sediment-water interfaces to reach equilibrium and bubble-free conditions were maintained during incubations. After the pre-incubation period, influent and effluent water samples were collected at each completion (~3 h) of three turnovers of the overlying water per experiment. Water samples were filtered through 25 mm GF/F glass microfiber filers (0.7 µm particle retention) immediately and stored frozen before measuring inorganic nutrients ($\text{NH}_4^+$, NO$_2^-$, NO$_3^-$ and PO$_4^{3-}$). Additional 60 ml water samples were collected and filtered for every overlying water turnover and stored frozen to measure dissolved $^{15}$NH$_4^+$ concentrations for sediment cores in TRTs 1 and 2 with $^{15}$NO$_3^-$ addition. For each of the three turnovers during incubation, replicate samples were collected in 12ml gas-tight exetainers (Labco Limited, Lampeter, Wales, UK) for gas analysis, and 200 µL of ZnCl$_2$ solution (50%
saturation concentration) was injected before capping to stop biological activities (Nielsen and Glud 1996). Dissolved gas samples were stored in a water bath at 4°C and analyzed for dissolved $^{28}\text{N}_2$, $^{29}\text{N}_2$ and $^{30}\text{N}_2$ on a membrane inlet mass spectrometer (MIMS) within one month (Kana et al. 1994). Dissolved oxygen concentrations were measured in influent waters and in every sediment core as the effluent waters at the end of each turnover using a dissolved oxygen meter (HQ40d, Hach, USA). Benthic fluxes of inorganic nutrients and dissolved gas ($\text{N}_2$ and $\text{O}_2$) were determined by the equation:

$$\text{Flux} = \frac{(C_e-C_i) \times \text{flow rate}}{\text{Core surface area}} \quad (1)$$

where $C_e$ and $C_i$ refer to effluent and influent concentrations ($\mu$M).

Denitrification is categorized as ‘direct denitrification’ that reduces external NO$_3^-$ to $\text{N}_2$ gas and ‘coupled nitrification-denitrification’ that uses NO$_3^-$ generated by in situ nitrification as electron acceptor. Direct denitrification and coupled nitrification-denitrification rates were calculated in TRTs 1 and 2 with 5 $\mu$M and 100 $\mu$M $^{15}$NO$_3^-$ additions following the IPT method outlined by Nielsen (1992). Direct denitrification rates ($D_w$) supported by $^{15}$NO$_3^-$ in overlying water were calculated from the sum of the produced $^{15}$N-$\text{N}_2$, while coupled nitrification-denitrification ($D_n$) was calculated from $D_w$ assuming denitrified $^{15}$NO$_3^-$ and $^{14}$NO$_3^-$ were uniformly mixed:

$$D_w = ^{14}_N^{15}N + 2 \times ^{15}_N^{15}N \quad (2)$$

$$D_n = D_w \times \frac{^{14}_N^{15}N}{2 \times ^{15}N^{15}N} \quad (3)$$

The int-SOM site did not have $D_n$ measurements because the production of $^{15}N^{15}N$ was zero and equation (3) was invalid.

Coupled nitrification-denitrification rates were also calculated directly in TRT 3 with 50 $\mu$M $^{15}$NH$_4^+$ addition in aerobic condition using the equation:
\[ D_{n'} = ^{14}\text{N}^{15}\text{N} + 2 \times ^{15}\text{N}^{15}\text{N} + \frac{C_{14}\text{NH}_4}{C_{15}\text{NH}_4} (^{14}\text{N}^{15}\text{N} + 2\times^{15}\text{N}^{15}\text{N}) \] (4)

where C14NH4 and C15NH4 represented the concentrations of \(^{14}\text{NH}_4^+\) and \(^{15}\text{NH}_4^+\) in the overlying water, which was measured using an autoanalyzer prior to and following the addition of \(^{15}\text{NH}_4^+\) into the inflow reservoir. This equation assumes that the added \(^{15}\text{NH}_4^+\) uniformly mixed with endogenous \(^{14}\text{NH}_4^+\) in the incubation system and there was equal probability that these two molecules were used in nitrification. \(^{15}\text{NH}_4^+\) concentrations in the overlying water column were stable with time in our experiments, indicating steady state conditions during incubation (Miller-Way and Twilley 1996). Thus, we used the concentration ratio of \(^{14}\text{NH}_4^+\) to \(^{15}\text{NH}_4^+\) in the overlying water column to represent the ratio at the sediment-water interface. This method may overestimate coupled nitrification-denitrification because anammox may occur in the anaerobic sediment layer that contributes certain amounts of \(^{14}\text{N}^{15}\text{N}\) to the effluent water.

We used OX/MIMS method in the measurement of effluent \(^{15}\text{NH}_4^+\) concentrations to determine rates of DNRA in TRTs 1 and 2 with \(^{15}\text{NO}_3^-\) additions (Yin et al. 2014). Briefly, an OX/MIMS calibration was prepared with a concentration gradient of 0, 0.1, 0.25, 0.5, 1.0, 2.5, 5, 10, 25 µM \(^{15}\text{NH}_4\text{Cl}\) and measured on MIMS. Each water sample (60 ml) was transferred to three gas-tight exetainers after samples were defrosted. One exetainer was run on MIMS as baseline \(^{15}\text{N}-\text{N}_2\), while the other two were measured on MIMS right after adding 200 µL hypobromite reagent to oxidize all \(^{\text{NH}_4}^+\) to \(^{\text{N}_2}\) gas. The difference of \(^{15}\text{N}-\text{N}_2\) between the baseline and each oxidized sample was from the oxidation of \(^{15}\text{NH}_4^+\) produced in DNRA. \(^{15}\text{NH}_4^+\) concentration in each water sample was then calculated from the standard calibration curve and DNRA rates were calculated based on the \(^{15}\text{NH}_4^+\) fluxes following equation (1).

Anammox rates were calculated from TRT 4 with 50 µM \(^{15}\text{NH}_4^+\) addition under anaerobic condition. We consider these rates as ‘potential’ anammox since we intentionally created anaerobic conditions, which are more favorable to anammox, to inhibit the
confounding signal of $^{29}$N$_2$ from coupled nitrification-denitrification. Under anaerobic condition the formulas of anammox with labeled $^{15}$NH$_4^+$ are as follows (Thamdrup and Dalsgaard 2002; Trimmer et al. 2003):

$$^{15}\text{NH}_4^+ + ^{14}\text{NO}_2^- \rightarrow 100\% ^{29}\text{N}_2 + 2 \text{H}_2\text{O}$$

(5)

$$5 ^{15}\text{NH}_4^+ + 3 ^{14}\text{NO}_3^- \rightarrow 75\% ^{29}\text{N}_2 \text{ and } 25\% ^{30}\text{N}_2 + 9\text{H}_2\text{O} + \text{H}^+$$

(6)

However, we only detected the production of $^{29}$N$_2$ as $^{30}$N$_2$ signal was negligible in our experimental sites. The anammox rates were then calculated as follows:

$$\text{Anammox} = ^{14}\text{N}^{15}\text{N} + \frac{^{14}\text{NH}_4}{^{15}\text{NH}_4} \times ^{14}\text{N}^{15}\text{N}$$

(7)

where C$^{14}$NH$_4$ and C$^{15}$NH$_4$ represented the concentrations of $^{14}$NH$_4^+$ and $^{15}$NH$_4^+$ in the overlying water.

Sediment bulk density and SOM content were measured in each core after incubations. In brief, the top 4 cm of sediment was collected using a piston core (2.4 cm internal diameter) and oven-dried at 60 °C to a constant mass. Bulk density was measured by dividing dry sediment mass by sediment volume (8.75 cm$^3$). Each dried sediment sample was ground to less than 250 μm and a 1 ± 0.01 g subsample was ignited at 550 °C for 2 h to estimate SOM content as % dry mass.

We used analysis of variance (ANOVA, p < 0.05) with repeated measures in each of the four treatments to test the influence of SOM content on each benthic process. Difference of benthic fluxes under reduced and enriched NO$_3^-$ concentrations in TRT 1 and 2 was tested using one-way ANOVA treating each experimental site as a block. The same method was also used to test the difference of coupled nitrification-denitrification rates measured in TRT 2 and 3. When the influences were significant, Tukey’s HSD test (p < 0.05) was then applied to do pairwise comparisons within experimental sites and letters designated significant.
differences. Data analyses were performed using JMP statistical software. Benthic fluxes were present as means with error bars of standard error (SE).

RESULTS

Experimental conditions

There were significant gradients in SOM concentration and bulk density among the three sites selected to test hypotheses in this study (Fig. 3.2). The mean SOM concentration in higher-SOM site was 22.5 %, which was significantly higher than SOM concentrations in lower-SOM (2.8 %) and int-SOM sites (5.9 %). In situ salinity in three sites varied from 0.2 to 0.4 with no obvious difference between surface water and porewater (Table 3.1). In situ NO$_3^-$ concentrations ranged from 13.3 to 46.4 µM in surface water compared to 0 to 2.4 µM in porewater. In situ NH$_4^+$ concentrations varied from 1.8 to 2.1 µM in surface water compared to 10.8 to 91.7 µM in porewater. In situ NO$_2^-$ and PO$_4^{3-}$ concentrations were low in both surface water and porewater in all three sites.

Incubation temperatures ranged from 19.0 to 22.4 °C and influent water was high in dissolved oxygen concentrations (7.6 to 8.4 g L$^{-1}$) in all treatments except for those bubbled with He for anoxic incubations (0.8 to 2.8 mg L$^{-1}$; Table 3.2). Influent $^{15}$NO$_3^-$ concentrations in TRTs 1 and 2 were controlled at 4.5 to 5.4 µM and 82.0 to 101.9 µM, respectively. Influent NO$_3^-$ in TRTs 3 and 4 were ambient waters from WLO with concentrations ranging from 53.1 to 116.1 µM. Influent NH$_4^+$ in TRTs 1 and 2 were ambient waters with low concentrations, while NH$_4^+$ in TRTs 3 and 4 were combinations of injected $^{15}$NH$_4^+$ (45 to 50 µM) and ambient $^{14}$NH$_4^+$. Influent NO$_2^-$ concentrations varied from 0 to 0.8 µM and PO$_4^{3-}$ concentrations varied from 0.4 to 3.1 µM in all treatments.
Figure 3.2. (A) Sediment organic matter content as % dry mass and (B) bulk density in the top 4 cm of sediment in each site (mean ±1 SE). Experimental sites include lower sediment organic matter (Lower-SOM), intermediate sediment organic matter (Int-SOM) and higher sediment organic matter (Higher-SOM). Letters designate significant differences among experimental sites using Tukey’s HSD test (p < 0.05).

**Benthic fluxes of dissolved oxygen and inorganic nutrients**

Benthic dissolved oxygen fluxes increased from lower-SOM to higher-SOM sites in TRTs 1-3 that represented aerobic incubation treatments (Table 3.3). The increase in overlying NO$_3^-$ concentrations from 5 to 100 µM (TRTs 1 and 2) did not generate significant difference in dissolved oxygen consumption. Benthic NO$_3^-$ fluxes indicated clear increase from lower-SOM to higher-SOM sites in TRT 2 (100 µM-$^{15}$NO$_3^-$), TRT 3 (aerobic-$^{15}$NH$_4^+$)
and TRT 4 (anaerobic-\(^{15}\)NH\(_4^+\)). NO\(_3^-\) fluxes with lower NO\(_3^-\) enrichment (5 \(\mu\)M) in TRT 1 were significantly lower than fluxes in all other treatments with much higher NO\(_3^-\) enrichments (Table 3.3). NO\(_2^-\) fluxes were higher in treatments with \(^{15}\)NH\(_4^+\) addition, especially in TRT 4 under anaerobic condition (ranged from 41 to 85 \(\mu\)mol m\(^{-2}\) h\(^{-1}\)). NH\(_4^+\) fluxes were mostly negative in TRTs 3 and 4 (\(^{15}\)NH\(_4^+\)additions under aerobic and anaerobic conditions) with int-SOM as the site with the highest NH\(_4^+\) uptake (Table 3.3). Benthic PO\(_4^{3-}\) fluxes were positive and much higher in TRT 4 under anaerobic condition compared to PO\(_4^{3-}\) fluxes in the other treatments with aerobic overlying water.

Table 3.1. Ambient surface water and porewater conditions at three experimental sites in the Wax Lake Delta (WLD), Louisiana. Experimental sites include lower sediment organic matter (lower-SOM), intermediate sediment organic matter (int-SOM) and higher sediment organic matter (higher-SOM). Note that int-OM site had no surface water during sampling.

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<tr>
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<th>Surface Water</th>
<th></th>
<th>Porewater</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower-SOM</td>
<td>Int-SOM</td>
<td>Higher-SOM</td>
<td>Lower-SOM</td>
<td>Int-SOM</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.3</td>
<td>NA</td>
<td>0.2</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>O(_2) (mg L(^{-1}))</td>
<td>5.7</td>
<td>NA</td>
<td>1.7</td>
<td>2.3</td>
<td>0.9</td>
</tr>
<tr>
<td>NO(_3) ((\mu)M)</td>
<td>46.4</td>
<td>93.3</td>
<td>13.3</td>
<td>2.4</td>
<td>0.2</td>
</tr>
<tr>
<td>NO(_2) ((\mu)M)</td>
<td>0.2</td>
<td>0.2</td>
<td>1.5</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>NH(_4) ((\mu)M)</td>
<td>2.1</td>
<td>0.9</td>
<td>1.8</td>
<td>91.7</td>
<td>10.8</td>
</tr>
<tr>
<td>PO(_4) ((\mu)M)</td>
<td>3.9</td>
<td>3.7</td>
<td>2.2</td>
<td>1.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>
Table 3.2. Incubation conditions of four treatments in three experimental sites in the Wax Lake Delta (WLD), Louisiana. Experimental sites include lower sediment organic matter (lower-SOM), intermediate sediment organic matter (int-SOM) and higher sediment organic matter (higher-SOM).

<table>
<thead>
<tr>
<th></th>
<th>TRT 1: 5 µM $^{15}$NO$_3$</th>
<th>TRT 2: 100 µM $^{15}$NO$_3$</th>
<th>TRT 3: Aerobic $^{15}$NH$_4$</th>
<th>TRT 4: Anaerobic $^{15}$NH$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (°C)</td>
<td>19.0</td>
<td>22.2</td>
<td>21.2</td>
<td>19.0</td>
</tr>
<tr>
<td>$O_2$ (mg L$^{-1}$)</td>
<td>8.4</td>
<td>8.1</td>
<td>8.1</td>
<td>8.4</td>
</tr>
<tr>
<td>NO$_3$ (µM)</td>
<td>5.4</td>
<td>5.2</td>
<td>4.5</td>
<td>97.9</td>
</tr>
<tr>
<td>NO$_2$ (µM)</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>NH$_4$ (µM)</td>
<td>0.6</td>
<td>0.4</td>
<td>NA</td>
<td>0.6</td>
</tr>
<tr>
<td>PO$_4$ (µM)</td>
<td>0.6</td>
<td>0.4</td>
<td>1.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Table 3.3. Benthic fluxes of dissolved oxygen and inorganic nutrients in four treatments in the Wax Lake Delta (WLD), Louisiana. Experimental sites include lower sediment organic matter (Lower-SOM), intermediate sediment organic matter (Int-SOM) and higher sediment organic matter (Higher-SOM). Treatment (TRT) 4 with anaerobic conditions had no record of O$_2$ fluxes. Higher-SOM sites in TRT 1 (5 µM $^{15}$NO$_3^-$) and 2 (100 µM $^{15}$NO$_3^-$) had no NH$_4^+$ measurements due to analytical errors.

<table>
<thead>
<tr>
<th>Flux</th>
<th>TRT 1: 5 µM $^{15}$NO$_3$</th>
<th>TRT 2: 100 µM $^{15}$NO$_3$</th>
<th>TRT 3: Aerobic $^{15}$NH$_4$</th>
<th>TRT 4: Anaerobic $^{15}$NH$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$ (g O$_2$ m$^{-2}$ d$^{-1}$)</td>
<td>-1.0</td>
<td>-1.2</td>
<td>-2.4</td>
<td>-0.8</td>
</tr>
<tr>
<td></td>
<td>(0.1)</td>
<td>(0.1)</td>
<td>(0.2)</td>
<td>(0.0)</td>
</tr>
<tr>
<td>NO$_3^-$ (µmol m$^{-2}$ h$^{-1}$)</td>
<td>1.6</td>
<td>-49.2</td>
<td>-19.9</td>
<td>-4.3</td>
</tr>
<tr>
<td></td>
<td>(4.0)</td>
<td>(8.6)</td>
<td>(9.9)</td>
<td>(21.6)</td>
</tr>
<tr>
<td>NO$_2^-$ (µmol m$^{-2}$ h$^{-1}$)</td>
<td>0.7</td>
<td>0.4</td>
<td>2.1</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>(0.6)</td>
<td>(0.2)</td>
<td>(0.5)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>NH$_4^+$ (µmol m$^{-2}$ h$^{-1}$)</td>
<td>53.6</td>
<td>3.7</td>
<td>NA</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>(17.2)</td>
<td>(0.7)</td>
<td>(47.7)</td>
<td>(2.7)</td>
</tr>
<tr>
<td>PO$_4^{3-}$ (µmol m$^{-2}$ h$^{-1}$)</td>
<td>7.2</td>
<td>4.3</td>
<td>-3.2</td>
<td>-6.8</td>
</tr>
<tr>
<td></td>
<td>(2.3)</td>
<td>(1.0)</td>
<td>(4.5)</td>
<td>(2.7)</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Nitrogen-15 dynamics

Gross denitrification rates ranged from 4 to 12 µmol N m⁻² h⁻¹ in TRT 1 (Fig. 3.3A, 5 µM ¹⁵NO₃⁻ addition), which were significantly (p<0.05) lower than the rates in corresponding sites in TRT 2 with 100 µM ¹⁵NO₃⁻ enrichment (68 to 276 µmol N m⁻² h⁻¹, Fig 3.3B). Gross denitrification increased 19-fold on average in response to the increase in NO₃⁻ concentrations from 5 to 100 µM, most of which was an increase in direct denitrification rates rather than coupled nitrification-denitrification rates. Direct denitrification, as the dominant benthic process in the experimental sites, was one to two magnitudes larger than coupled nitrification-denitrification. In TRT 2, coupled nitrification-denitrification only accounted for 4.0 % of gross denitrification in lower-SOM site, 14.4 % in int-SOM site and 1.4 % in higher-SOM site, respectively. Higher-SOM site had the highest mean gross denitrification rate of 230 µmol N m⁻² h⁻¹ compared to lower-SOM and int-SOM sites in TRT 2 with 100 µM ¹⁵NO₃⁻ additions. About 99% of the N removed in high-SOM site was from direct denitrification which used ¹⁵NO₃⁻ from the overlying water column. Coupled nitrification-denitrification (Dn’) rates directly measured from ¹⁵NH₄⁺ addition (TRT 3, Fig 3.4A) were not significantly different to estimated rates (Dn) from ¹⁵NO₃⁻ addition in TRT 2 (Fig. 3.3B). Both results from TRT 2 and 3 (Dn and Dn’) indicate that the int-SOM site was the most efficient site on coupled nitrification-denitrification among the three sites under higher NO₃⁻ enrichment (100 µM).

