ASSESSMENT AND VALUATION OF NITROGEN MITIGATION ECOSYSTEM SERVICES IN NATURAL AND RESTORED WETLANDS OF THE TEXAS COASTAL BEND

A Thesis

by

LYDIA HAYES

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

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ABSTRACT

Eutrophication leads to poor water quality, hypoxia, and biodiversity loss in aquatic ecosystems, which is a major issue in the Gulf of Mexico and its surrounding estuaries. It is largely caused by excess nutrients in aquatic environments. With population rising along the Texas coast, there is an increase in nitrogen-based nutrients output, through wastewater and agricultural runoff. It is critical to reduce nutrients input to nitrogen-limited waters to improve water quality and eliminate eutrophication. Wetlands are important ecosystems that offer many ecosystem services including nitrogen mitigation. Denitrification is the main pathway for removing excessive nitrogen-based nutrients in wetland sediments. In this study, nitrogen mitigation was quantified through potential denitrification measured in five wetland sites, two restored and three natural ones. Our results showed that the age of wetlands is a major factor regulating denitrification rates, with lowest average annual rates found in two restored sites, Egery Flats and the Nueces Bay restored marsh (11.46 and 10.85 kg $N \cdot ha^{-2} \cdot yr^{-1}$, respectively). Significantly higher rates were found in natural wetland sites, with mean annual rates of 22.5, 29.39, and 39.27 kg $N \cdot ha^{-2} \cdot yr^{-1}$ measured for the Aransas River Estuary, Oso Bay marsh, and the Naval Airbase Bridge, respectively. Temperature was another influencing factor for denitrification rates in three of the five sites. The seasonal denitrification rates measured in this study were used to quantify the economic value of nitrogen mitigation ecosystem services in the two restored wetland sites. The replacement cost was \$36,565$\cdot yr^{-1}$, and \$8,125$\cdot yr^{-1}$, for Egery Flats and Nueces Bay restored marsh, respectively, which is an equivalent value of \$13.55$\cdot$kg N^{-1} removed.

DEDICATION

I dedicate this work to my parents, David and Kathy Hayes, along with my sisters, Natalie Taylor, and Joanna Hayes, for their never-ending support, and for getting me to where I am. I also dedicate this work to Nicholas Guastella, for empowering me and encouraging me to achieve my goals.

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TABLE OF CONTENTS

LIST OF FIGURES

FIGURES PAGE

LIST OF TABLES

1. Introduction

1.1 Wetland Ecosystem Services

Wetlands are essential ecosystems that provide many ecosystem services including water quality improvement, pollution mitigation, nutrient cycling, carbon sequestration, climate regulation, buffering zones, fisheries habitat, recreation opportunities and nitrogen mitigation (Gren et al., 1995; Woodward and Wui 2001; Yang et al., 2008; Canfield et al., 2010; Zhang et al., 2016). Ecosystem services are defined as benefits provided by an ecosystem that fulfills a specific purpose with value (Chen et al., 2009; Vymazal 2011). Human activity highly impacts aquatic ecosystems through nitrogen pollution from agricultural runoff, fossil fuel combustion, and wastewater effluent. Nitrogen mitigation is the removal of nitrogen from the environment and is the ecosystem service focus of this study.

As of the 2010 Census, more than 6 million people reside on the Texas coast and this number is expected to increase by 50 percent by the year 2050 (Texas Shores, 2013). Coastal development associated with rising populations has been causing degradation of natural wetlands and in turn a loss of ecosystem services. As population increases the magnitude of sources of nitrogen including agricultural runoff, fossil fuel combustion, and wastewater effluent will also increase, taking a toll on the economy by causing environmental pollution of water resources (Shahi et al., 2013).