DNRA rates measured in this study using the continuous flow-through incubation system varied from 0 to 65 µmol N m⁻² h⁻¹ with the higher DNRA fluxes (mean = 32 ± 6 µmol N m⁻² h⁻¹) observed in the higher-SOM site in TRT 2 with 100 µM-¹⁵NO₃⁻ addition (Fig. 3.3D). The proportion of DNRA to total NO₃⁻ loss through dissimilatory pathways varied from 0 to 12% in TRT 2, the rest of which is contributed by denitrification. In TRT 1 with 5 µM-¹⁵NO₃⁻ addition (Fig.3.3C), the relative importance of DNRA increased to 28% in
lower-SOM site and to 21% in higher-SOM site, respectively. Anammox rates varied from 0 to 38 \( \mu \text{mol N m}^{-2} \text{h}^{-1} \) with an increase from lower-SOM to higher-SOM sites (Fig. 3.4B).

Figure 3.3. Denitrification and dissimilatory nitrate reduction to ammonium (DNRA) rates for cores in treatments (TRT) 1 and 2 using different concentrations of nitrate enrichment: 5 \( \mu \text{M} \, ^{15}\text{NO}_3^- \) addition as shown in grey columns (A and C, respectively) and 100 \( \mu \text{M} \, ^{15}\text{NO}_3^- \) addition as shown in blue columns (B and D, respectively). Striped bars in figure A and B indicate direct denitrification (Dw), and solid bars in figure A and B represent coupled nitrification-denitrification (Dn). Error bars reflect standard errors in experimental sites. Experimental sites include lower sediment organic matter (lower-SOM), intermediate sediment organic matter (int-SOM) and higher sediment organic matter (higher-SOM).
Figure 3.4. (A) Coupled nitrification-denitrification rates (Dn') directly measured in cores treated with 50 µM 15NH4+ addition under aerobic condition (TRT 3), and (B) potential anammox rates measured from cores treated with 50 µM 15NH4+ addition under anaerobic condition (TRT 4). Error bars reflect standard errors in experimental sites. Experimental sites include lower sediment organic matter (lower-SOM), intermediate sediment organic matter (int-SOM) and higher sediment organic matter (higher-SOM).

DISCUSSION

Benthic N dynamics respond to NO3− enrichment

Benthic N dynamics in coastal ecosystems are closely related to NO3− concentrations in overlying waters (White and Reddy 1999; Fixen and West 2002; Rabalais et al. 2002; Canfield et al. 2010; Koop-Jakobsen and Giblin 2010; Hardison et al. 2015). Benthic denitrification is stimulated due to the anthropogenic increase of bioavailable N in coastal systems, while nitrogen fixation, as an energetically expensive process, is reported to decrease (Howarth et al. 1988; Mulholland et al. 2001; Capone et al. 2008; Scott et al. 2008;
Koop-Jakobsen and Giblin 2010; Hardison et al. 2015). Our results indicated that both direct denitrification and coupled nitrification-denitrification in WLD were promoted by increasing NO$_3^-$ concentrations, which is supported by other studies in coastal wetlands and estuaries (Hamersley and Howes 2005; Drake et al. 2009; Koop-Jakobsen and Giblin 2010; Scaroni et al. 2011). Sites with higher SOM were more sensitive to increased NO$_3^-$ concentrations compared to those with lower SOM, as demonstrated by an increase of 2.3 µmol N m$^{-2}$ h$^{-1}$ in direct denitrification for each µM increase in NO$_3^-$ concentration. Coupled nitrification-denitrification was relatively insignificant compared to direct denitrification across the SOM gradient, and the relative importance of coupled nitrification-denitrification to gross denitrification decreased as overlying NO$_3^-$ concentration increased. Nitrification requires NH$_4^+$ and labile organic carbon under aerobic conditions while denitrification requires NO$_3^-$ and labile organic carbon under anaerobic conditions (Eyre and Ferguson 2009; Damashek and Francis 2018). Increasing NO$_3^-$ loading directly favored direct denitrification rather than coupled nitrification-denitrification and, thus, decreased the relatively importance of coupled nitrification-denitrification to gross denitrification.

DNRA rates measured in this study using continuous flow-through incubation system varied from 0 to 65 µmol N m$^{-2}$ h$^{-1}$, which is comparable to rates in Florida (11-242 µmol N m$^{-2}$ h$^{-1}$), Alabama (0-237 µmol N m$^{-2}$ h$^{-1}$) and Texas (0-79 µmol N m$^{-2}$ h$^{-1}$) for sediments in estuarine ecosystems (An and Gardner 2002; Scott et al. 2008; Gardner and McCarthy 2009; Koop-Jakobsen and Giblin 2010; Bernard et al. 2015). Increased overlying NO$_3^-$ loading stimulated DNRA only under conditions of higher SOM. The relative importance of DNRA to total NO$_3^-$ loss through dissimilatory pathways was not enhanced by NO$_3^-$ fertilization as denitrification was more sensitive to increased NO$_3^-$ loading than DNRA in WLD. Based on previous research, DNRA is comparable or even larger than denitrification in many saline estuarine and coastal ecosystems since sulfide from sulfate reduction in saline systems.
significantly inhibits nitrification and denitrification (Gardner et al. 1997; An and Gardner 2002; Koop-Jakobsen and Giblin 2010; Bernard et al. 2015; Hardison et al. 2015). Unlike saline coastal regions, WLD is a tidal freshwater ecosystem with salinities ≤ 0.4 that provides a favorable condition for denitrification rather than DNRA. The lower contribution of DNRA compared to denitrification in total NO$_3^-$ loss indicates that NO$_3^-$ reduction to N$_2$ gas in WLD can potentially reduce eutrophication as river waters connect with deltaic floodplain wetlands. We emphasize that DNRA rates measured here might be conservative because a portion of $^{15}$NH$_4^+$ released through DNRA might exchange with absorbed $^{14}$NH$_4^+$ on sediment particles and be retained in sediments rather than exchanging with overlying water (Rosenfeld 1979; Gardner et al. 2006). Also, the occurrence of coupled nitrification-denitrification might use some of the released $^{15}$NH$_4^+$, which could underestimate DNRA rates (Gardner and McCarthy 2009; Yin et al. 2014).

Benthic N dynamics respond to SOM content

During the early stages of deltaic development from subtidal to intertidal and supratidal hydrogeomorphic zones, SOM content increases with decreased bulk density from younger to older chronosequence zones over decadal time scales (Henry and Twilley 2014; Li et al. 2020). Together with increases in soil surface elevation and SOM content is the zonation of vegetation communities from wetland fringe to interior and from inland to open water (Ma et al. 2018). The significant inverse relation between bulk density and SOM concentrations across hydrogeomorphic and chronosequence zones supports evidence that ecological succession of deltaic vegetation communities is a significant feedback in soil development as coastal deltaic floodplains emerge and increase in elevation (Bevington and Twilley 2018; Twilley et al., 2019; Ma et al. 2018). Vegetative succession, riverine flooding events and shifts in hydrogeomorphic zones lead to distinct SOM concentrations in our three experimental sites, each of which reflects different benthic N dynamics (Li et al. 2020).
Previous studies have found that denitrification rates are highly correlated with SOM concentrations in active coastal deltaic floodplains (Henry and Twilley 2014; Li et al. 2020). Direct denitrification measured here showed an increase with SOM content in TRT 2 with 100 µM $^{15}$NO$_3^-$ addition (Fig. 3.5A), but coupled nitrification-denitrification did not exhibit such pattern with SOM concentration (Fig. 3.5B). Coupled nitrification-denitrification rates ($Dn'$) directly measured from $^{15}$NH$_4^+$ addition were not significantly different to estimated rates ($Dn$) from $^{15}$NO$_3^-$ addition based on $^{29}$N$_2/^{15}$N$_2$ production ratio, indicating the influence of anammox in the aerobic incubation of TRT 3 was negligible. Both measurements indicated that coupled nitrification-denitrification from the int-SOM site was higher than rates from lower-SOM and higher-SOM sites. The lower NH$_4^+$ release in TRTs 1 and 2 and higher $^{14}$NH$_4^+$ uptake in TRTs 3 and 4 in int-SOM site also support the variation trend that int-SOM was more efficient in coupled nitrification-denitrification than other sites. Previous studies indicate that nitrification rates are stimulated by increase in SOM, but only to a threshold value of SOM (Caffrey et al. 2003; Wankel et al. 2011; Damashek and Francis 2018). Further addition in SOM past the threshold induces elevated benthic metabolism, which depletes benthic oxygen and inhibits nitrification, like the situation in our higher-SOM site (Caffrey et al. 1993; Cornwell et al. 1999). The relatively low coupled nitrification-denitrification rates in the lower-SOM site were very likely due to limited labile organic carbon, while the low coupled nitrification-denitrification rates in the higher-SOM site was due to minimal aerobic sediment zone as a result of increasing benthic metabolism.
Figure 3.5. Relationship of different nitrogen fluxes with sediment organic matter content (% dry mass) as follows: (A) direct denitrification, (B) coupled nitrification-denitrification (both Dn and Dn'), (C) DNRA and (D) anammox to sediment organic matter content in cores with overlying water enriched with 100 µM $\text{NO}_3^-$ (either $^{15}\text{NO}_3^-$ or $^{14}\text{NO}_3^-$). Error bars reflect standard errors in experimental sites. Green circles represent lower-SOM site, red circles represent int-SOM site and blue circles indicate higher-SOM site. Solid circles in figure B represent coupled nitrification-denitrification (Dn) estimated from 100 µM $^{15}\text{NO}_3^-$ addition. Open circles represent coupled nitrification-denitrification rates (Dn') directly measured in cores treated with 50 µM $^{15}\text{NH}_4^+$ addition under aerobic condition.
In our study, DNRA is positively correlated with SOM content under TRT 2 with 100 µM $^{15}$NO$_3^-$ addition (Fig. 3.5C), which is in agreement with previous research results explaining higher organic carbon as a prerequisite to stimulate DNRA (Hardison et al. 2015). Previous research document that DNRA is highly correlated with labile organic carbon to NO$_3^-$ ratio because DNRA, which obtains more electrons per NO$_3^-$ molecule than denitrification, is more competitive in anaerobic environments where organic carbon is enriched and NO$_3^-$ is limited (Tiedje et al. 1983; Algar and Vallino 2014; Hardison et al. 2015). DNRA rate in higher-SOM site under lower (5 µM) NO$_3^-$ enrichment was much smaller than the corresponding rate under higher (100 µM) NO$_3^-$ enrichment in our study, which seems inconsistent with previous research results. However, lower NO$_3^-$ enrichment increased the relative importance of DNRA to total NO$_3^-$ loss through dissimilatory pathways in WLD. For example, the relative importance of DNRA in higher-SOM site increased from 12% to 21% as overlying NO$_3^-$ concentrations decreased from 100 µM to 5 µM.

**Anammox rates respond to SOM content**

Our results confirm the presence of anammox in the newly emergent coastal deltaic floodplain. The proportion of potential anammox to N$_2$ loss in WLD sediments ranged from 4% to 17%, suggesting that denitrification, rather than anammox, accounted for the bulk of N$_2$ loss in the tidal freshwater ecosystem. The contribution of anammox estimated in WLD might be overestimated because anammox rates measured here were potential rates with more favorable environment of higher NH$_4^+$ input and low dissolved oxygen concentrations. However, the proportion of anammox to N$_2$ loss measured here is consistent with previous research results in estuarine and coastal sediments with the proportion of anammox ranged from 0 to 14% (Thamdrup and Dalsgaard 2002; Dalsgaard et al. 2003; Trimmer et al. 2003; Koop-Jakobsen and Giblin 2009; Brin et al. 2014; Lisa et al. 2014).
The significance of anammox to N cycling in coastal ecosystems is related to SOM concentrations and nutrient input (Thamdrup and Dalsgaard 2002; Trimmer et al. 2003; Risgaard-Petersen et al. 2004; Brin et al. 2014). Some reports find that anammox is greater as SOM increases with higher bacterial activity and higher NH$_4^+$ supply from SOM mineralization (Meyer et al. 2005; Lisa et al. 2014). Some research describe anammox as inversely related to SOM for the reason that denitrification is favored in higher SOM concentrations and thus outcompetes anammox (Thamdrup and Dalsgaard 2002; Trimmer et al. 2003; Jin et al. 2012). Based on our data, anammox rates in int-SOM and higher-SOM sites were significantly higher (p<0.05) than rates in lower-SOM site and the correlation between SOM and anammox was positive (Fig. 3.5D). The positive correlation between anammox and SOM may be due to higher NO$_2^-$ release through incomplete denitrification and nitrification and/or higher NH$_4^+$ release through organic matter mineralization along SOM gradient (Meyer et al. 2005; Engström et al. 2005b; Lisa et al. 2014).

Additions of $^{15}$NH$_4^+$ under anaerobic conditions stimulated NO$_2^-$ effluxes from sediments to the overlying water column. Higher NO$_2^-$ effluxes may be due to incomplete nitrification and/or denitrification. If NO$_2^-$ effluxes were due to incomplete nitrification, most of NO$_2^-$ should be labeled as $^{15}$N and these labeled $^{15}$NO$_2^-$ molecules would react with $^{15}$NH$_4^+$ and form $^{30}$N$_2$. However, there was no obvious $^{30}$N$_2$ efflux in cores from TRT 4 with $^{15}$NH$_4^+$ addition under anaerobic condition, indicating that NO$_2^-$ effluxes were mainly $^{14}$NO$_2^-$ from incomplete denitrification. Increased NO$_2^-$ effluxes through incomplete denitrification might further stimulate anammox. This speculation is supported by similar patterns between NO$_2^-$ effluxes and the proportion of anammox to N$_2$ loss as follows: int-SOM site had the highest NO$_2^-$ effluxes and the highest proportion of anammox (85 µmol m$^{-2}$ h$^{-1}$ and 17%, respectively) followed by higher-SOM (59 µmol m$^{-2}$ h$^{-1}$ and 9%) and lower-SOM sites (41 µmol m$^{-2}$ h$^{-1}$ and 4%).
There are still several uncertainties in our understanding of anammox in response to SOM in WLD. For example, anaerobic conditions in the anammox incubation (TRT 4) slightly changed with oxygen concentrations varied from 2.8 to 0.8 mg L\(^{-1}\) from lower-SOM to higher-SOM sites, which might impact anammox rates. Increase in anammox might be related to increasingly anaerobic conditions which favored anammox. The combination of higher SOM to more complete anaerobic conditions may also be evident in the field, given higher respiration rates and longer water residence time in the area with higher SOM concentration. The comparison of laboratory and field conditions as to how anaerobic conditions behave at sites with higher SOM will need further investigations to generalize any patterns of anammox in coastal deltaic floodplains.

**Comparing denitrification rates from the IPT and N\(_2\):Ar methods**

The gross denitrification rates under 100 µM \(^{15}\)NO\(_3\)^\(-\) addition varied from 87 to 229 µmol m\(^{-2}\) h\(^{-1}\), which were lower than net denitrification rates measured one year earlier using the N\(_2\):Ar method in the similar experimental locations in WLD (Fig. 3.6, 198 to 911 µmol m\(^{-2}\) h\(^{-1}\), Li et al. 2020). First of all, denitrification rates measured from the N\(_2\):Ar method, unlike gross denitrification, are net denitrification rates that represent a combination of processes that produce N\(_2\) (anammox and denitrification) and a process that consumes N\(_2\) (N\(_2\) fixation). Higher net denitrification rates from the N\(_2\):Ar method might be due to the interference of higher anammox rates and lower N\(_2\) fixation occurred during incubations, which contributed positive net denitrification rates. Secondly, these two sets of denitrification results are from two different years (except for the int-SOM site) and the exact locations of three sites in this research are slightly different to the corresponding ones in Li et al. (2020). As reported, biogeochemical heterogeneity over time and space is common in wetland sediments (Mermillod-Blondin et al. 2003; Michaud et al. 2003; Wenzhöfer and Glud 2004; Stockdale et al. 2009). Denitrification rates measured in different years and slightly different locations
may be different due to physical changes in sediments with different benthic communities and biogeochemical processes (Michaud et al. 2003).

Figure 3.6. Denitrification rates measured from the isotope pairing technique (IPT) with 100 μM $^{15}$NO$_3^-$ addition to the overlying water (blue bars) compared to net denitrification rates measured using the N$_2$:Ar method (red bars) in Li et al., 2020 in the similar experimental locations. Error bars reflect standard errors in experimental sites.

In addition, differences in methodology may account for the major disparity in denitrification rates since the IPT and N$_2$:Ar methods are based on quite different assumptions (Eyre et al. 2002; Cook et al. 2006; Ferguson and Eyre 2007). IPT assumes that the added isotopes mix homogenously with endogenous NO$_3^-$ without changing the natural uptake of NO$_3^-$ during denitrification. The IPT calculated gross denitrification rates based on the production of labelled $^{15}$N-N$_2$ gas and a ratio of released $^{29}$N$_2^{/30}$N$_2$ to quantify coupled nitrification-denitrification. However, the N$_2$:Ar method attributes all the concentration differences of dissolved N$_2$ between inflow and outflow to denitrification, whereas the dissolved N$_2$ concentrations are very sensitive to gas bubbles in overlying waters, temperature and air pressure changes during incubations (Eyre et al. 2002). N$_2$:Ar method is
frequently reported to give two to four folds higher denitrifications rates than the IPT method (Eyre et al. 2002; Cook et al. 2006; Ferguson and Eyre 2007). The relatively smaller gross denitrification rates measured by IPT demonstrated that previous estimate that WLD could remove 10% to 27% of total NO$_3^-$ loading to the delta using the N$_2$:Ar method may be overestimated (Li et al. 2020). Nevertheless, the capacity of NO$_3^-$ removal in WLD continuous to increase as WLD grows from subaqueous to emergent delta that expands the deltaic area to process riverine NO$_3^-$ prior to export to coastal ocean (Li et al. 2020). The newly formed deltaic zone with mainly mineral sedimentation, like our lower-SOM site, usually has low SOM and longer flooding duration because of lower soil surface elevation (Bevington and Twilley 2018; Li et al. 2020). Though the sites with lower SOM have relatively low denitrification rates, frequent inundation makes this area fully contact with riverine NO$_3^-$ and remove potential amount of riverine NO$_3^-$. Except for annual progradation of WLD, there is a shift in proportion of hydrogeomorphic zones from subtidal to intertidal and supratidal zones in response to organic matter accumulation as emergent vegetation expands with increased soil elevation (Li et al. 2020). Increased SOM during deltaic development facilitates denitrification more than DNRA in WLD, which further contributes to permanent NO$_3^-$ removal. Thus, coastal deltaic floodplains like WLD are important depositional landscapes to permanently remove excess riverine nutrients and reduce coastal eutrophication through direct denitrification.

CONCLUSION

We incubated intact sediment cores from sites with different SOM concentrations that represented distinct succession stages of coastal deltaic floodplains using continuous flow-through system. Direct denitrification, coupled nitrification-denitrification and DNRA were measured simultaneously, indicating the coexistence of these processes in sediment-water interface in coastal deltaic floodplains. Significant anammox rates were observed in intact
core incubations under continuous flow-through system without the interference of coupled nitrification-denitrification. We found that direct denitrification was the major process of NO$_3^-$ removal whereas coupled nitrification-denitrification, DNRA and anammox were one to two magnitudes smaller than denitrification in the tidal freshwater ecosystem of WLD. The significantly higher direct denitrification and coupled nitrification-denitrification rates under higher-NO$_3^-$ enrichment (TRT 2) compared to rates under lower-NO$_3^-$ enrichment (TRT 1) demonstrated that N$_2$ production through denitrification was strongly stimulated by increased NO$_3^-$ loading in coastal deltaic floodplains. DNRA rates were enhanced by enriched NO$_3^-$ loading only in higher-SOM site. Increased NO$_3^-$ concentrations caused direct denitrification rates to increase to levels higher than coupled nitrification-denitrification and DNRA. Thus, the relative importance of coupled nitrification-denitrification to gross denitrification and the relative importance of DNRA to total NO$_3^-$ loss through dissimilatory pathways decreased with increasing NO$_3^-$ concentrations.

We investigated the responses of direct denitrification, coupled nitrification-denitrification, DNRA and anammox among three different ranges in SOM as a function of biotic feedback in WLD. Direct denitrification and DNRA increased as SOM increased from younger mineral-dominated subtidal sediments to older supratidal sediments with higher biotic feedback, but coupled nitrification-denitrification was higher in the site with intermediate SOM rather than the site with higher SOM. As SOM increased, the relative importance of DNRA to total NO$_3^-$ loss through dissimilatory pathways increased but the proportion of DNRA to total NO$_3^-$ loss remained low ($\leq$ 14% under enriched NO$_3^-$ addition). Potential anammox also increased with SOM concentrations. Continuous dominance of direct denitrification and increased rates of potential anammox among the increased SOM gradient indicated that the active coastal deltaic floodplain of WLD plays a significant role in permanent N removal to N$_2$ gas before riverine water export to coastal ocean. The N removal
capacity in WLD continuously increases as WLD grows from subaqueous to emergent delta that expands the deltaic area to process riverine NO$_3^-$.

The variation of benthic N dynamics with increasing NO$_3^-$ loading and SOM accumulation in coastal deltaic floodplains in this study provides better understanding of N cycling during the early stages of deltaic development in tidal freshwater ecosystems. Further studies examining N$_2$ fixation in response to increasing NO$_3^-$ loading and SOM enrichment will improve our understanding of how emerging coastal deltaic floodplains react to high NO$_3^-$ loading under different stages of deltaic succession. More anammox studies are required before drawing sound conclusion of the general patterns of anammox in response to SOM in coastal deltaic floodplains.
CHAPTER 4. HETEROTROPHIC NITROGEN FIXATION IN RESPONSE TO NITRATE LOADING AND SEDIMENT ORGANIC MATTER IN AN EMERGING COASTAL DELTAIC FLOODPLAIN WITHIN THE MISSISSIPPI RIVER DELTA PLAIN

INTRODUCTION

Agricultural fertilization has dramatically increased nitrogen (N) loading into riverine, estuarine and coastal ecosystems, which significantly alters benthic N dynamics in response to the change of nitrate (NO$_3^-$) availability (Canfield et al. 2010; Koop-Jakobsen and Giblin 2010). Historical NO$_3^-$ concentration was about 10 µM in 1900s in the Mississippi River Basin, which was 5 to 10 times lower than current NO$_3^-$ concentrations ranging from 54 to 106 µM (Goolsby et al. 2000; Rabalais et al. 2002). Before humans developed industrial processes to convert N$_2$ gas to bio-reactive N for agricultural use, biological N$_2$ fixation (both autotrophic and heterotrophic) was the key process providing bio-reactive N while denitrification was approximately balanced with N$_2$ fixation (Delwiche 1970; Galloway et al. 1995; Vitousek et al. 1997). With anthropogenic increase in bio-reactive N input to aquatic ecosystems, the energetically expensive process of heterotrophic N$_2$ fixation was often assumed negligible in eutrophic ecosystems (Howarth et al. 1988; Herbert 1999; Capone et al. 2008). However, recent studies indicate that heterotrophic N$_2$ fixation could be an important source of bio-reactive N in many coastal and marine ecosystems using newly developed research methods of stable isotope incubations and the direct measurement of N$_2$ fluxes (Gardner et al. 2006; Newell et al. 2016a). Other observations suggest that increasing N loading can switch heterotrophic marine sediments from being a net sink to being a net source of N$_2$ gas, and the dominance of denitrification rather than N$_2$ fixation reduces eutrophication of the coastal ocean (Fulweiler et al. 2007).

Heterotrophic N$_2$ fixation is performed by diazotrophic bacteria that break down the triple bond in N$_2$ and fix N into ammonia using the nitrogenase enzyme in concert with other
cofactors and enzymes (Postgate 1970; Klotz and Stein 2008). The nitrogenase is a complex enzyme comprising a heterotetrameric core and a dinitrogenase reductase subunit that is encoded by \textit{nifH}. A variety of \textit{nifH} sequences exist in estuarine and coastal sediments, which makes \textit{nifH} an ideal gene for molecular analyses of heterotrophic N-fixing microorganisms (Zehr et al. 2003; Damashek and Francis 2018). The abundance of \textit{nifH} gene determines the microbial potential for heterotrophic N\textsubscript{2} fixation rates in wetland sediments (Dias et al. 2012). N\textsubscript{2} fixation rates and diazotrophic community are related to the availability of dissolved inorganic N (DIN = NH\textsubscript{4}\textsuperscript{+} + NO\textsubscript{3}\textsuperscript{-} + NO\textsubscript{2}\textsuperscript{-}) and organic carbon (Fulweiler et al. 2007; Scott et al. 2008; Dias et al. 2012). N\textsubscript{2} fixation can be significantly repressed when NH\textsubscript{4}\textsuperscript{+} concentration is higher than 1 mM in the rhizosphere sediments of seagrass (Welsh et al. 1997; Welsh 2000) or when NO\textsubscript{3}\textsuperscript{-} concentration is higher than 10 µM in the ocean (Mulholland et al. 2001). However, the repression is not universal as many heterotrophic diazotrophic bacteria are not sensitive to increasing concentrations of DIN (McCarthy et al. 2016). Organic matter has no consistent influence on N\textsubscript{2} fixation as some research indicate that heterotrophic N\textsubscript{2} fixation rate is higher in organic-enriched sediments (Howarth et al. 1988; McCarthy et al. 2016) while some other studies show that N\textsubscript{2} fixation is facilitated when organic quantity is low in estuarine and marine ecosystems (Fulweiler et al. 2007, 2013).

Recent studies highlight the significance of heterotrophic N\textsubscript{2} fixation in different estuarine and coastal ecosystems with rates varying from 12 to 650 µmol N m\textsuperscript{-2} h\textsuperscript{-1} (Gardner et al. 2006; Fulweiler et al. 2007; McCarthy et al. 2016). However, fewer studies have documented the potential role of N\textsubscript{2} fixation in coastal deltaic floodplains at the interface of land and oceans, including mechanisms controlling N\textsubscript{2} fixation and the abundance of the diazotrophic community. Coastal deltaic floodplains form at the mouth of major river basins where NO\textsubscript{3}\textsuperscript{-} removal occurs before riverine nutrients export to the ocean (Bevington and
Twilley 2018; Twilley et al. 2019). Recent research at Wax Lake Delta (WLD) demonstrates that benthic NO$_3^-$ uptake and net denitrification rates increased with greater sediment organic matter (SOM) concentrations as a function of biotic feedback associated with coastal deltaic floodplain development (Li et al. 2020). Net NO$_3^-$ uptake and N$_2$ gas release at sediment-water interfaces in WLD are due to the dominance of direct denitrification compared to other N pathways like coupled nitrification-denitrification, anaerobic ammonium oxidation (anammox) as well as N$_2$ fixation (Li and Twilley, in review). Heterotrophic N$_2$ fixation rates may vary along the gradient of SOM concentrations, interfering the trend of denitrification rates estimated from net N$_2$ fluxes in response to SOM increase. Quantifying N$_2$ fixation rates at different SOM concentrations as a function of biotic feedback associated with deltaic succession is necessary to better understand the variation of benthic N cycles under different stages of delta development. Also, the evaluation of N$_2$ fixation is critical to know how much of the removed N in deltaic floodplains is associated with upstream N enrichment.

There is evidence that the dominant N pathway has switched from N$_2$ fixation to denitrification in WLD in response to an increase of riverine NO$_3^-$ (from 2 to > 60 µM) that shifts net N$_2$ fluxes from negative (uptake from water column to sediment) to positive (release from sediment to water column; Henry and Twilley 2014). This change of the net N$_2$ fluxes could be because the increased NO$_3^-$ concentration either inhibited N$_2$ fixation or facilitated both processes but favored denitrification more than N$_2$ fixation. If it is the first situation, it is uncertain whether N$_2$ fixation is totally suppressed or partially inhibited at 100 µM of overlying NO$_3^-$ concentration. Investigating N$_2$ fixation and the relative contribution of N$_2$ fixation and denitrification to net N$_2$ fluxes under the impacts of increasing NO$_3^-$ concentrations are necessary to clarify the role of coastal deltaic floodplains in benthic N cycling in response to decadal changes in river fertilization.
We studied WLD, a young (47 years) coastal deltaic floodplain, to investigate the response of N\textsubscript{2} fixation and denitrification to increased NO\textsubscript{3}\textsuperscript{-} loading and SOM. WLD receives a large amount of riverine NO\textsubscript{3}\textsuperscript{-} (3300 to 8600 Mg of N) per year as 23 to 54% of eutrophic riverine water discharge from primary channels enters the interdistributary islands of WLD (Lane et al. 2002; Hiatt and Passalacqua 2015; Li et al. 2020). The coastal deltaic floodplain in this large delta estuary removes 10 to 27% of riverine NO\textsubscript{3}\textsuperscript{-} through denitrification prior to export to the ocean (Li and Twilley, in review; Li et al. 2020). The patterns of benthic nutrient fluxes and pathways of N cycling vary in WLD in response to an increase in SOM resulting from morphological development with delta age, which makes WLD an ideal system to study the significance of N\textsubscript{2} fixation under different SOM and nutrient loading conditions (Li and Twilley, in review; Li et al. 2020).

In this research, incubations of intact sediment cores with \textsuperscript{30}N\textsubscript{2} tracer were conducted to measure heterotrophic N\textsubscript{2} fixation directly. The abundance of \textit{nifH} gene and δ\textsuperscript{15}N of the total N in incubated sediments were measured to support the occurrence of N\textsubscript{2} fixation. Simultaneous measurements of denitrification and N\textsubscript{2} fixation are difficult because denitrification releases N\textsubscript{2} gas whereas N\textsubscript{2} fixation consumes N\textsubscript{2} gas. Here we used estimated denitrification rates from Redfield stochiometric ratios to compare with directly measured N\textsubscript{2} fixation rates as previous research in WLD indicated that estimated benthic denitrification rates using the stochiometric ratio fitted measured denitrification rates (Li et al. 2020). We evaluated benthic N budgets with a major focus on N\textsubscript{2} fixation and denitrification under different NO\textsubscript{3}\textsuperscript{-} concentrations at the earlier and later successional stages of delta development in WLD. Specific research questions addressed include the following. 1) How will increasing NO\textsubscript{3}\textsuperscript{-} concentrations in overlying water impact heterotrophic N\textsubscript{2} fixation rates under different SOM concentrations? 2) How does the relative importance of N\textsubscript{2} fixation and denitrification change with the increasing NO\textsubscript{3}\textsuperscript{-} loading and SOM concentrations?
METHODS

WLD forms at the mouth of the Wax Lake Outlet (WLO) in coastal Louisiana within the Atchafalaya Basin in the Mississippi River Delta (Fig. 3.1). The delta is river-dominated with the land growth rate of 2.62 km² per year (Edmonds et al. 2011; Paola et al. 2011; Twilley et al. 2019). WLD is primarily composed of mineral sediments with an increasing gradient of SOM associated with morphological development along the chronosequence from younger to older deltaic area (Bevington and Twilley 2018; Li et al. 2020). WLD provides a natural lab of deltaic processes to investigate the relative important of N₂ fixation and denitrification in response to increasing NO₃⁻ loading and SOM concentrations.

Field sampling and experiments were conducted at three experimental sites representing lower sediment organic matter (lower-SOM), intermediate sediment organic matter (int-SOM) and higher sediment organic matter (higher-SOM) in WLD (Fig. 3.1). The lower-SOM site is a younger subtidal hydrogeomorphic site with mainly mineral sedimentation at the earlier successional stage of delta development. The int-SOM and higher-SOM sites at the later successional stage of delta development are older experimental sites located more near delta apex with higher soil elevation and SOM concentrations. The int-SOM site is located near the fringe along a primary channel of WLD and exposed to frequent flood pulses of inorganic sedimentation, thus its SOM concentration is relatively lower than the higher-SOM site located within the interior of the island. Detailed descriptions of these experimental sites are shown in Li and Twilley (in review). The int-SOM and higher-SOM and lower-SOM sites were sampled on June 22, July 6 and July 20, respectively, in 2019. Unfortunately, one week before the field sampling of the lower-SOM site a hurricane (Barry) occurred 70km to the west of WLD. Though the hurricane might interfere benthic activity in the lower-SOM, we still collected samples and incubated cores the same way as we treated the other two sites. We compared the post-hurricane sediment property and
benthic fluxes in the lower-SOM site in summer 2019 to the corresponding results measured in the same site in summer 2018 (Li and Twilley, in review) to evaluate the possible hurricane effects to benthic N dynamics.

Duplicate surface water (right below the air-water interface) and porewater (4 cm below the sediment-water interface) samples were collected and filtered (GF/F glass microfiber filters, 0.7 µm particle retention) in each of the three experimental sites for the analysis of inorganic nutrients. Concentrations of NO$_3^-$, nitrite (NO$_2^-$), ammonium (NH$_4^+$) and phosphate (PO$_4^{3-}$) were analyzed on a segmented flow solution IV autoanalyzer (OI analytical, College Station, Texas). Triplicate ambient samples of the top 4 cm sediments were sampled in each site in the field using a piston core (2.4 cm internal diameter), then oven dried at 60°C to constant mass and ground to less than 250 µm for the isotope ratio analysis of total $^{15}$N in sediments using an isotope ratio mass spectrometer (Sercon 20/20 ANCA-GLS). In situ water temperature, salinity and dissolved O$_2$ concentrations were measured using a portable YSI salinity-conductivity-temperature meter (model 30, YSI Incorporated, Yellow Springs, Ohio) and a dissolved oxygen meter (HQ40d, Hach, USA).

Nine intact sediment cores (about 10 cm internal diameter) with 10 ± 1 cm depth of sediments and 10 ± 1 cm of overlying water were collected from each experimental site and sealed with silicone-greased bottoms and detachable lids. Cores were then stored in a cooler at in situ temperature and transported to the lab within 4h. In the lab, the nine sediment cores from each site were randomly assigned to three treatments of NO$_3^-$ concentrations at 0 (TRT 1), 10 (TRT 2) and 100 (TRT 3) µM in incubation solutions. The incubation solutions were riverine waters collected from WLO and filtered using a five-stage filtration system (30, 20, 5, 1, and 0.2 µm) several days before the sampling date. These filtered waters flowed through packed column of NO$_3^-$-specific resin (ResinTech SIR-100-HP, West Berlin, New Jersey) to remove all background NO$_3^-$, then stored in 25L gas-tight Tedlar bags (Keika Ventures) at
4 °C until incubations. Three Tedlar bags of water were brought to room temperature and injected with 120 ml of $^{30}$N$_2$ gas (98%, Cambridge isotope laboratories) per Tedlar bag at room temperature and atmospheric pressure 24h before every incubation event. All bags with water and injected $^{30}$N$_2$ gas were shaken gently for 5 min every 3 to 4 h until the beginning of an incubation event. Extra gas bubbles in Tedlar bags were gently squeezed out and different amounts of KNO$_3$ ($^{14}$N) were added to the three bags to make water NO$_3^-$ concentrations at 0, 10 and 100 µM, respectively, right before the incubations. An extra bag of water was prepared with the similar amount of $^{30}$N$_2$ injection under the same condition to incubate three blank cores with only 10 cm of treated water during every incubation event. The incubations of blank cores were used to correct all possible gas diffusion and interferences not related to benthic activities. There are three incubation events in total (each one focused on one experimental site) and one of the three NO$_3^-$ concentrations (0, 10 and 100 µM) were assigned to the blank core incubation in each incubation event to correct the possible change in NO$_3^-$ concentrations not related to benthic activities.