A substantial amount of excess nitrogen (N) in the form of nitrate, nitrite, and ammonium flows from wastewater treatment plants (WWTP) without N removal capabilities (secondary treatment) into important waterways including rivers, streams, and estuaries which can lead to soil acidification, eutrophication, and hypoxia in coastal environments. Desimone and Howes (1996) found the effluent of a secondary WWTP in Cape Cod, MA to contain concentrations of 671 µM, 54 µM, and 1927 µM, for nitrate, nitrite, and ammonium, respectively. In Hong Kong, a secondary

WWTP was found the effluent contained 310 μM, 5 μM, and 250 μM, for nitrate, nitrite, and ammonium, respectively (Arachana et al., 2016). Another study in Southern California found the effluent of a secondary WWTP contained 584 μM, 95 μM, and 1743 μM, for nitrate, nitrite, and ammonium, respectively (McLaughlin et al., 2017). Soil acidification, eutrophication, and hypoxia in coastal environments are serious problems leading to habitat degradation, decreased biodiversity, fish kills, and mortality to shellfish in aquatic systems (Fowler et al., 1998; Rabalais et al., 2002; Galloway et al., 2004; Richardson et al., 2004; Mitsch et al., 2005; DeLaune et al., 2005; Jenkins et al., 2010; Davidson et al., 2012; Coban et al., 2015; Bruesewitz et al., 2017). These hypoxic zones along the coast of Texas have increased over the past three decades, which is related to N pollution and a large loss of wetlands in the coastal area (Rabalais et al., 2002; Mitsch et al., 2005; Lindau et al., 2008).

1.2 Stable Nitrogen Isotopes ($\delta^{15}N$)

N stable isotope ratios ($\delta^{15}N$) in nitrate, nitrite, and ammonium help differentiate among N sources and processes such as synthetic fertilizer, sewage, soil N, atmospheric N, denitrification and nitrification origins (Bottcher et al., 1990; Kendall 1998; BryantMason et al., 2013), which have different $\delta^{15}N$ (‰) (Table 1). Animal and human waste have been found to have a $\delta^{15}N\text{-}NO_3^-$ range from +10 to +20‰ (Kreitler 1975; Kreitler 1979; Kendall et al., 2007). A study of WWTP inputs into the Aransas River near Skidmore, Texas found particulate organic nitrogen $\delta^{15}N$ values of 17‰, which indicates the influence of wastewater effluent into the river (Mooney 2009). Different nitrogen cycling processes have different fractionation factors leading to unique $\delta^{15}N$, i.e., the fractionation factor of nitrification ranges from -38 to -14‰, from -1 to +1‰ for ammonification, and from +15 to +30‰ for denitrification (Kendall et al., 2007). Using $\delta^{15}N$ in nitrate, nitrite, and ammonium can aid in identifying specific sources of excess nitrogen and cycling processes in the environment, which can aid in understanding how N moves through the system.

Table 1. Table of known stable nitrogen isotope ($\delta^{15}N$) sources and processes.

Source or Process	$\delta^{15}N-NO_3^-$ (‰)	$\delta^{15}N-NH_4^+$ (‰)	$\delta^{15}N-NO_2^-$ (‰)	Bulk $\delta^{15}N$ (‰)	PON- $\delta^{15}N$ (‰)	Location	Reference
Secondary WWTP Effluent	+5.32	+12.78	-	-	-	Southern California, USA	McLaughlin et al. 2017
Secondary WWTP Effluent	+4.9	-	-	-	-	Ulaanbaatar, Mongolia	Itoh et al. 2011
Animal and Human Waste	+10 to +20	+10 to +25	-	-	-		Kreitler 1975; Kreitler 1979; Heaton 1986; Kendall et al. 2007
River Downstream Secondary WWTP	-	-	-	-	17	Skidmore, Texas	Mooney 2009
Spring Water	-6.7	-	-	-	-	Ulaanbaatar, Mongolia	Itoh et al. 2011
Soil Organic Nitrogen	-2 to +10	-2 to +5	-	+4 to +9	-	Seven Country Average	Heaton 1986; Kendall et al. 2007
Soil Organic Matter	-	-	-	+2 to +7	-	Coastal Sediments	Heaton 1986; Kendall et al. 2007
NH_4^+ Fertilizer	-	-	-	-5 to +5	-	USA, Australia, France, South Africa	Heaton 1986
NO_3^- Fertilizer	-5 to +3	-10 to +5	-	-5 to +7	-	USA, Australia, France, South Africa	Heaton 1986; Kendall et al. 2007
Atmospheric NH_4^+	-	-	-	-15 to 0	-	Jülich, Germany	Freyer 1978; Heaton 1986
Atmospheric NO_3^-	-	-	-	-12 to +2	-	Jülich, Germany	Freyer 1978; Heaton 1986
Wet Deposition	+3.1	-	-	-	-	USA	Hastings et al. 2003; Elliot et al. 2006; Kendall et al. 2007
Nitrification			-44.6			Leipzig, Germany	Coban et al. 2015
Nitrification	-38 to -14	+5 to +45	-	-	-		Kendall et al. 2007
Ammonification	-	-	-	-1 to +1	-		Kendall et al. 2007
Denitrification	+15 to +30	-	-	-	-		Kendall et al. 2007
Nitrate Mineralization	+4 to +9	-	-	-	-		Heaton 1986
Ammonium Mineralization	-	-40 to -15	-	-	-		Kendall et al. 2007
Volatized Ammonia of Urea and Manure	-	-20	-	-	-	Agricultural land	Kendall et al. 2007