The ambient overlying water in blank and sediment cores were gently replaced with treatment solutions and installed into a continuous flow-through system in a dark chamber at room temperature (Miller-Way and Twilley 1996; Li et al. 2020). We adjusted the flow rate of influent solutions over the cores to make the overlying water residence time inside each core at about 3 h. A 10 h pre-incubation period was conducted to allow fluxes at sediment-water interfaces to approach an equilibrium. Following the pre-incubation period, influent and effluent solutions were sampled at the completion of a single water residence time for a total of three turnovers per incubation event. Duplicate influent and effluent water samples were collected and filtered through 25 mm GF/F glass microfiber filters (0.7 µm particle retention) in each sampling event. Samples were frozen (-20°C) until analyzed for NH$_4^+$, NO$_2^-$, NO$_3^-$ and PO$_4^{3-}$ concentrations on a flow solution IV autoanalyzer. Duplicate samples
for influent and effluent dissolved gas analyses were collected into 12ml gas-tight exetainers (Labco Limited, Lampeter, Wales, UK) with 200 µL addition of ZnCl₂ solution (50% saturation concentration, Nielsen and Glud 1996). Gas samples were stored underwater at 4°C until analyzed for ²⁸N₂, ²⁹N₂ and ³⁰N₂ within one month on a membrane inlet mass spectrometer (MIMS) with a copper column heated to 600°C (Kana et al. 1994; Eyre et al. 2002). Dissolved oxygen concentrations of influent and effluent waters were measured using a Hach HQ30 LDO probe at the end of each water residence time.

Benthic fluxes of dissolved gas and inorganic nutrients were calculated using the equation:

\[
\text{Flux} = \frac{([C_e - C_i] - [C_{be} - C_{bi}]) \times \text{flow rate}}{\text{core surface area}}
\]  

(1)

where Ce and Ci are effluent and influent concentrations (µM) of a sediment core whereas Cbe and Cbi are averaged effluent and influent concentrations of blank cores in the corresponding incubation event. Denitrification rates were estimated based on a stoichiometric assumption that the molar oxygen (O):N ratios of sediment fluxes follow the Redfield composition (O:N = 138:16). Denitrification rates refer to the discrepancy between the measured fluxes of DIN (NH₄⁺ + NO₃⁻ + NO₂⁻) and the estimated fluxes of DIN based on benthic oxygen consumptions multiplied by the Redfield ratio of 16/138 (Cowan et al. 1996; Cornwell et al. 1999; Li et al. 2020). We used the estimated rates of denitrification because previous research in WLD indicated good consistency between estimated benthic denitrification rates using the stoichiometric ratio and measured denitrification rates (Li et al. 2020). N₂ fixation rates were calculated from the sum of ²⁸N₂, ²⁹N₂ and ³⁰N₂ fluxes minus the estimated denitrification rates.

After the incubations, duplicate samples of the top 4cm sediments in each sediment core were collected using a piston core (2.4 cm internal diameter). One set of sediment
samples (totally 27 samples from three experimental sites) was frozen for DNA extraction
and quantitative polymerase chain reaction (qPCR) analysis. The other set of sediments was
oven-dried at 60°C to constant mass to determine bulk density (g/cm³) using dry sediment
mass divided by wet sediment volume (8.75 cm³). SOM concentrations (% dry mass) were
determined by grinding each dried sediment sample to less than 250 µm, weighting out 1 ±
0.01 g subsample and igniting at 550 ºC for 2 h. Certain amounts (based on instrument
limitation) of the dried and powdered sediment samples were weighed into tin capsules and
analyzed together with the ambient sediment samples collected in the field on the isotope
ratio mass spectrometer for δ¹⁵N Air of total N (%o, the deviation of the ¹⁵N/¹⁴N ratio in a
sample from the corresponding isotope ratio in the reference material of air-N₂).

Sediment samples for DNA extraction were ground in liquid N₂ and preserved at -
80°C. DNA and RNA were extracted from 2 ± 0.01 g sediment per sample using the RNeasy
PowerSoil total RNA kit and RNeasy PowerSoil DNA elution kit (Qiagen). Quality and
quantity of the DNA and RNA extracts were checked spectrophotometrically (nanodrop ND-
2000C, Thermo Scientific). RNA was found degraded due to mis-preservation, and thus, we
only quantified the key functional gene of nifH in DNA. We used the PolF (TGC GAY CCS
AAR GCB GAC TC) and PolR (ATS GCC ATC ATY TCR CCG GA) primers to amplify
the 361-bp nifH fragment (Poly et al. 2001). A standard curve of qPCR was made by
amplifying nifH gene using conventional PCR technique, followed by purification, cloning
and serial plasmid dilutions of the PCR product extracted from agarose gel (Fan 2013). We
used a 20 µL reaction mixture including 2 µL template DNA (about 10 to 40 ng µL⁻¹ after 10
times dilution), 1 µL of each primer (10 µM), 6 µL real time-PCR grade water and 10 µL
PowerUp SYBR green master mix. Quantitative real-time PCR was performed on a CFX
Connect™ Real-time system (Bio-RAD) with the thermocycling conditions including 5 min
at 95°C, 35cycles of 30 s at 95°C, 30 s at 55°C and 40 s at 72°C. A melting curve analysis
was done after the amplification by heating the products from 50°C to 95°C at a rate of 0.5°C s⁻¹, the results of which confirmed the specificity of the amplification. All qPCR analyses were performed in two 96-well plates with each of the 7 standards in triplicate, samples in triplicates, a no-template control in each plate, and several repeated samples between plates to check the consistency between two assays. The efficiencies for standard curves ranged from 97% to 101% and the R² values were over 0.99. The gene copy numbers were calculated based on nanograms of amplicon following the equation:

\[
\text{Gene copy number} = \frac{\text{Amount (ng)} \times \text{Abundance (}6.022 \times 10^{23} \text{ mol}^{-1}\text{)}}{\text{bp (361)} \times \text{ng g}^{-1}(10^{9}) \times \text{g mol}^{-1} \times \text{of bp (660)}}
\]  

(2)

One-way analysis of variance (ANOVA) was used to test the significance of SOM and bulk density among the experimental sites. The significance of inorganic nutrient and dissolved gas fluxes as well as N₂ fixation and denitrification rates among the treatments and experimental sites were tested using repeated measures ANOVAs with three sampling events in each core as the repeated measures. Two-way ANOVA was used to test the difference of \(\text{nifH}\) copy numbers per gram of dry sediment in response to the interaction between study sites and \(\text{NO}_3^-\) treatments. Tukey’s HSD post hoc test with all pairwise comparisons was used when differences were significant at a 95% confidence level. The difference of the \(\delta^{15}\text{N}_{\text{Air}}\) values of total N between the ambient sediments and incubated sediments under different treatment in every experimental site was tested using ANOVA followed by Dunnett’s test at \(p < 0.05\). Data analyses were conducted using JMP software and results were presented as means with error bars of standard error (SE).
RESULTS

Characteristics of experimental sites and lab incubations

There was a strong decrease in sediment bulk density from 1.2 to 0.2 g cm\(^{-3}\) and increase in SOM concentration from 4.5 to 20.6\% from the lower-SOM to higher-SOM sites (Fig. 4.1). The study area is a tidal freshwater wetland with salinity < 0.2 in surface waters and 0.2 to 0.5 in porewaters (Table 4.1). NO\(_3^\-)\) concentrations of \textit{in-situ} surface water ranged from 58.0 µM at the lower-SOM site to 6.6 µM at the higher-SOM site, which were higher than NO\(_3^\-)\) concentrations in porewater in the corresponding site. NH\(_4^+\) concentrations were lower in the \textit{in-situ} surface water (0.5 to 5.4 µM) but higher in the porewater (41.3 to 182.6 µM). NO\(_2^-\) and PO\(_4^{3-}\) concentrations of the \textit{in-situ} surface water and porewater were low in all the experimental sites. Lab incubations were conducted in a water bath controlling the temperature at 19.5 to 21.9 °C with influent water saturated with dissolved oxygen (Table 4.2). All sediment cores were incubated under similar physical and chemical conditions except for the three different treatments of influent NO\(_3^-\) concentrations.
Figure 4.1. (a) Bulk density and (b) sediment organic matter concentrations (SOM) in the top 4 cm of sediment in the three experimental sites representing lower (Lower-SOM), intermediate (Int-SOM) and higher (Higher-SOM) sediment organic matter concentrations (mean ±1 SE, n = 9). Letters designate significant differences among experimental sites using Tukey’s HSD test (p < 0.05).
Table 4.1. Ambient surface water and porewater conditions in experimental sites including lower sediment organic matter (lower-SOM), intermediate sediment organic matter (int-SOM) and higher sediment organic matter (higher-SOM) in Wax Lake Delta (WLD), Louisiana. There was no measurement of dissolved O₂ concentration in porewater samples.

<table>
<thead>
<tr>
<th></th>
<th>Surface Water</th>
<th>Porewater</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower-SOM</td>
<td>Int-SOM</td>
</tr>
<tr>
<td>Salinity</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>O₂ (mg L⁻¹)</td>
<td>4.6</td>
<td>5.2</td>
</tr>
<tr>
<td>NO₃⁻ (µM)</td>
<td>58.0</td>
<td>56.7</td>
</tr>
<tr>
<td>NO₂⁻ (µM)</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>NH₄⁺ (µM)</td>
<td>0.5</td>
<td>2.3</td>
</tr>
<tr>
<td>PO₄³⁻ (µM)</td>
<td>2.7</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 4.2. Incubation conditions of intact sediment cores at different overlying NO₃⁻ concentrations in each of the three experimental sites representing lower, intermediate, and higher sediment organic matter (SOM) concentrations in Wax Lake Delta (WLD), Louisiana. The three treatment of overlying NO₃⁻ concentrations were: TRT 1 with 0 µM NO₃⁻ enrichment, TRT 2 with 10 µM NO₃⁻ enrichment and TRT 3 with 100 µM NO₃⁻ enrichment.

<table>
<thead>
<tr>
<th></th>
<th>Lower-SOM</th>
<th>Int-SOM</th>
<th>Higher-SOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRT 1</td>
<td>TRT 2</td>
<td>TRT 3</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>19.5</td>
<td>19.5</td>
<td>19.5</td>
</tr>
<tr>
<td>O₂ (mg L⁻¹)</td>
<td>9.7</td>
<td>9.5</td>
<td>9.4</td>
</tr>
<tr>
<td>NO₃⁻ (µM)</td>
<td>0.0</td>
<td>9.1</td>
<td>85.9</td>
</tr>
<tr>
<td>NO₂⁻ (µM)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>NH₄⁺ (µM)</td>
<td>0.9</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>PO₄³⁻ (µM)</td>
<td>0.2</td>
<td>0.2</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Table 4.3. Benthic fluxes of dissolved oxygen and inorganic nutrients at different overlying NO$_3^-$ concentrations in each of the three experimental sites representing lower, intermediate, and higher SOM concentrations in Wax Lake Delta (WLD), Louisiana. The three treatment of overlying NO$_3^-$ concentrations were: TRT 1 with 0 µM NO$_3^-$ enrichment, TRT 2 with 10 µM NO$_3^-$ enrichment and TRT 3 with 100 µM NO$_3^-$ enrichment.

<table>
<thead>
<tr>
<th>Flux</th>
<th>Lower-SOM</th>
<th>Int-SOM</th>
<th>Higher-SOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TRT 1</td>
<td>TRT 2</td>
<td>TRT 3</td>
</tr>
<tr>
<td>O$_2$ (g O$_2$ m$^2$ d$^-1$)</td>
<td>-0.3</td>
<td>-0.5</td>
<td>-0.5</td>
</tr>
<tr>
<td>(SE)</td>
<td>(0.0)</td>
<td>(0.0)</td>
<td>(0.2)</td>
</tr>
<tr>
<td>NO$_3^-$ (µmol m$^2$ h$^-1$)</td>
<td>21.7</td>
<td>7.3</td>
<td>-157.6</td>
</tr>
<tr>
<td>(SE)</td>
<td>(2.4)</td>
<td>(3.3)</td>
<td>(44.8)</td>
</tr>
<tr>
<td>NO$_2^-$ (µmol m$^2$ h$^-1$)</td>
<td>1.6</td>
<td>1.8</td>
<td>4.6</td>
</tr>
<tr>
<td>(SE)</td>
<td>(0.4)</td>
<td>(0.4)</td>
<td>(1.6)</td>
</tr>
<tr>
<td>NH$_4^+$ (µmol m$^2$ h$^-1$)</td>
<td>19.6</td>
<td>20.5</td>
<td>59.3</td>
</tr>
<tr>
<td>(SE)</td>
<td>(8.2)</td>
<td>(5.3)</td>
<td>(28.3)</td>
</tr>
<tr>
<td>PO$_4^{3-}$ (µmol m$^2$ h$^-1$)</td>
<td>1.1</td>
<td>-7.4</td>
<td>-7.1</td>
</tr>
<tr>
<td>(SE)</td>
<td>(0.5)</td>
<td>(0.6)</td>
<td>(3.1)</td>
</tr>
</tbody>
</table>

**Sediment oxygen consumption and benthic nutrient fluxes**

Mean rates of sediment oxygen consumption were not significantly different among the three NO$_3^-$ treatments for each site, but there was a significant increase (p < 0.0001) from lower to higher SOM sites for each NO$_3^-$ concentration (Table 4.3). Benthic NO$_3^-$ fluxes were significantly different among both the three NO$_3^-$ treatments (p < 0.0001) and sites (p = 0.005). Benthic NO$_3^-$ uptakes increased with increasing NO$_3^-$ loading from TRT 1 to TRT 3 from 21.7 to -157.6 µmol m$^2$ h$^-1$ in the lower-SOM site, from 10.0 to -260.0 µmol m$^2$ h$^-1$ in the int-SOM site and from -13.5 to -213.9 µmol m$^2$ h$^-1$ in the higher-SOM site. NO$_3^-$ fluxes in the int-SOM and higher-SOM sites were more negative (uptake from water columns to sediments) than fluxes in the lower-SOM site in each of the three NO$_3^-$ treatments. NH$_4^+$ fluxes were positive (release from sediments to water columns) with an increased NO$_3^-$ from TRT 1 to TRT 3 in each of the experimental sites. The higher-SOM site was significantly
higher in benthic NH$_4^+$ release (32.7 to 112.5 µmol m$^{-2}$ h$^{-1}$, p = 0.002) than the other two sites (19.0 to 59.3 µmol m$^{-2}$ h$^{-1}$) in each NO$_3^-$ treatment. NO$_2^-$ fluxes in TRT 1 and 2 (0.7 to 1.8 µmol m$^{-2}$ h$^{-1}$) with lower NO$_3^-$ additions were significantly lower (p < 0.0001) than the fluxes of 4.6 to 14.4 µmol m$^{-2}$ h$^{-1}$ in TRT 3 with 100 µM NO$_3^-$ addition. There was no clear trend of NO$_2^-$ fluxes among the SOM gradient of the three sites. The benthic fluxes of PO$_4^{3-}$ did not show clear patterns among the treatments or experimental sites.

**Nitrogen fluxes in response to N$_2$ fixation and denitrification**

$^{30}$N$_2$ fluxes were mostly negative under different NO$_3^-$ treatments in the three experimental sites, indicating the occurrence of N$_2$ fixation that consumed $^{30}$N$_2$. The int-SOM site showed significantly higher $^{30}$N$_2$ uptake especially under TRTs 1 and 2 with lower NO$_3^-$ addition (0 and 10 µM, Fig. 4.2a). In contrast, the lower-SOM site had significantly lower $^{30}$N$_2$ uptake (p < 0.0001) regardless of the overlying NO$_3^-$ concentrations. Net fluxes of the sum of $^{28}$N$_2$, $^{29}$N$_2$ and $^{30}$N$_2$ were mostly positive under different treatments in the three experimental sites (Fig. 4.2b). Net fluxes of the sum of $^{28}$N$_2$, $^{29}$N$_2$ and $^{30}$N$_2$ indicated a combined signal of denitrification and N$_2$ fixation as positive fluxes demonstrated a dominance of denitrification that produces N$_2$ whereas negative fluxes demonstrated a dominance of N$_2$ fixation rather than denitrification. The lower-SOM site had positive net fluxes of $^{28}$N$_2$, $^{29}$N$_2$ and $^{30}$N$_2$ with significantly higher fluxes in TRT 3 with 100 µM NO$_3^-$ addition compared to the other two NO$_3^-$ treatments (Fig. 4.2b). In the int-SOM site, increasing overlying NO$_3^-$ concentrations from 0 and 10 µM to 100 µM shifted the negative net N$_2$ fluxes (-130.6 to -140.6 µmol N m$^{-2}$ h$^{-1}$) to positive fluxes (199.4 µmol N m$^{-2}$ h$^{-1}$). A similar pattern was observed in the higher-SOM site as net N$_2$ fluxes switched from negative to positive with the increase in overlying NO$_3^-$ concentrations.
Figure 4.2. (a) Benthic fluxes of $^{30}\text{N}_2$ and (b) net fluxes of $^{28+29+30}\text{N}_2$ at the sediment-water interface across the three treatments of NO$_3^-$ addition in the experimental sites representing lower (Lower-SOM), intermediate (Int-SOM) and higher (Higher-SOM) sediment organic matter concentrations (mean ±1 SE, n = 9). Letters designate significant differences among the interaction of experimental sites and treatments using Tukey’s HSD test (p < 0.05).

Heterotrophic $\text{N}_2$ fixation rates in the lower-SOM site (0 to 21.0 µmol N m$^{-2}$ h$^{-1}$) were significantly lower (p < 0.05) than the other two sites regardless of overlying NO$_3^-$ treatments (Fig. 4.3a). Denitrification rates in the lower-SOM site increased from 18.5 to 141.4 µmol N m$^{-2}$ h$^{-1}$ as overlying NO$_3^-$ concentrations increased from 0 to 100 µM (Fig. 4.3b). $\text{N}_2$ fixation rates in the int-SOM site ranged from 189.2 to 437.3 µmol N m$^{-2}$ h$^{-1}$ with a lower $\text{N}_2$ fixation rate in TRT 3 with 100 µM NO$_3^-$ addition compared to the other two NO$_3^-$ treatments.

Denitrification rates in the int-SOM site increased significantly (p < 0.0001) with the increased NO$_3^-$ concentrations from TRT 1 to TRT 3. The range of denitrification rates from 189.1 to 377.1 µmol N m$^{-2}$ h$^{-1}$ in the int-SOM site were comparable with the $\text{N}_2$ fixation rates.
in this site. The higher-SOM site had a decrease in N\textsubscript{2} fixation rates from 283.6 to 52.6 µmol N m\textsuperscript{-2} h\textsuperscript{-1} and an increase in denitrification rates from 238.1 to 350.7 µmol N m\textsuperscript{-2} h\textsuperscript{-1} as overlying NO\textsubscript{3}\textsuperscript{-} concentration increased from 0 to 100 µM.

We detected the presence of \textit{nifH} gene in all three experimental sites under different NO\textsubscript{3} treatments (Fig 4.4). The \textit{nifH} copy numbers per gram of dry sediment revealed
significant difference (p < 0.0001) in diazotrophic community abundances across the three experimental sites with different SOM concentrations. However, no significant difference of nifH abundance was detected among the three treatments of NO₃⁻ addition within each site during the 20 h period of incubation. δ¹⁵N_Air values significantly increased (p < 0.0001) from the lower-SOM site (4.9 ‰) to the higher-SOM site (7.7 ‰) in the ambient sediment samples collected in the field (orange columns in Fig. 4.5). The δ¹⁵N_Air values after incubations were slightly higher than the respective pre-incubation values in the ambient sediments, especially under lower overlying NO₃⁻ additions (0 and 10 µM). But only the lower-SOM site at 0 µM NO₃⁻ addition (p = 0.026) and cores in the higher-SOM site at 0 and 10 µM NO₃⁻ additions (p = 0.0042 and 0.0051, respectively) indicated significant differences to the corresponding ambient sediments based on statistical test.

![Graph showing nifH copy numbers per gram of sediment](image)

**Figure 4.4.** NifH copy numbers in DNA extraction per gram of dry sediment using RT-qPCR (mean ±1 SE, n = 3). Letters designate significant differences among the interaction of experimental sites and treatments using Tukey’s HSD test (p < 0.05). Sites include lower (Lower-SOM), intermediate (Int-SOM) and higher (Higher-SOM) sediment organic matter concentrations.
Fig. 4.5. $\delta^{15}$N$_{Air}$ values of total nitrogen in the top 4 cm layer of incubated sediments compared to the reference sediments collected in the field in the respective experimental site (mean ±1 SE, n = 3). The asterisk indicated a significant difference between the reference and the incubated sediments from a certain treatment in each experimental site using ANOVA followed by Dunnett’s test at p < 0.05. Sites include lower (Lower-SOM), intermediate (Int-SOM) and higher (Higher-SOM) sediment organic matter concentrations.

**DISCUSSION**

**Nitrogen fixation under the effect of nitrate loading and SOM concentrations**

Heterotrophic N$_2$ fixation rates are frequently excluded in benthic N budgets as previous indirect measurements indicated that N$_2$ fixation rates are not significant in coastal ecosystems (Howarth et al. 1988; Damashek and Francis 2018). However, recent research revealed that N$_2$ fixation is a significant part of N cycle in estuarine and coastal sediments using improved methods of isotope enrichments (either NO$_3^-$ or $^{30}$N$_2$) with intact-core incubations and nif$H$ quantification (An et al. 2001; Fulweiler et al. 2007; Newell et al. 2016a). Direct measurement of N$_2$ fixation that fixes N$_2$ to bio-reactive N is necessary to define the role of coastal deltaic floodplains in processing eutrophic riverine water.

We evaluated sediment N$_2$ fixation directly with the $^{30}$N$_2$ tracer addition in response to increasing overlying NO$_3^-$ concentrations in the emerging WLD with different SOM concentrations representing different stages of morphological development. The rates of N$_2$
fixation from 0 to 437 µmol N m\(^{-2}\) h\(^{-1}\) measured in this study were comparable to heterotrophic N\(_2\) fixation rates of 12 to 650 µmol N m\(^{-2}\) h\(^{-1}\) reported in other estuarine and coastal ecosystems (Gardner et al. 2006; Fulweiler et al. 2007; McCarthy et al. 2016). The occurrence of N\(_2\) fixation in the research area was also supported by the measured nifH gene abundances in the three experimental sites. WLD is a tidal freshwater ecosystem with lower sulfate concentration that facilitates the growth of diazotrophic bacteria and leads to higher capacity in heterotrophic N\(_2\) fixation (Marino et al. 2003; Howarth and Marino 2006). \(\delta^{15}\)N\(_{\text{Air}}\) results showed slight accumulation of \(^{15}\)N in incubated sediments from the three sites, especially when overlying NO\(_3^-\) concentrations were lower (0 and 10 µM). The increased signal of \(\delta^{15}\)N\(_{\text{Air}}\) in the top layer of sediments may be more significant if the 20 h incubation is extended to a longer duration (Newell et al. 2016a).

**Figure 4.6.** Heterotrophic N\(_2\) fixation rates relative to NO\(_3^-\) concentrations in overlying water column in each experimental site. Error bars refer to standard error with n = 9. Sites represent lower (Lower-SOM), intermediate (Int-SOM) and higher (Higher-SOM) sediment organic matter concentrations.

Heterotrophic N\(_2\) fixation rates were negatively correlated with overlying NO\(_3^-\) concentrations in the sites with intermediate and higher SOM concentrations (Fig. 4.6), which supports the research result that increasing N loading repressed N\(_2\) fixation in wetland sediments (Scott et al. 2008; Moseman-Valtierra et al. 2010). The slopes of fitted equations
indicated that N₂ fixation decreased by 0.6% (slope/intercept) in the higher-SOM site and by 0.5% in the int-SOM site for each µM increase of overlying NO₃⁻ concentration. For example, N₂ fixation was suppressed by 50% to 60% when NO₃⁻ concentrations increased from 0 to 100 µM, only under conditions where SOM concentrations are higher than 6.5%. Historically, the net flux of N₂ in the int- and higher-SOM sites was controlled by N₂ fixation as an important source of bio-reactive N when overlying NO₃⁻ concentration was 10 µM or even lower in the early 1900s (Goolsby et al. 2000). Currently higher NO₃⁻ concentrations around 100 µM suppresses N₂ fixation, but the inhibited N₂ fixation still equals to 28% to 50% of total bio-reactive N loss via denitrification when SOM concentrations are higher than 6.5%. Thus, we propose that a positive net N₂ flux across sediment-water interface at higher NO₃⁻ concentrations does not preclude the possible occurrence of N₂ fixation. N₂ fixation, though decreasing with increasing NO₃⁻ loading, is not totally suppressed under higher NO₃⁻ concentrations (100 µM). Net N₂ fixation rates estimated from the net uptake of N₂ flux may underestimate the significance of N₂ fixation as N₂ fixation can occur even under higher NO₃⁻ concentrations.

The fitted lines between N₂ fixation and overlying NO₃⁻ concentrations (Fig. 4.6) demonstrate that the int-SOM and higher-SOM sites had higher potential in N₂ fixation than the lower-SOM site under different NO₃⁻ concentrations from 0 to 100 µM. Such variation of N₂ fixation as higher rates occurred with higher SOM was reported in other aquatic sediments (Howarth et al. 1988; McCarthy et al. 2016). The increased abundance of nifH gene from lower-SOM to higher-SOM sites also supported the finding that the site with higher SOM had higher potential of N₂ fixation than the lower-SOM site. It is reasonable that the site with lower SOM had lower N₂ fixation and nifH abundance as labile organic carbon is an important carbon source for heterotrophic diazotrophs to produce nitrogenase enzyme and fix N (Romero et al. 2012; Fan 2013; McCarthy et al. 2016). Even though the nifH gene was
present in the lower-SOM site, N₂ fixation rates were low in this site. This situation is possible as the presence of \textit{nifH} gene does not necessarily mirror N₂ fixation rates (Zehr et al. 2001; Piehler et al. 2002; Bentzon-Tilia et al. 2015). The abundance of \textit{nifH} gene only indicates the potential capacity of the experimental site to fix N₂ but not represent the actual amount of \textit{nifH} gene expressed under \textit{in situ} conditions during experimental incubations (Howarth and Marino 2006; Bentzon-Tilia et al. 2015).

It is noteworthy that the absence of N₂ fixation and significantly lower abundance of \textit{nifH} gene in the lower-SOM site might be related to a hurricane event in WLD. The lower-SOM site is a subtidal site with lower sediment surface elevation (-0.61 m NAVD88) and is inundated year round (Li et al. 2020). Submersed aquatic vegetation and benthic microalgae dominate the earlier successional zones of delta development in this area. Hurricanes in WLD can cause substantial mineral sedimentation to the delta, which changes the initial bulk density and SOM concentrations (Bevington et al. 2017). However, the bulk density (1.2 g cm⁻³) and SOM concentration (4.5 %) in the lower-SOM site measured after the hurricane in 2019 were not significantly different with the bulk density (1.5 g cm⁻³) and SOM (2.9 %) measured in summer 2018 (Li and Twilley, in review). The hurricane event induced a sudden increase of surface water salinity from 0.2 to 4.3 within 11 h (data from CRMS 0464 station on the east side of WLD) then dropped back to 0.2 by the date we sampled this area. Our estimated denitrification rates of 141 ± 44.3 \( \mu \text{mol N m}^{-2} \text{h}^{-1} \) using stochiometric ratio after Hurricane Barry in summer 2019 are similar to denitrification rates of 197 ± 17 \( \mu \text{mol N m}^{-2} \text{h}^{-1} \) (100 \( \mu \text{M overlying NO}^-_3 \)) using \( \text{N}_2 \text{-Ar} \) method in summer 2017 (Li et al. 2020) and 115 ± 6.4 \( \mu \text{mol N m}^{-2} \text{h}^{-1} \) (100 \( \mu \text{M overlying NO}_3^- \)) in summer 2018 using isotope pairing technique (Li and Twilley, in review). In summary, we propose that the lower soil surface elevation site represents minor benthic disturbance from Hurricane Barry and is representative of lower-SOM treatment as used in this study. The absence of N₂ fixation and significantly lower
abundance of \( nifH \) gene in the lower-SOM site is very likely to due to lower SOM concentrations in this site rather than the hurricane effects.

**Denitrification and its correlation with \( N_2 \) fixation**

Estimated denitrification rates ranged from 141 to 377 \( \mu \text{mol N m}^{-2} \text{ h}^{-1} \) based on the stochiometric ratio of benthic fluxes, and are generally consistent with the measured denitrification rates of 87 to 229 \( \mu \text{mol N m}^{-2} \text{ h}^{-1} \) at the same study area under 100 \( \mu \text{M NO}_3^- \) addition using isotope pairing technique (Li and Twilley, in review). The significantly higher denitrification rates in the int-SOM and high-SOM sites compared to the lower SOM site are consistent to the increasing trend of \( \delta^{15}N_{\text{Air}} \) values in the ambient sediments from the lower-SOM to higher-SOM sites (orange columns in Fig. 4.5). The natural abundance of \( ^{15}N \) in ambient sediments reflects a long-term isotopic fractionation with a preferential consumption of lighter \( ^{14}N \) and residual of \( ^{15}N \) during denitrification, anammox and/or volatilization (Robinson 2001; Reis et al. 2019). For experimental sites with the same N source, sites with higher denitrification rates usually had higher \( \delta^{15}N_{\text{Air}} \) values in total N content as more \( ^{14}N \) was released from sediments back to atmosphere through denitrification (Bryantmason et al. 2013; Reis et al. 2019). As such, our result of \( \delta^{15}N_{\text{Air}} \) values increasing from the lower-SOM to higher-SOM sites in the ambient sediment samples indicates that the area with higher SOM concentrations had greater bio-reactive N loss to the atmosphere, which supports the finding that higher SOM facilitates denitrification in coastal deltaic floodplain (Li and Twilley, in review; Li et al. 2020).

Heterotrophic \( N_2 \) fixation was positively correlated with denitrification in each \( \text{NO}_3^- \) treatment (Fig. 4.7), which was probably because that the increased SOM provided favorable conditions for both \( N_2 \) fixation and denitrification (Howarth et al. 1988; Henry and Twilley 2014; Li et al. 2020). Greater SOM can increase benthic metabolism and expand an anaerobic zone, which then enhance benthic denitrification (Cornwell et al. 1999; Boynton et al. 2018).
On the other hand, higher SOM provided a greater source of labile organic carbon for heterotrophic diazotrophs to generate nitrogenase enzyme for N₂ fixation (Romero et al. 2012; Fan 2013; McCarthy et al. 2016). The slope of the fitted equation was 1.7 when overlying NO₃⁻ concentration was 0 µM, indicating that N₂ fixation generally outcompeted denitrification under oligotrophic (low NO₃⁻ concentration) conditions especially when SOM concentrations were no less than 6.5%. The slope of fitted equation decreased to 0.9 when overlying NO₃⁻ concentration increased to 10 µM, which means sediment N₂ fixation was barely sufficient to offset bio-reactive N loss via benthic denitrification. However, when the overlying NO₃⁻ concentration increased to 100 µM, the slope of the fitted equation decreased to 0.7 and the intercept became more negative (-92.3), demonstrating that N₂ fixation was less significant to bio-reactive N loss via denitrification in the eutrophic system. In summary, though N₂ fixation and denitrification both increased with increasing SOM concentration, the relative importance of these two processes were impacted mostly by overlying NO₃⁻ concentration as increasing NO₃⁻ gradually switched the dominance of N₂ fluxes from N₂ fixation to denitrification in a coastal deltaic floodplain.

Figure 4.7. Heterotrophic N₂ fixation rates relative to denitrification rates among the three sites at lower (Lower-SOM), intermediate (Int-SOM), and higher (Higher-SOM) sediment organic matter concentrations in each treatment of NO₃⁻ addition. F ratio and p values from ANOVA test were shown below each equation.
Nitrogen budgets in the emerging coastal deltaic floodplain

We evaluated benthic N budgets with a major focus on N₂ fixation and denitrification under lower and higher overlying NO₃⁻ concentrations at the earlier and later successional stages of coastal deltaic floodplain development. Benthic fluxes at the earlier successional stage of WLD were based on the results measured from the lower-SOM site, whereas benthic fluxes at the later successional stage were based on the averaged results from the int-SOM and higher-SOM sites. NH₄⁺ production from ammonification was calculated from sediment oxygen consumptions divided by the stochiometric ratio of O: NH₄⁺ (13.25) in each experimental site (Cowan et al. 1996). Under historically lower NO₃⁻ concentration (10 µM), ammonification rates increased from 92 to 369 µmol N m⁻² h⁻¹ in study areas from earlier to later successional stages, which were higher than respective NH₄⁺ release rates from sediments to overlying water columns (13 to 41 µmol N m⁻² h⁻¹; Fig 4.8a). The difference in NH₄⁺ flux between ammonification and sediment release to overlying water might represent N buried in wetland sediments and/or converted to NO₃⁻ via nitrification. N₂ fixation occurred under both successional stages of delta development but the later successional stage with higher SOM concentrations had higher N₂ fixation rate (309 µmol N m⁻² h⁻¹) than the earlier successional stage with lower SOM concentration (21 µmol N m⁻² h⁻¹). Denitrification varied from 41 to 314 µmol N m⁻² h⁻¹ along the increased SOM gradient from earlier to later successional stages, but net NO₃⁻ uptake rates from overlying water to deltaic sediments were no larger than 74 µmol N m⁻² h⁻¹, indicating that less than 24% of the removed N via denitrification was from overlying NO₃⁻ loading directly. Instead, the majority of N removed via denitrification might be from fixed N through heterotrophic N₂ fixation and/or SOM ammonification under historically lower overlying NO₃⁻ concentration.

Under currently higher NO₃⁻ concentration (100 µM) due to anthropogenic fertilization, ammonification rates were similar to the respective rates at lower overlying
NO$_3^-$ concentration (Fig. 4.8b). NH$_4^+$ flux across sediment-water interface slightly increased compared to the rates at lower NO$_3^-$ loading but were still smaller than the respective ammonification rates. N$_2$ fixation rates were inhibited under both successional stages with lower and higher SOM concentrations (3 and 144 µmol N m$^{-2}$ h$^{-1}$, respectively) under higher overlying NO$_3^-$ concentration compared to the corresponding rates under historically lower overlying NO$_3^-$ concentration. However, denitrification rates were facilitated under higher overlying NO$_3^-$ concentration, resulting in an increased significance of denitrification compared to N$_2$ fixation in dominating benthic N fluxes in coastal deltaic floodplain. Net NO$_3^-$ uptake rates from overlying water to deltaic sediments increased to 158 µmol N m$^{-2}$ h$^{-1}$ at the earlier successional stage and 237 µmol N m$^{-2}$ h$^{-1}$ at the later successional stage due to the increased overlying NO$_3^-$ concentration. The comparable rates between denitrification and benthic NO$_3^-$ uptake under higher overlying NO$_3^-$ concentration demonstrate that deltaic sediments were an important sink of riverine NO$_3^-$ as over 65% of the removed N via denitrification was from riverine NO$_3^-$ loading directly.