1.3 Nitrogen Mitigation Ecosystem Service and Denitrification

Wetlands can remove some of this excess nitrogen through the microbially-mediated process of denitrification, where nitrate and nitrite are ultimately reduced to nitrogen gas (Lindau et al., 2008). This process is important for nitrogen mitigation because it represents a direct removal of fixed nitrogen (i.e. nitrate and nitrite) from the water and sediment releasing nitrogen gas into the atmosphere (Groffman 1991; DeLaune et al., 2005; Lindau et al., 2008).

There have been many studies quantifying denitrification in wetlands as an N mitigation service, but very few have been conducted in wetlands along the Texas Coastal Bend, and even fewer have monetized this N mitigation ecosystem service (Appendix 1) (Groffman and Tiedje 1989; Richardson et al. 2004; Lindau et al., 2008; Jenkins et al., 2010; Bruesewitz et al., 2017). Denitrification quantification has been done in several locations in the United States and globally, exploring many different environments such as restored and natural wetlands, as well as distinguishing between freshwater and marine systems (Groffman and Tiedje 1989; Behrendt et al., 1999; Dehnhardt 2002; Richardson et al. 2004; DeLaune et al. 2005; Lindau et al., 2008; Jenkins et al., 2010; Wolf et al., 2011; Theriot et al., 2013; Song et al., 2014; Bruesewitz et al., 2017).

Factors affecting denitrification in natural wetlands have been documented in many studies in the United States and worldwide. Some of the factors that were investigated were temperature, nutrient availability, carbon availability, water flow rate, and soil composition. One study in the Copano Bay in Texas addressed denitrification in sediments that are influenced by wastewater during a prolonged drought. This study measured potential denitrification in the West Copano Bay and in the Aransas Tidal River in July of 2011, using membrane inlet mass spectrometry. The mean potential denitrification rate in the West Copano Bay sediments were 3.25 mg $N \cdot m^{-2} \cdot hr^{-1}$, and the mean potential in the Aransas Tidal River sediment was 1.5 mg $N \cdot m^{-2} \cdot hr^{-1}$. The study determined that severe drought may decrease anthropogenic N input into coastal systems due to less runoff from land area into water systems (Bruesewitz et al., 2017).

There have been many studies investigating denitrification outside of Texas in the United States, and in other parts of the world. One study in Michigan documented denitrification in forest soils. This study found a mean potential denitrification rate for poorly drained sand to be 199 g $N{\cdot}ha^{-2}{\cdot}d^{-1}$, and in well drained sand to be 1,251 g $N{\cdot}ha^{-2}{\cdot}d^{-1}$. This study tested denitrification seasonally in intact cores using acetylene blocking and added nitrate and found that there was a lot of variation in rates during the summer, and lower variation during the spring and fall (Groffman and Tiedje 1989). A natural freshwater wetland in Wisconsin, near the Mississippi River, has an area of 10,425 ha. A denitrification study was done in the summer of 2001 using sediment slurries and acetylene blocking and found mean denitrification to be 1.97 $\mu g\ N{\cdot}cm^{-2}{\cdot}hr^{-1}$. The N loss during the summer months was calculated to be 26.6 t $N{\cdot}day^{-1}$. The researchers determined that denitrification rates were temperature-dependent, and influenced by nutrient enrichment, and carbon (C) availability (Richardson et al. 2004). Another study on nitrogen reduction in the Elbe River Floodplains in Germany done by Dehnhardt (2002), measured nitrogen reduction on an area of 1,800 ha and extrapolated up to 15,000 ha. Nitrogen reduction was estimated to be 3,000 tons per year with a rate of 200 kg $N{\cdot}ha^{-2}{\cdot}yr^{-1}$ using a statistical model developed by Behrendt et al. (1999).