Balancing the N budgets in the emerging coastal deltaic floodplain can serve as an analog of benthic N dynamics during different stages of deltaic development in continental margins of major rivers around the world. The evaluation of N budgets at different SOM concentrations representing different stages of deltaic development helps to clarify the change of N$_2$ fixation and denitrification with ecological succession associated with SOM accumulations. Comparison of N budgets between historically low and currently higher NO$_3^-$ loadings advances our understanding of how benthic N dynamics of N$_2$ fixation and denitrification have been altered by significant increase in riverine NO$_3^-$ due to anthropogenic fertilization. However, benthic N dynamics were more complex than analyzed here since other sources of N like groundwater input, atmospheric deposition and autotrophic N$_2$ fixation may also play an important role in N input in the natural ecosystem (Nixon et al. 123
Also, except for N\textsubscript{2} fixation and denitrification evaluated in this research, other co-occurred N pathways like coupled nitrification-denitrification and dissimilatory nitrate reduction to ammonium (DNRA) and the possible occurrence of anammox may alter benthic N dynamics in coastal deltaic floodplains (Li and Twilley, in review). Further analysis of model simulation that accounts for all the possible N input and output as well as natural hydrodynamic conditions may provide a more clear and accurate evaluation of benthic N budget in coastal deltaic floodplains in continental margins of major rivers.

Figure 4.8. Nitrogen budgets at the sediment-water interface under (a) oligotrophic condition with historically lower NO\textsubscript{3}\textsuperscript{-} concentration (10 µM) and (b) eutrophic condition with currently higher NO\textsubscript{3}\textsuperscript{-} concentration (100 µM) in study areas representing different successional stages of coastal deltaic floodplain development. The earlier successional stage has lower sediment organic matter (SOM) concentrations (based on results from the lower-SOM site) and the later successional stage has relatively higher SOM concentrations (based on results from the int-SOM and higher-SOM sites). Summarized rates for all four stages of ecosystem development are presented in µmol N m\textsuperscript{-2} h\textsuperscript{-1}. Negative values indicate uptake from overlying water column to deltaic sediments. Ammonification rates were evaluated based on sediment oxygen consumptions divided by the stochiometric ratio of O: NH\textsubscript{4}\textsuperscript{+} (13.25) in each experimental site (Cowan et al. 1996).
CONCLUSION

We evaluated heterotrophic N\textsubscript{2} fixation rates in a newly emergent coastal deltaic
floodplain in Mississippi River Delta using continuous flow-through incubations with \textsuperscript{30}N\textsubscript{2}
enrichment. The occurrence of heterotrophic N\textsubscript{2} fixation was supported by the presence of
\textit{nifH} gene and the increased δ\textsuperscript{15}N of total N in sediment cores after incubation. The results
indicated that increasing NO\textsubscript{3}\textsuperscript{−} loading decreased N\textsubscript{2} fixation rates and increased
denitrification rates at each SOM concentration in wetland sediments. However, the
decreased N\textsubscript{2} fixation rates under higher NO\textsubscript{3}\textsuperscript{−} concentration (100 µM) still equal to 28% to
50% of bio-reactive N loss via denitrification, demonstrating the importance of N\textsubscript{2} fixation as
a bio-reactive N source in benthic N cycling. Both N\textsubscript{2} fixation and denitrification increased
with SOM concentration, but the relative importance of these two processes was impacted
mostly by overlying NO\textsubscript{3}\textsuperscript{−} concentration as increasing NO\textsubscript{3}\textsuperscript{−} gradually switched a dominance
of N\textsubscript{2} fixation to a dominance of denitrification in benthic N cycling in a coastal deltaic
floodplain. The evaluation of benthic N budgets focusing on N\textsubscript{2} fixation and denitrification
reveals that N\textsubscript{2} fixation was comparable to denitrification under historically lower NO\textsubscript{3}\textsuperscript{−}
concentrations (10 µM). The majority removed N (≥ 76 %) via denitrification at historically
lower NO\textsubscript{3}\textsuperscript{−} concentrations was from heterotrophic N\textsubscript{2} fixation and/or SOM ammonification
rather than riverine NO\textsubscript{3}\textsuperscript{−} loading. In contrast, currently higher overlying NO\textsubscript{3}\textsuperscript{−} concentration
(100 µM) makes denitrification the dominant benthic N pathway compared to N\textsubscript{2} fixation and
over 65% of the removed N via denitrification was from riverine NO\textsubscript{3}\textsuperscript{−} loading. This study
highlights the importance of N\textsubscript{2} fixation and clarifies the variation mechanism of N\textsubscript{2} fixation
and denitrification in a newly emergent coastal delta in response to increased NO\textsubscript{3}\textsuperscript{−} loading.
We propose that the quantification of heterotrophic N\textsubscript{2} fixation is necessary to evaluate
coastal N budget not only in oligotrophic environment but also in eutrophic environment.
CHAPTER 5. HURRICANE EFFECTS ON BENTHIC NITROGEN CYCLING IN AN EMERGING COASTAL DELTAIC FLOODPLAIN WITHIN THE MISSISSIPPI RIVER DELTA PLAIN

INTRODUCTION

Anthropogenic fertilization dramatically increases bio-reactive nitrogen (N) loading to rivers and coastal oceans, which decreases coastal water quality, causes coastal eutrophication and generates harmful algal blooms (Rabalais et al. 2002; Paerl et al. 2002; Erisman et al. 2008; Diaz 2009; Canfield et al. 2010; Steffen et al. 2015; Damashek and Francis 2018). Coastal deltaic floodplains forming at the mouth of major river basins have potential capacity to remove excess riverine nitrate (NO$_3^-$) prior to export to coastal oceans (Henry and Twilley 2014; Li and Twilley 2020; Li et al. 2020). Recent research reported that about 10 to 27% of riverine NO$_3^-$ loading to a newly emergent coastal deltaic floodplain could be permanently removed through denitrification before exporting to the oceans (Li and Twilley 2020; Li et al. 2020). The N removal capacity is related to sediment organic matter (SOM) concentrations as denitrification rates increase with elevated SOM concentrations from younger to older consequence from subtidal to supratidal hydrogeomorphic zones in the delta (Li et al. 2020).

However, SOM concentrations in deltaic floodplains are not only a function of morphological development and delta age, but also depend on natural disturbance events such as hurricanes and floods (Turner et al. 2006; Bevington 2016; Bevington and Twilley 2018; Li et al. 2020). Hurricanes are one of the most common natural events that create a variety of disturbances to estuarine and coastal environments along the Gulf of Mexico (GoM; Conner et al. 1989; Davis et al. 2004; Williams et al. 2008; Morton and Barras 2011; Liu et al. 2014; Carle and Sasser 2016). The episodic and energetic events of hurricanes alter wetland hydrodynamics, vegetation coverage and sedimentation, which may further impact the capacity of coastal wetlands in processing riverine pollutions and nutrients (Michener et al.

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1997; Davis et al. 2004; Turner et al. 2006; Deng et al. 2010; Liu et al. 2014; Wang et al. 2016). For example, hurricanes resuspend inorganic sediments from shallow inshore and offshore zones and redeposit them into coastal wetlands, which decreases organic matter concentrations in the top layer of sediments (Walker 2001; Turner et al. 2006; Castañeda-Moya et al. 2010; Bevington et al. 2017; Liu et al. 2018). The decreased SOM at the sediment-water interface may inhibit benthic denitrification and alter the relative importance of denitrification to other N processes like coupled nitrification-denitrification and dissimilatory nitrate reduction to ammonium (DNRA). Nevertheless, few research studies have investigated hurricane effects on benthic N cycling in a coastal deltaic floodplain.

Except for changes in SOM due to hurricane effects, saltwater intrusion and salt spray during a hurricane event also causes higher salt stress to vegetations and benthic microbes (Blood et al. 1991; Michener et al. 1997; Wang et al. 2016). An increase of salinity from 0.9 to 3.6 after a hurricane event is reported to generate substantial mortality of freshwater vegetations in the Mississippi River Delta (Chabreck and Palmisano 1973; Lacoul and Freedman 2006). The activities of denitrifying and nitrifying bacteria may be inhibited due to hurricane effects, therefore impacting benthic nitrification and denitrification (Blood et al. 1991; Wang et al. 2016). Increased sulfide concentrations from sulfate reduction following saltwater intrusion are reported to favor DNRA more than nitrification and denitrification in estuarine and coastal ecosystems (Michener et al. 1997; An and Gardner 2002; Koop-Jakobsen and Giblin 2010; Bernard et al. 2015). In addition, hurricanes could increase fluxes of labile organic carbon and nutrients leaching from dead vegetation and litter to overlying water column (Davis et al. 2004). Higher nutrient concentrations following hurricanes may increase chlorophyll \( a \) concentrations in coastal waters (Fogel et al. 1999; Huang et al. 2011). In summary, the impacts of a hurricane on coastal wetland ecosystems are diverse and complex, which are related to not only the track and magnitude of a hurricane event, but also
the morphological and ecological characteristics of disturbed wetlands (Davis et al. 2004; Wang et al. 2016). More field research of how benthic sediment characteristics may change benthic fluxes in response to hurricane disturbances is needed to better understand hurricane effects on nutrient biogeochemistry in coastal deltas.

We investigate short-term hurricane effects on benthic N cycling in Wax Lake Delta (WLD) using intact sediment core incubations with $^{15}$NO$_3^-$ enrichment before and after Hurricane Barry in 2019. As a newly emergent coastal deltaic floodplain, WLD experienced more than 46 tropical storms and hurricanes passing within 400 km distance since 1973 (Carle and Sasser 2016). The frequent disturbances from tropical storms and hurricanes make WLD an ideal system to study the impacts of hurricanes on benthic nutrient cycling in active coastal deltaic floodplains. We investigated the change of benthic biogeochemical characteristics (salinity, bulk density, chlorophyll $a$ and organic and inorganic nutrients) under pre versus post Hurricane Barry conditions in the coastal deltaic floodplain of WLD. In addition, we evaluated benthic N processes of direct denitrification, coupled nitrification-denitrification and DNRA 25 days after Hurricane Barry. These benthic N processes were compared with corresponding rates measured in summer 2018 to clarify the response of benthic N dynamics to hurricane events. We present the hypotheses that allochthonous mineral sedimentation from Hurricane Barry may decrease SOM concentrations in disturbed deltaic floodplain, which further inhibits denitrification and changes the relative importance of denitrification, coupled nitrification-denitrification and DNRA.

**METHOD**

**Study Area**

WLD is a newly emergent coastal deltaic floodplain since 1973 in the Atchafalaya Coastal basin within the Mississippi River Delta (Fig. 5.1b). As a river-dominated delta with weak influences of tides and waves, WLD subaerial land grows at a rate of 2.62 km$^2$ yr$^{-1}$ with
minor anthropogenic manipulation from navigation (Edmonds et al. 2011; Paola et al. 2011; Shaw and Mohrig 2014; Twilley et al. 2019). The delta is primarily composed of mineral sediments with an increasing gradient of sediment organic matter (SOM) associated with morphological development along the chronosequence from younger to older deltaic area (Bevington and Twilley 2018; Li et al. 2020). Sediment stoichiometry demonstrates that the productivity in WLD is primarily limited by N based on N:phosphorus (P) molar ratios less than 16 (Henry and Twilley 2014; Aarons 2019; Twilley et al. 2019; Li et al. 2020).

Hurricane Barry

Hurricane Barry made its landfall 70 km to the west of WLD at southern Louisiana on July 13th, 2019 as a category 1 hurricane with peak wind speed of 65 kt (1kt = 1.85 km h⁻¹; Fig. 5.1a). The cyclone of Barry was asymmetric through landfall as most of its associated heavy rains occurred in the south and east part of the hurricane track. Heavy rainfall and storm surge led to additional flooding occurring along the banks of Atchafalaya River near Morgan City, Louisiana. Hurricane Barry induced substantial mortality of semi-aquatic and aquatic macrophytes across the WLD (Fig. 5.2). Hourly wind speed, wind direction and water level in July 2019 were measured at the Amerada Pass gauge of National Oceanic and Atmospheric Administration (NOAA 8764227; https://tidesandcurrents.noaa.gov). The gauge is approximately 10 km southeast of WLD in the Atchafalaya Bay. Hourly surface water salinity data were downloaded from the Coastal Resources Monitoring System (CRMS) 0464 station on the east side of WLD (https://www.lacoast.gov/crms/Home.aspx). Salinity data are missing from 07/17 to 07/23 because of instrument failure.
Figure 5.1.(a) Hurricane Barry track with wind scales taken from anemometers above the standard 10 m observation height (data were from the NOAA Weather Prediction Center and national Hurricane Center), and (b) map of Wax Lake Delta (WLD), Louisiana, with (c) the location of study sites in Mike Island. Elevation records are from USGS Atchafalaya 2 project LiDAR Survey 2012 digital elevation model (4m resolution).
Figure 5.2. Landscape change in the intertidal area of Mike Island in WLD after Hurricane Barry. Post-hurricane photo is taken on July 20th, which is seven days after Hurricane Barry. The intertidal area is dominated by the emergent floating-leaf vegetation of *Nelumbo lutea*. 
Site description and lab incubation

Field sampling and lab incubations were done at three experimental sites serving as lower (lower-SOM), intermediate (int-SOM) and higher (higher-SOM) sediment organic matter concentrations on Mike Island of WLD 25 days after Hurricane Barry (Fig. 5.1c). The lower-SOM site (29°28’48.4”N, 91°26’53.4”W) is located at the distal portion of the delta with lower elevation (-0.6 m NAVD 88) and younger mineral sedimentation (Bevington and Twilley 2018). The int-SOM site (29°30’47.9”N, 91°26’33.4”W) is on the west side levee of Mike Island along a primary channel of WLD whereas the higher-SOM site (29°30’21.2”N, 91°26’18.5”) is located more interior of the island with greater biotic feedback from *Colocasia esculenta* (Li and Twilley 2020). *In situ* surface water and porewater samples were sampled 4 cm within the air-water interface and sediment-water interface, respectively, in each experimental site. Water samples were filtered through GF/F glass microfiber filters with 0.7 µm particle retention in lab and analyzed for NO$_3^-$, nitrite (NO$_2^-$), ammonium (NH$_4^+$) and phosphate (PO$_4^{3-}$) concentrations on a segmented flow solution IV autoanalyzer (OI analytical, College Station, Texas). The *in situ* nutrient concentrations in surface water and porewater samples after Hurricane Barry were compared to the respective nutrient concentrations measured one month before Hurricane Barry in Li et al. (in review). However, there was no recent measurements of nutrient concentrations in the lower-SOM site, so we used the observed results in summer, 2018 (Li and Twilley, in review) as the pre-hurricane records in this site.

Triplicate sediment cores (10 cm internal diameter) were collected with 10 ± 1 cm depth of sediments and 10 ± 1 cm depth of ambient water from each of the three sites and moved to the lab within 4 h. Incubation water was collected from Wax Lake Outlet at the Calumet boat launch, LA and filtered through a five-stage filtration system (30, 20, 5, 1, and
0.2 µm) at lab to remove suspended particles and microbes in overlying waters. The ambient NO$_3^-$ in the incubation water was removed by flowing the water through a column of packed NO$_3^-$-specific resin (ResinTech SIR-100-HP, West Berlin, New Jersey). About 100 µM of labelled $^{15}$NO$_3^-$ was then enriched to the incubation water ($^{15}$NO$_3^-$, 99%, Cambridge stable isotope laboratories).

The nine sediment cores from three experimental sites were incubated in a water chamber in dark at 21.3 °C with the treated incubation water flowing through the systems at a speed of about 4 ml min$^{-1}$. The overlying water residence time inside each core was controlled at 3 h and the incubation conditions were clearly described in Li and Twilley (in review). After 10 h of pre-incubation, influent and effluent water samples were collected and filtered for NO$_3^-$, NO$_2^-$, NH$_4^+$ and PO$_4^{3-}$ analyses at every 3 h time interval for three times of sampling events. Duplicate influent and effluent samples were also collected into 12ml gas-tight exetainers (Labco Limited, Lampeter, Wales, UK) with the injection of 200 µL ZnCl$_2$ solution (50% saturation concentration, Nielsen and Glud 1996) in each sampling event. Exetainers were then sealed and stored underwater at 4°C for the analysis of dissolved $^{28}$N$_2$, $^{29}$N$_2$ and $^{30}$N$_2$ on a membrane inlet mass spectrometer (MIMS; Kana et al. 1994). Dissolved oxygen concentrations of influent and effluent waters were recorded with a Hach HQ30 dissolved oxygen probe at each sampling event. Benthic fluxes of nutrients and dissolved gases (N$_2$ and O$_2$) were calculated based on the equation:

$$\text{Flux}=\frac{(C_e-C_i) \times \text{flow rate}}{\text{Core surface area}}$$

(1)

where $C_e$ and $C_i$ refer to the effluent and influent concentrations of a certain compound in a sediment core.

We classified denitrification to direct denitrification that converts NO$_3^-$ from overlying water columns to N$_2$ gas and coupled nitrification-denitrification that consumes
NO$_3^-$ oxidized from NH$_4^+$ through nitrification. Direct denitrification rates were calculated by summing the $^{15}$N-N$_2$ released from the sediment-water interface and coupled nitrification-denitrification rates were calculated based on the equation outlined by Nielsen (1992) and Li and Twilley (in review). DNRA rates were determined by the production of $^{15}$NH$_4^+$ during the incubation using OX/MIMS method described in Yin et al. (2014). Direct denitrification, coupled nitrification-denitrification, and DNRA rates were measured 25 days after Hurricane Barry and compared to the corresponding rates measured in summer 2018 in Li and Twilley (in review) to determine the influence of hurricane disturbance to benthic N cycling.

After the three sampling events, the top 4 cm layer of sediments was collected by a smaller piston core with 1.4 cm internal diameter and segmented at 2 cm depth intervals in each sediment core. Samples were then stored frozen in dark for fluorometric determination of chlorophyll $a$ following the method of Arar and Collins (1997). Briefly, chlorophyll $a$ was extracted from wet sediment samples (3.1 ml) with 45 ml of 90% acetone and the sonicated for 30 s before 24 h storage at -20 °C in dark. Then chlorophyll $a$ concentrations were determined fluorometrically (Turner Designs TD-700) using the HCl acidification method (Arar and Collins 1997). We also collected another set of top 4 cm sediment in each core using a larger piston core (2.4 cm internal diameter) and oven dried these samples at 60 °C to a constant mass. Bulk density (g cm$^{-3}$) was then determined by dividing dry sediment mass by wet sediment volume. The dried sediment samples were ground to less than 250 μm and 1 ± 0.01 g subsamples were ignited at 550 °C for 2 h to analyze for SOM concentrations (% dry mass). Bulk density and SOM concentration results in the int- and higher-SOM sites after Barry were compared to the corresponding results measured one month before Hurricane Barry whereas the post-hurricane records in the lower-SOM site were compared to the results in summer 2018.
We did pairwise comparisons of least squares means pre versus post Hurricane Barry in each experimental site using Student's t test (p < 0.05). One way analysis of variance (ANOVA) was used when tested the significance of each parameter among the three experimental sites under a certain sampling time (either pre or post Barry). Statistical analyses were done using JMP software and data were presented as means with error bars representing standard error (SE).

RESULTS

Response of biogeochemical characteristics to hurricane disturbance

Wind direction at the Amerada Pass gauge during Hurricane Barry deflected clockwise from orienting toward the northwest to orienting to the southwest with maximum wind speed increased to 13.5 m s\(^{-1}\) (Fig. 5.3a). The passage of Hurricane Barry 70 km to the west of WLD resulted in a 2 m increase in water level (Fig. 5.3b). The storm surge elevated surface water salinity from 0.2 to 4.3 within 11 h during the hurricane. After Hurricane Barry, water level and salinity returned to pre-existent conditions in several days.

Bulk density and SOM concentration results did not show obvious difference between 0 to 2 cm and 2 to 4 cm layers of sediments, so we averaged these two layers of results when presenting results. The lower-SOM and int-SOM sites did not have significant difference in bulk density or SOM concentration in pre versus post Hurricane Barry conditions (Fig. 5.4). However, Hurricane Barry significantly increased sediment bulk density in the higher-SOM site from 0.21 to 0.47 g cm\(^{-3}\) (p < 0.0001) and decreased the SOM concentration from 20.6 to 9.4 % (p < 0.0001). The chlorophyll \(a\) concentrations of the top 2 cm sediments indicated significant increases in response to Hurricane Barry (Fig. 5.5). Twenty-five days after Barry, the chlorophyll concentrations were 5 times greater in the lower-SOM site, 1 time greater in the int-SOM site and 3 times greater in the higher-SOM site compared to the corresponding pre-hurricane results in the top 2 cm sediments.
Figure 5.3. (a) Time-series of wind speed and wind direction at Amerada Pass gauge 10 km southeast to the WLD (NOAA 8764227) in July 2019. (b) Corresponding water levels (m NAVD 88) at Amerada Pass gauge and surface water salinity at Coastal Resources Monitoring System (CRMS) 0464 station on the east of WLD. Water salinity data had some missing values due to instrument failure.
Figure 5.4. (a) Averaged bulk density and (b) sediment organic matter (SOM) concentrations from 0 to 4 cm layer of sediments pre versus post hurricane Barry among the experimental sites. The pre-hurricane results in the int- and higher-SOM sites were measured one month before Hurricane Barry in 2019 while the pre-hurricane results in the lower-SOM site were from summer 2018 (Li and Twilley, in review). The post-hurricane results were measured 25 days after Barry in all experimental sites. Asterisks designate significant differences from pairwise comparisons of least squares means pre versus post hurricane Barry using Student’s t test results in each site (p < 0.05).

Surface water salinity 25 days after Hurricane Barry was similar to pre-hurricane salinity in each experimental site in WLD (0.2 to 0.3, Table 5.1). Porewater salinity was the same as surface water salinity before Hurricane Barry in each of the three sites. But porewater salinity increased from 0.3 to 0.5 in the lower-SOM site and from 0.2 to 0.7 in the higher-SOM site 25 days post Hurricane Barry. Porewater salinity in the int-SOM site did not change between pre versus post Barry conditions. In-situ NH$_4^+$ concentrations in overlying
surface waters were low and not obviously changed in response to the hurricane. Porewater \( \text{NH}_4^+ \) concentrations, which were much higher than \( \text{NH}_4^+ \) concentrations in surface water, indicated significant increases after Hurricane Barry in the int-SOM and higher-SOM site. The porewater \( \text{NH}_4^+ \) concentrations increased from 41.3 to 142.1 \( \mu \text{M} \) (a 3.4-fold increase) in the int-SOM site and from 97.7 to 442.6 \( \mu \text{M} \) (a 4.5-fold increase) in the higher-SOM site 25 days after Hurricane Barry. Porewater \( \text{NH}_4^+ \) concentrations in the lower-SOM site increased to 182.6 \( \mu \text{M} \) 7 days post Hurricane Barry (Supplemental Table B1) then dropped to 54.5 \( \mu \text{M} \) 25 days post Hurricane Barry. \( \text{PO}_4^{3-} \) concentrations in \textit{in situ} surface water and porewater were low in both pre and post hurricane Barry conditions with slight decreases in response to Hurricane Barry in each site. There was no consistent variation of \( \text{NO}_3^- \) or \( \text{NO}_2^- \) concentrations in either \textit{in situ} surface water or porewater samples.

![Graph](image-url)

**Figure 5.5.** Chlorophyll concentrations in the 0 to 2cm layer of sediments pre versus post hurricane Barry among the experimental sites. The pre-hurricane results in the int-and higher-SOM sites were measured one month before Hurricane Barry in 2019 while the pre-hurricane results in the lower-SOM site were from summer 2018 (Li and Twilley, in review). The post-hurricane results were measured 25 days after Barry in all experimental sites. Asterisks designate significant differences from pairwise comparisons of least squares means at pre versus post Hurricane Barry using Student’s t test results in each site (p < 0.05).
Table 5.1. Variation of ambient surface water and porewater conditions pre versus post Hurricane Barry at three experimental sites representing lower, intermediate, and higher SOM concentrations in Wax Lake Delta (WLD), Louisiana. The pre-hurricane results in the int-and higher-SOM sites were measured one month before Hurricane Barry in 2019, but the pre-hurricane results in the lower-SOM site were from summer 2018 in Li and Twilley (2020). The post-hurricane results were measured 25 days after Barry in all experimental sites.

<table>
<thead>
<tr>
<th></th>
<th>Lower-SOM</th>
<th>Int-SOM</th>
<th>Higher-SOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Salinity (mg L⁻¹)</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>O₂ (µM)</td>
<td>5.7</td>
<td>4.9</td>
<td>5.2</td>
</tr>
<tr>
<td>NO₃ (µM)</td>
<td>46.4</td>
<td>44.6</td>
<td>56.7</td>
</tr>
<tr>
<td>NO₂ (µM)</td>
<td>0.2</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>NH₄ (µM)</td>
<td>2.1</td>
<td>1.5</td>
<td>2.3</td>
</tr>
<tr>
<td>PO₄ (µM)</td>
<td>3.9</td>
<td>2.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Lower-SOM</th>
<th>Int-SOM</th>
<th>Higher-SOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Salinity (mg L⁻¹)</td>
<td>0.3</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>O₂ (µM)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>NO₃ (µM)</td>
<td>2.4</td>
<td>26.3</td>
<td>5.5</td>
</tr>
<tr>
<td>NO₂ (µM)</td>
<td>0.2</td>
<td>2.6</td>
<td>0.8</td>
</tr>
<tr>
<td>NH₄ (µM)</td>
<td>91.7</td>
<td>54.5</td>
<td>41.3</td>
</tr>
<tr>
<td>PO₄ (µM)</td>
<td>1.5</td>
<td>0.2</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Response of Benthic fluxes to hurricane disturbance**

Sediment cores in each experimental site were incubated under similar physical and chemical conditions in pre versus post Hurricane Barry experiments (Table 5.2). Benthic fluxes of dissolved O₂ in the higher-SOM site significantly decreased from -2.8 to -1.8 g O₂ m⁻² d⁻¹ from pre to post Hurricane Barry periods (p < 0.0001), but dissolved O₂ fluxes in the lower-SOM and int-SOM sites following the hurricane were similar to pre-hurricane conditions (Table 5.3). Benthic NO₃⁻ uptake in the lower-SOM site significantly increased from -4.3 to -134.2 µmol m⁻² h⁻¹ while NO₃⁻ uptake in the higher-SOM site significantly decreased from -731.0 to -297.2 µmol m⁻² h⁻¹ after Hurricane Barry (p < 0.0001). Benthic
NO$_3^-$ fluxes at the int-SOM site did not significantly change in response to Barry disturbance. NO$_2^-$ fluxes significantly increased after Hurricane Barry compared to pre-hurricane results in each of the three experimental sites (p < 0.05). NH$_4^+$ fluxes demonstrated significant increases from 21.5 to 39.2 µmol m$^{-2}$ h$^{-1}$ in the lower-SOM site and from 70.0 to 139.4 µmol m$^{-2}$ h$^{-1}$ in the higher-SOM site 25 days after Hurricane Barry compared to the pre-hurricane results (p < 0.05). The increase of NH$_4^+$ fluxes in the int-SOM site (from 4.7 to 21.7 µmol m$^{-2}$ h$^{-1}$) was not significant. Benthic PO$_4^{3-}$ fluxes had no clear pattern in pre versus post hurricane conditions among the three experimental sites.

Table 5.2. Incubation conditions pre versus post Hurricane Barry in the experimental sites representing lower, intermediate, and higher SOM concentrations in Wax Lake Delta (WLD), Louisiana. The pre-hurricane incubations were done in summer 2018 and the post-hurricane incubations were done 25 days after Hurricane Barry in summer 2019.

<table>
<thead>
<tr>
<th></th>
<th>Lower-SOM</th>
<th></th>
<th>Int-SOM</th>
<th></th>
<th>Higher-SOM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Temp (°C)</td>
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<td>21.3</td>
<td>21.8</td>
<td>21.4</td>
<td>21.6</td>
</tr>
<tr>
<td>O$_2$ (mg L$^{-1}$)</td>
<td>9.4</td>
<td>9.9</td>
<td>8.3</td>
<td>10.0</td>
<td>9.5</td>
</tr>
<tr>
<td>NO$_3$ (µM)</td>
<td>85.9</td>
<td>104.0</td>
<td>86.0</td>
<td>104.7</td>
<td>86.2</td>
</tr>
<tr>
<td>NO$_2$ (µM)</td>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.1</td>
</tr>
<tr>
<td>NH$_4$ (µM)</td>
<td>1.0</td>
<td>2.0</td>
<td>1.4</td>
<td>2.0</td>
<td>2.1</td>
</tr>
<tr>
<td>PO$_4$ (µM)</td>
<td>1.2</td>
<td>0.7</td>
<td>0.6</td>
<td>0.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Direct denitrification rates decreased significantly from 109.9 to 61.5 µmol N m$^{-2}$ h$^{-1}$ in the lower-SOM site and from 226.3 to 123.9 µmol N m$^{-2}$ h$^{-1}$ in the higher-SOM site after Hurricane Barry compared to the corresponding pre-hurricane rates (p < 0.0002, Fig. 5.6). The trend was opposite in the Int-SOM site as direct denitrification rates increased from 74.1 to 136.8 µmol N m$^{-2}$ h$^{-1}$ after Hurricane Barry. Coupled nitrification-denitrification rates decreased in each of the three experimental sites after Hurricane Barry. DNRA rates increased in the lower-SOM and int-SOM sites after Barry compared to the corresponding pre-hurricane rates, but the rates were still low after the increases (5.9 and 4.3 µmol N m$^{-2}$ h$^{-1}$, respectively). DNRA rates in the higher-SOM site significantly decreased from 32.3 to 14.7
µmol N m\(^{-2}\) h\(^{-1}\) after Hurricane Barry (p = 0.009), but the higher-SOM was still the most efficient site in DNRA after Hurricane Barry as it was before the hurricane.

Table 5.3. Benthic fluxes of dissolved oxygen and inorganic nutrients in the experimental sites pre versus post Hurricane Barry. Three sites representing lower, intermediate, and higher SOM concentrations in Wax Lake Delta (WLD), Louisiana. The pre-hurricane incubations were done in summer 2018 (Li and Twilley 2020a) and the post hurricane incubations were done 25 days after Barry. Asterisks designate significant differences from pairwise comparisons of least squares means at pre versus post hurricane conditions using Student’s t test results for each flux in each site (p < 0.05). There were no NH\(_4^+\) fluxes in the higher-SOM site before Hurricane Barry.

<table>
<thead>
<tr>
<th>Flux</th>
<th>Lower-SOM</th>
<th>Int-SOM</th>
<th>Higher-SOM</th>
<th>Stat</th>
<th>Lower-SOM</th>
<th>Int-SOM</th>
<th>Higher-SOM</th>
<th>Stat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Stat</td>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Stat</td>
<td></td>
</tr>
<tr>
<td>O(_2) (g O(_2) m(^{-2}) d(^{-1}))</td>
<td>-0.8</td>
<td>-0.9</td>
<td></td>
<td></td>
<td>-1.5</td>
<td>-1.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO(_3^-) (µmol m(^{-2}) h(^{-1}))</td>
<td>-4.3</td>
<td>-134.1</td>
<td>*</td>
<td></td>
<td>-393.8</td>
<td>-310.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NO(_2^-) (µmol m(^{-2}) h(^{-1}))</td>
<td>4.1</td>
<td>10.8</td>
<td>*</td>
<td></td>
<td>1.7</td>
<td>18.3</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>NH(_4^+) (µmol m(^{-2}) h(^{-1}))</td>
<td>21.5</td>
<td>39.2</td>
<td>*</td>
<td></td>
<td>4.7</td>
<td>21.7</td>
<td>70.0*</td>
<td>139.4</td>
</tr>
<tr>
<td>PO(_4^{3-}) (µmol m(^{-2}) h(^{-1}))</td>
<td>-6.8</td>
<td>3.5</td>
<td>*</td>
<td></td>
<td>0.3</td>
<td>-2.5</td>
<td>-8.3</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

* The pre-hurricane NH\(_4^+\) flux was from summer 2017 (Li et al. 2020) as there was no available NH\(_4^+\) flux in the higher-SOM site in summer 2018.
Figure 5.6. (a) Direct denitrification, (b) coupled nitrification-denitrification and (c) dissimilatory nitrate reduction to ammonium (DNRA) rates at pre versus post Hurricane Barry among the experimental sites. The pre-hurricane results were from summer 2018 (Li and Twilley 2020a) and the post-hurricane results were measured 25 days after Barry in all experimental sites. Asterisks designate significant differences from pairwise comparisons of least squares means at pre versus post Barry conditions using Student’s t test results in each site (p < 0.05).
DISCUSSION

Hurricane effects on soil properties

Our results suggest that the effects of Hurricane Barry on soil characteristics of the coastal deltaic floodplain are spatially different. The higher-SOM site located in the east side of the island with greater macrophyte density (*Colocasia esculenta*) than the other two sites indicated significant response to Hurricane Barry. In the higher-SOM site, the significant increase in bulk density and decrease in SOM in the top 4 cm layer of sediment were associated with allochthonous mineral sedimentation attributed to Hurricane Barry (Liu et al. 2018). Storm surge, wind, waves and currents associated with hurricanes can resuspend significant amounts of mineral sediments from shallow inshore and offshore zones and redeposit them to coastal wetlands as the storm surge moves towards the wetlands (Walker 2001; Bevington et al. 2017). The consistent increase of bulk density and decrease of SOM between 0 to 2 cm and 2 to 4 cm layers of sediments demonstrated that the hurricane-related deposition in the higher-SOM site equaled to or exceeded 4 cm in depth. The ≥ 4 cm sediment deposition in the higher-SOM site resulting from Hurricane Barry was more than 3 times greater than the long-term annual elevation increase of 1.1 cm yr$^{-1}$ in WLD as measured at the CRMS site (29°31’36.7”N, 91°26’52.9”W) from 2010 to 2019. The sediment deposition in the higher-SOM site due to Hurricane Barry was also comparable to recent measurements of annual accretion rate in Louisiana coastal marshes varying from 1.6 to 5.9 cm per year (Cahoon et al. 2011).

The allochthonous mineral sedimentation associated with hurricane events provides an appreciable sediment subsidy to deltaic floodplain wetlands, which help to maintain the wetland elevation relative to sea level rise and subsidence (Turner et al. 2006; Tweel and Turner 2012; Liu et al. 2018). However, hurricanes are rare events and the long-term
contribution of hurricane derived sediments to coastal deltaic floodplains is much smaller than the contribution of river floods in the study area (Bevington et al. 2017).

The lower-SOM and int-SOM sites did not show as significant response of bulk density and SOM to Hurricane Barry as it was in the higher-SOM site. This site-to site variation in response to Barry disturbance may be related to the hydrogeomorphic characteristics of the disturbed wetland and/or the direction of storm surge (Davis et al. 2004; Castañeda-Moya et al. 2010; Wang et al. 2016). Firstly, the int-SOM site is located on the west-side levee of Mike Island near the island apex along a primary channel of WLD. High density of trees (Salix nigra) in this area might help to shield benthic zones from serious disturbance of Hurricane Barry (Li and Twilley 2020). Also, the lower-SOM site is a younger subtidal site with mineral sedimentation and frequent inundation (Li and Twilley 2020; Li et al. 2020). The relatively lower soil surface elevation (-0.61 m NAVD88) and higher water level in the lower-SOM site might buffer direct wind damage to benthic zone and lead to small benthic disturbance from Hurricane Barry. The other possibility is that Hurricane Barry created allochthonous mineral sedimentation to the lower-SOM site. However, these allochthonous sediments had similar biogeochemical characteristics to the autochthonous mineral-dominated sediments in the lower-SOM site so that we did not detect significant difference of bulk density or SOM between pre versus post hurricane.