Excess nitrogen pollution from agricultural and wastewater runoff is a known cause of coastal hypoxia which can cause degradation of natural wetlands. There have been many studies looking at wetland restoration as a means of improving nitrogen mitigation. These studies investigated the effects of nitrate concentrations, diffusion rate of nitrate into the sediment temperature dependency, carbon availability, soil composition, organic matter accumulation over time, and wetland age (DeLaune et al., 2005; Lindau et al., 2008; Wolf et al., 2011; Mitsch et al 2012; Song et al., 2014).

One such study was done in the Mississippi Alluvial Valley (MAV) floodplain, where 75% of the flood plain has been converted to agricultural land and the remaining 25% remains wetland. Measurements for potential denitrification were completed using the denitrification enzyme assay method and found that low elevation sites had a mean denitrification potential of 28.8 kg $N \cdot ha^{-2} \cdot yr^{-1}$. This study modeled future denitrification potential in restored wetlands in the MAV. As the restored wetland ages, they show total nitrate mitigated increasing from 36 kg $N \cdot ha^{-2} \cdot yr^{-1}$ in year 5 to just under 68 kg $N \cdot ha^{-2} \cdot yr^{-1}$ in year 90, the end of the modeled study period (Jenkins et al., 2010). In another study, denitrification was measured in a restored bald cypress swamp near the Atchafalaya River Basin in Louisiana. This swamp receives water from the Mississippi and Atchafalaya Rivers which are high in nitrogen concentration. The researchers measured potential denitrification in surface sediments using acetylene blocking and nitrate amendment and found the mean denitrification rates were 207.9, 386.6, and 682.0 g $N \cdot ha^{-2} \cdot d^{-1}$ at 8, 22, and 30°C, respectively. This study showed that restored wetland areas have the potential to remove a significant amount of nitrate from the environment (Lindau et al. 2008).

Another study in the Louisiana area measured denitrification in Davis Pond, a restored wetland downstream of a diversion of the Mississippi River. The wetland was designed to control the flow of river water and nutrients into the Barataria Bay estuary, minimizing loss, enhancing vegetation, and improving wildlife habitats. The area of this pond is 3,700 ha. This study measured nitrogen removal in sediments collected in April 2003 through acetylene blockage in surface soil (0-5cm) and a mean rate of denitrification of 31 mg $N \cdot m^{-2} \cdot d^{-1}$ was measured. This value was extrapolated up to show a removal of 1,350 kg $N \cdot ha^{-2} \cdot d^{-1}$ is removed by the entire marsh area (DeLaune et al. 2005). This study states that controlled hydrology optimizes the nitrogen removal pathways.

A study looking at restored riparian wetlands along the Mississippi River a few miles north of Memphis, TN looked at four wetland sites, three restored sites and one natural site, to compare restored and natural wetlands. The restoration involved putting notches in dikes that had previously been placed along the river to restore hydrology between the Mississippi River and the 11-mile secondary channel in Loosahatchie Bar. Each site had three 25 m^2 sections that were monitored for a year. The natural wetland site had a mean potential denitrification rate of 294.9 g N_2O-N·m^{-2}·d^{-1}, while the three restored sites had mean rates that were much lower of 11.8, 9.3, and 3.0 g N_2O-N·m^{-2}·d^{-1}. The study determined that the blocking of water flow altered sediment composition and biogeochemical properties that affected the rates of denitrification in the restored wetland. Some of the factors that may affect denitrification are decreased organic matter, increased soil oxygen from infrequent flooding events, and decreased nutrient loading. The researchers determined that it may take longer than two years to improve the biogeochemical processes in the restored wetlands (Theriot et al., 2013).