**Hurricane effects on biotic communities and benthic nutrients**

WLD is a tidal freshwater ecosystem dominated by freshwater submersed aquatic vegetation in subtidal hydrogeomorphic zone, *Nelumbo lutea* in intertidal zone and *Colocasia esculenta* in supratidal zone (Bevington and Twilley 2018; Ma et al. 2018). The freshwater macrophytes are susceptible to cyclones and cannot tolerate elevated salinity conditions (Chabreck and Palmisano 1973; Lacoul and Freedman 2006; Ibáñez et al. 2012; Vaurasi and Kant 2016). Previous research reported that an increase of surface water salinity from 0.9 to
3.6 after a hurricane caused *Panicum hemitomon* (a common and native grass in freshwater wetlands in North America) to disappear in 3 weeks and absent for one year in the Mississippi River Delta (Chabreck and Palmisano 1973; Lacoul and Freedman 2006). Similarly, in WLD, an observed increase in surface water salinity from 0.2 to 4.3 during Hurricane Barry lead to substantial mortality of *Nelumbo lutea, Colocasia esculenta* and other plants as shown in Fig. 5.2.

Observed increases of porewater NH$_4^+$ concentrations and benthic NH$_4^+$ fluxes after Hurricane Barry were linked to microbial NH$_4^+$ regeneration of substantial dead vegetation and litter resulting from the hurricane (Blood et al. 1991; Steudler et al. 1991; Michener et al. 1997; Williams et al. 2008). In addition, increased soil NH$_4^+$ pool could be associated with the decreased efficiency of NH$_4^+$ immobilization because of macrophyte mortality and root damage in the system (Blood et al. 1991; Michener et al. 1997). The increased benthic NO$_2^-$ fluxes after Barry was related to the increase of benthic NH$_4^+$ concentrations as NO$_2^-$ is the intermediate product when converts NH$_4^+$ to NO$_3^-$ via nitrification (Reddy and DeLaunce 2008). The responses of benthic NO$_3^-$ fluxes to Hurricane Barry were different in different sites and this might be due to site-to-site variation of hurricane effects on SOM concentrations and N dynamics in WLD.

Chlorophyll *a* concentration at the sediment-water interface was an indicator of benthic algae abundance (Nelson et al. 1999). Benthic chlorophyll *a* concentrations are related to light, nutrient availability as well as salinity and temperature stress (Day et al. 2012; McGlathery et al. 2012). Post-hurricane blooms usually occur in overlying water columns in coastal region when suspended sediment concentrations drop back to pre-hurricane levels but nutrient concentrations still remain high after a hurricane (Miller et al. 2006; Zang et al. 2020). Similar to blooms in water column, we detected blooms of benthic algae in WLD 25 days after Barry landfall. Chlorophyll *a* concentrations in the top 2 cm layer
of sediment significantly increased by 1 to 5 times compared to pre-hurricane concentrations in the three experimental sites, especially the lower-SOM and higher-SOM sites. The post-hurricane blooms were firstly related to increased fluxes of inorganic nutrients leaching from dead vegetation and litter after Hurricane Barry (Fogel et al. 1999; Huang et al. 2011). Elevated porewater NH$_4^+$ concentrations and increased benthic NH$_4^+$ fluxes detected after Hurricane Barry in the experimental sites might increase benthic chlorophyll $a$ concentrations. Secondly, substantial mortality of macrophytes across the delta during Hurricane Barry penetrate more light into benthic areas without dense macrophyte shadow, which could stimulate the growth of benthic algae (Pennock 1985).

**Hurricane effects on benthic N cycling**

Benthic N cycling in different sites responded differently to Hurricane Barry. The higher-SOM site, as the most severely disturbed site among the three sites, halved direct denitrification, coupled nitrification-denitrification and DNRA rates after Hurricane Barry. The decrease in benthic N dynamics in the higher-SOM site was associated with the significantly reduced SOM concentrations after Hurricane Barry. Recent research indicated that direct denitrification and DNRA rates were positively correlated with sediment organic matter concentrations in coastal deltaic floodplains under higher overlying NO$_3^-$ concentrations (solid circles in Fig. 5.7; Li et al. 2020). After Hurricane Barry, direct denitrification rates (open circles in Fig. 5.7a) follow the pre-hurricane linear correlation with SOM concentrations. The largest variation occurs in the higher-SOM site with significant decrease in SOM concentrations after Hurricane Barry. Direct denitrification rates also decreased in the higher-SOM site due to the decrease of SOM concentrations after Hurricane Barry, but the decreased direct denitrification rates still fit in the pre-hurricane linear correlation model. We detected a similar pattern of the correlation between DNRA and SOM concentrations pre vs post Hurricane Barry (Fig. 5.7b). After Barry, DNRA rates reduced due
to the decreased SOM concentrations in the higher-SOM site, but this reduction follows the pre-hurricane linear correlation between DNRA and SOM concentrations. Based on dummy variable regression analysis setting pre vs post hurricane conditions as the dummy variable, we conclude that Hurricane Barry did not significantly alter the correlation between SOM concentrations and benthic N pathways like direct denitrification and DNRA (p = 0.93 and 0.16, respectively). Instead, Hurricane Barry only reset the deltaic succession stage of the higher-SOM site by decreasing its SOM concentrations and then decreasing benthic N dynamics that related to the SOM reduction.

Figure 5.7. (a) Direct denitrification and (b) DNRA rates as a function of sediment organic matter concentrations before Hurricane Barry (filled circles) based on Li and Twilley (2020). The observed results post Hurricane Barry were added as open circles but not involved in the linear correlation model in each figure.
The decreased denitrification rates in the higher-SOM site after Barry were associated with decreased SOM concentration resulting from mineral sedimentation that reduced benthic N mineralization and dissolved oxygen consumption (Caffrey et al. 1993; Cai and Sayles 1996; Cornwell et al. 1999; Eyre and Ferguson 2009). The decrease of DNRA rates with reduced SOM concentrations was because that DNRA requires labile organic carbon as electron donor to convert NO$_3^-$ to NH$_4^+$ (Tiedje et al. 1983; Algar and Vallino 2014; Hardison et al. 2015).

The decreased coupled nitrification-denitrification together with the elevated benthic NO$_2^-$ efflux demonstrated a possible occurrence of incomplete nitrification after Barry. The aerobic process of nitrification involves two steps of oxidation: the first step converts NH$_4^+$ to NO$_2^-$ by *Nitrosomonas* and the second step oxides NO$_2^-$ to NO$_3^-$ through *Nitrobacter* (Reddy and DeLaune 2008). The activity of *Nitrobacter* is sensitive to pH conditions and could be inhibited by increased concentration of NH$_4^+$ and unionized NH$_3$ (Roseberg et al. 1986; Reddy and DeLaune 2008; White and Reddy 2009). The observed increase of soil NH$_4^+$ pool in every experimental site and the possible change of pH conditions after Hurricane Barry (Michener et al. 1997) were likely to inhibit the oxidation of NO$_2^-$ to NO$_3^-$ and cause the decrease of coupled nitrification-denitrification and the accumulation of benthic NO$_2^-$.

We want to emphasize that some variations of benthic N processes after Hurricane Barry compared to the corresponding pre-hurricane rates measured one year ago may attribute to conventional biogeochemical heterogeneity over time (Mermillod-Blondin et al. 2003; Michaud et al. 2003; Wenzhöfer and Glud 2004; Stockdale et al. 2009). Benthic N processes measured in different years may be slightly different due to deltaic succession in wetlands that changes benthic communities and biogeochemical dynamics (Michaud et al. 2003).
Our short-term study of hurricane effects on benthic N cycling demonstrates the decreasing capacity of coastal wetlands to process eutrophic riverine water and mitigate coastal eutrophication after hurricane events. The observed decrease of direct denitrification, coupled nitrification-denitrification together with increased NH$_4^+$ effluxes induced by Hurricane Barry in most experimental sites reduced the capacity of WLD to remove excess riverine NO$_3^-$. The reduced efficiency in permanent N removal in coastal deltaic floodplains and heavier nutrient inputs with flash flooding from estuaries associated with hurricane events could decrease coastal water quality and deteriorate coastal eutrophication (Paerl et al. 2001; Shelby et al. 2005; Williams et al. 2008). In addition, hurricane induced sedimentations alter deltaic succession by changing SOM concentrations (Bevington et al. 2017), then alter benthic N dynamics. Further research on the long-term hurricane effects and recovery benthic biogeochemistry in a disturbed coastal wetland are important to clarify the response and recovery ability of a coastal deltaic floodplain to natural disturbance of hurricanes.

CONCLUSION

We investigated hurricane effects on sediment properties, benthic chlorophyll $a$ concentrations and N dynamics in the newly emergent coastal deltaic floodplain of WLD. Bulk density, SOM, Chlorophyll $a$ concentrations as well as surface water and porewater nutrient concentrations were measured in three experimental sites with distinct SOM concentrations 25 days post Hurricane Barry and compared to respective results evaluated one month before Hurricane Barry. Direct denitrification, coupled nitrification-denitrification and DNRA rates were measured using intact sediment core incubations 25 days post Hurricane Barry and compared to the corresponding rates evaluated one year ago. We found that the effect of Hurricane Barry on soil characteristics was spatially different as the higher-SOM site was the only site indicating significantly increased bulk density and decreased SOM after the hurricane event. Coupled with the decrease of SOM concentration in the
higher-SOM site, we detected around 50% decreases in direct denitrification, coupled nitrification-denitrification and DNRA in this site. Hurricane Barry did not significantly alter the correlation between SOM concentrations and benthic N pathways like direct denitrification and DNRA. We observed benthic algae bloom 25 days post Hurricane Barry with significant increases of chlorophyll $a$ concentrations in the top 2 cm layer of sediments in all three sites in WLD. The post-hurricane algae bloom appeared to be related to the observed increase of porewater NH$_4^+$ concentrations and increased NH$_4^+$ fluxes from sediments to overlying water columns after Hurricane Barry. In addition, substantial mortality of macrophytes due to Hurricane Barry penetrated more light to benthic areas without dense macrophyte shadow, which could stimulate the growth of benthic algae. In summary, the short-term study of hurricane effects on benthic N cycling demonstrates that the capacity of WLD to remove excess riverine NO$_3^-$ decreased after Hurricane Barry because of the observed decrease of direct denitrification, coupled nitrification-denitrification and increased NH$_4^+$ effluxes in most experimental sites. Hurricane Barry reset the deltaic succession stage of the higher-SOM site by decreasing its SOM concentrations and then decreasing benthic N dynamics that related to the SOM reduction.
CHAPTER 6. SYNTHESIS AND CONCLUSIONS

This dissertation research evaluates the capacity of an emerging coastal deltaic floodplain to process excess riverine NO$_3^-$ and explores the response of benthic N dynamics to deltaic succession, anthropogenic fertilization and hurricane events. Firstly, I investigated spatial and seasonal patterns of benthic fluxes and estimated annual N removal in the newly emergent WLD using continuous flow-through incubations of intact sediment cores. The results indicated that WLD could remove 10 to 27% of total NO$_3^-$ load to WLD, most of which was converted to N$_2$ through denitrification. The older intertidal hydrogeomorphic zone with relatively higher sediment organic matter (SOM) was the most efficient area in annual N removal. The subtidal zone had the lowest denitrification rates associated with lower SOM concentration, but has the largest spatial extent and the longest flood duration, and therefore contributed over half of the N removal in WLD. As a small prograding coastal deltaic floodplain under early stages of delta development, WLD demonstrated an increasing capacity of N removal of 0.2 to 2% per year before riverine NO$_3^-$ is exported to coastal oceans. These results highlight the contribution of the coastal deltaic floodplains in processing elevated riverine NO$_3^-$ at continental margins with coastal ocean. This chapter provides a basic understanding of how riverine nutrients could be processed under the ecological succession of a young emergent delta created from a major river diversion. To our knowledge, it is the first assessment on benthic fluxes as a function of hydrogeomorphology and delta age since emergence in coastal deltaic floodplains.

Secondly, I evaluated the relative importance of denitrification, coupled nitrification-denitrification, DNRA and anammox as a function of biotic feedback to soil development (higher, intermediate, and lower SOM). In addition, I investigated the response of benthic N processes to decadal changes in elevated riverine NO$_3^-$ concentrations due to anthropogenic fertilization. Isotope pairing technique (IPT) was used to calculate direct denitrification and
coupled nitrification-denitrification. DNRA rates were measured by OX-MIMS method and potential anammox rates were measured by anaerobic incubations with 50 µM $^{15}$NH$_4^+$ addition. Direct denitrification was the dominant N pathway in the tidal freshwater ecosystem, which was one to two orders of magnitude larger than coupled nitrification-denitrification, DNRA, and anammox. Gross denitrification increased 19-fold on average in response to the increase in overlying NO$_3^-$ concentrations from 5 to 100 µM, most of which was an increase in direct denitrification rates rather than coupled nitrification-denitrification. The relative importance of DNRA to total NO$_3^-$ loss through dissimilatory pathways decreased with increasing NO$_3^-$ concentrations. Anammox rates varied from 0 to 38 µmol N m$^{-2}$ h$^{-1}$ with an increase from lower-SOM to higher-SOM sites. The quantification of individual N processes responding to increasing SOM and NO$_3^-$ loading provides a better understanding of benthic N dynamics during the early stages of deltaic development in continental margins of major rivers around the world.

Thirdly, N$_2$ fixation and its correlation with denitrification responding to elevated SOM due to ecological succession as well as increased NO$_3^-$ loading due to agricultural fertilization were investigated. $^{30}$N$_2$ gas was enriched into influent overlying water of the experimental flow-through core system to capture the signal of N$_2$ fixation directly. Denitrification was also estimated based on benthic fluxes of dissolved inorganic N (NH$_4^+$ + NO$_3^-$ + NO$_2^-$) using the Redfield stoichiometric ratios. I quantified the abundance of nifH functional gene using Real-Time qPCR and measured $\delta^{15}$N of the total N in incubated sediments to support the occurrence of N$_2$ fixation. Heterotrophic N$_2$ fixation rates were negatively correlated with NO$_3^-$ concentrations in overlying water in each SOM concentration, but N$_2$ fixation was not completely repressed under higher NO$_3^-$ concentrations. Heterotrophic N$_2$ fixation was positively related with SOM, which is consistent with the correlation between the abundance of nifH functional gene and SOM.
concentrations. Though N₂ fixation and denitrification both increased with SOM concentration, the relative importance of these two processes were impacted mostly by overlying NO₃⁻ concentration as increasing NO₃⁻ gradually switched benthic N cycling from a dominance of N₂ fixation to a dominance of denitrification in the coastal deltaic floodplain. Based on this evaluation, I speculate that N₂ fixation was comparable to denitrification under historically lower NO₃⁻ concentrations (10 µM). When NO₃⁻ concentrations in the river were historically low, the source of N (≥ 68 %) removed via denitrification was from heterotrophic N₂ fixation and/or SOM remineralization (ammonification coupled to nitrification) rather than riverine NO₃⁻ loading. In contrast, the current enriched river waters with higher NO₃⁻ concentration (100 µM) results in denitrification dominating benthic N pathway compared to N₂ fixation and over 61% of the N removed via denitrification is from riverine NO₃⁻ loading. This research emphasizes that the quantification of heterotrophic N₂ fixation is necessary to evaluate coastal N budgets not only in oligotrophic environment but also in eutrophic environments.

The last section of my research focuses on the influence of Hurricane Barry to benthic N dynamics and sediment characteristics in the disturbed coastal deltaic floodplain of WLD. We incubated sediment cores 25 days after the occurrence of Hurricane Barry using IPT method and compared denitrification, coupled nitrification-denitrification and DNRA results with the rates we measured in the same sites before the hurricane event in the third chapter. The influence of Hurricane Barry indicated strong site-to-site variations on sediment characteristics. The higher-SOM site was the most severely disturbed site among the three sites with significantly increased bulk density and decreased SOM. Associated with the decreased SOM, the higher-SOM site demonstrated approximate 50% decrease in direct denitrification, coupled denitrification and DNRA. The lower- and int-SOM sites indicated significant increase of DNRA and decrease of coupled nitrification-denitrification after
Barry. The observed benthic algae blooms 25 days post Hurricane Barry might be related with the increase of porewater NH$_4^+$ concentrations and increased NH$_4^+$ fluxes from sediments to overlying water columns after the hurricane. Also, more light penetration to benthic areas because of substantial mortality of macrophytes might be another reason that benthic algae blooms occurred at these sites. I concluded that the NO$_3^-$ removal capacity in WLD decreased after Hurricane Barry because of the observed decrease of denitrification, enhanced proportion of DNRA to total NO$_3^-$ loss and increased NH$_4^+$ effluxes in most experimental sites as a result of decrease in SOM with deposition of mineral sediment.

In summary, this research defines the dynamic role of an emerging coastal deltaic floodplain in reducing eutrophication to coastal oceans. The assessment on benthic fluxes as a function of hydrogeomorphology and delta age since emergence provides an analog of nutrient fluxes in response to deltaic development in coastal deltaic floodplains. Quantifying N processes as a function of SOM and NO$_3^-$ loading provides a better understanding of benthic N dynamics during the early stages of deltaic development in continental margins of major rivers around the world. This study highlights the importance of heterotrophic N$_2$ fixation in coastal deltaic floodplain and emphasizes the necessity of N$_2$ fixation quantification to evaluate coastal N budget, particularly in response to N enrichment to river waters. Also, I found that hurricane events could decrease the N removal capacity in a coastal deltaic floodplain in a short period due to mineral sedimentation from storm surge. Further research on the long-term hurricane effects and recovery of benthic nutrient biogeochemistry in a disturbed coastal wetland are important to clarify the response and recovery ability of a coastal deltaic floodplain to natural disturbance of hurricanes.
APPENDIX A. COPYRIGHT INFORMATION

Chapter 2

Benthic fluxes of dissolved oxygen and nutrients across hydrogeomorphic zones in a coastal deltaic floodplain within the Mississippi River delta plain

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Publication: Biogeochemistry
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Supplemental Table B1. *In situ* water and sediment characteristics in the lower-SOM site seven days post Hurricane Barry (07/20/2020).

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VITA

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