A study done in Virginia comparing natural wetlands and created wetlands of various ages showed that younger created wetlands have lower denitrification potential than older created wetlands, as well as natural wetlands. This study was done using the denitrification enzyme assay method. The younger wetlands, three and four years old, showed significantly less soil development than older created wetlands between seven and ten years old. Older created wetlands between seven and ten years old showed similar soil development as natural wetlands, and similar denitrification potential. The 12.9 ha three year old wetland had an average denitrification rate of about 35 μmol N_2O-N kg·dw^{-1}·d^{-1}, the 0.9 ha four year old wetland had a rate of 30 μmol N_2O -N kg·dw^{-1}·d^{-1}, the 20.2 ha seven year old wetland had a rate of 85 μmol N_2O -N kg·dw^{-1}·d^{-1}, the 50.6 ha ten year old wetland had a rate of 60 μmol N_2O -N kg·dw^{-1}·d^{-1} and the two natural wetland sites

(2,000 ha and 290 ha) both had rates that averaged around 75 μmol N_2O -N $kg \cdot dw^{-1} \cdot d^{-1}$ (Wolf et al., 2011). Another study on created wetlands in Columbus, Ohio, measured denitrification from 2004-2009. They found that denitrification followed seasonal patterns, but the rates were highly variable. Two one-hectare wetlands were created between 1993-1994. The *in situ* denitrification rates were measured using an *in situ* acetylene blocking method. The denitrification rates in the shallow marsh sites averaged 316 $\mu g \cdot m^{-2} \cdot hr^{-1}$ (Song et al., 2014).

Natural denitrification restoration projects have been proposed to reduce the amount of nutrient loading into major waterways (Lindau et al., 2008) including wetland restoration and implementation of created wetlands, river water diversions, and riparian buffer restoration. Many wetland restoration projects cause disturbance to existing sediments which may temporarily reduce denitrifying microbial populations. Created wetlands have a less complex soil structure and lower microbial diversity than natural wetlands. Over time, total organic matter and nitrogen will increase in created wetlands, and sediment will develop a more complex microbial community, thus increasing N cycle development. This development may take five to ten years to have a similar function to the natural wetlands (Wolf et al., 2011). The development of increased N cycling will bridge the gap between restored and natural wetlands, making restored and created wetlands more similar in function to natural wetlands (Richardson et al., 2004; Jenkins et al., 2010; Wolf et al., 2011). Restoration projects can help to improve nitrate reduction through denitrification and biological uptake by plants and microorganisms through landscape planning, adjusting water depth, and velocity to increase the contact time between the sediment and the nutrients allowing more time for denitrification to occur (Mitsch et al., 2005; Bruesewitz et al., 2017).

1.4 Economic Evaluation of Nitrogen Mitigation

There have been many studies done to provide a monetary value for ecosystem services in the southeast region of the United States near the Gulf of Mexico (Appendix 2). Providing economic evaluation for services that do not have a traditional market value can aid in cost justification for conservation and restoration efforts. Many ecosystem services lack a conventional market; therefore, these services go undervalued (Salem and Mercer 2012). There have been many methods to assign an economic value to ecosystem services including willingness to pay, contingent valuation, market pricing, and production pricing (Loomis 1992; Breaux et al., 1995; Jenkins et al., 2010; Piehler and Smyth 2011; Pollack et al., 2013; Schmidt et al., 2014).

Methods commonly used to assign a monetary value to nitrogen mitigation ecosystem services are the benefit transfer and replacement cost methods. The benefit transfer method uses estimates calculated from a previous study that is being applied to the current policy site, and the replacement cost method involves determining the value of an ecosystem service based on the cost to replace the service with a manmade alternative (Salem and Mercer 2012; Pollack et al., 2013). To monetize an ecosystem service the following three steps must be accomplished 1) identify the ecosystem service, 2) quantify the service, and 3) monetize or assign value to the service (Jenkin et al., 2010).

There are several examples of the benefit transfer and replacement cost methods being used in the region surrounding the Gulf of Mexico. One such study in 1994 in Thibodaux, Louisiana used the replacement cost method to assess a four million gallon day (mgd) secondary wastewater treatment plant that needed to add tertiary treatment to meet discharge standards. To meet the discharge standards the WWTP could either add a sand filtration system or discharge the effluent into a natural wetland system. The option to discharge into a natural wetland was chosen. The wetland area of 570 acres received 19.9 g $N \cdot m^{-2} \cdot yr^{-1}$. Through biochemical processes including denitrification, plant assimilation, and soil burial, 72-85% of nitrogen was removed, which is equivalent to a removal range of 14.3 to 16.9 g $N \cdot m^{-2} \cdot yr^{-1}$. The study estimated a 30-year life span for the conventional plant that uses sand filtration. The cost savings for using the wetland system ranged from \$448,000 to \$504,000 which is equivalent to between \$785 and \$885 per acre. That would be \$26.16 to \$29.50 per acre per year for N removal (Breaux et al., 1995).

Another study by Pollack et al. (2013) assessed the monetary value of N mitigation provided by oyster reefs in the Mission-Aransas Estuary, TX, USA using a similar replacement cost method to the Thibodaux, Louisiana example. The study used the cost of building and maintaining a wastewater treatment plant with a biological N removal process to assign a monetary value to the N mitigation provided by oyster reefs. This is done by comparing the capacity to remove nitrogen by the WWTP and the oyster reefs. The capacity to remove nitrogen is calculated by measuring the amount of water filtered by the oysters, and the amount of nitrogen removed by the reefs, as well as measuring the amount of water that is processed by the WWTP, and the amount of nitrogen removed from the influent to the effluent. Then the capital and maintenance cost of the WWTP is used to determine the cost to replace the oyster reef nitrogen removal capacity with a manmade WWTP. The Back-River Wastewater Treatment Plant in Maryland processes 180 mgd. This plant removes 5,569,810 kg $N \cdot yr^{-1}$, or 22.4 mg $N \cdot L^{-1}$. The oyster reef in the study comprises an area of 18.11 km^2 and removes 9,100 kg $N \cdot yr^{-1}$, and 502.5 kg $N \cdot km^{-2}$ through denitrification. The replacement cost for this denitrification is \$74,788$\cdot yr^{-1}$. or \$8.33$\cdot$kg N^{-1} (Pollack et al., 2013).

Another study of N mitigation conducted in the USA by Jenkins et al. (2010) looked at restored wetlands in the Mississippi Alluvial Valley (MAV) using the benefit transfer method. The dollar value for nitrogen mitigation was determined from a previous study that used the productivity method (PM), which estimates the monetary value of ecosystem products bought and sold in commercial markets. The PM method was used to determine the value of nitrogen removal credit trading in the Mississippi Delta. Ribaudo et al. (2005) used modeling to determine a value of \$10.50·lb N^{-1} removed for nitrogen removal credits. Nitrogen removal credit trading occurs when farmers voluntarily install N removal wetlands, and the amount of nitrogen that is removed can be sold as "credits" to municipal or industrial facilities that need to meet regulatory standards but cannot achieve them at the facility. The study by Jenkins et al. (2010) converted the value of the nitrogen removal credits to \$·kg N^{-1}, and then inflated the value to \$2008 for an ending value of \$25.27·kg N^{-1}. This value was then applied to the study area, in the MAV. The researchers then annualized the data to a value of \$1,248 US\$2008·ha^{-2}·yr^{-1} (Jenkins et al. 2010).

In McIntosh County, Georgia a study valued nitrogen mitigation in forest wetlands, freshwater wetlands, brackish marsh, and salt marsh. The mean N removal in the forested wetland was 0.350 t N·ha^{-2} and the mitigation value was \$1,248·ha^{-2}·yr^{-1}, the freshwater marsh was 0.067 t N·ha^{-2} and the mitigation value was \$19·ha^{-2}·yr^{-1}, brackish marsh was 0.066 t N·ha^{-2} and the mitigation value was \$27·ha^{-2}·yr^{-1}, and salt marsh 0.033 t N·ha^{-2} and the mitigation value was \$112·ha^{-2}·yr^{-1}. The forested wetland has a higher monetary value than the other wetland sites because they were found to have a higher nitrogen removal capacity. This valuation was accomplished using the benefit transfer method with a value of \$25.27·kg N^{-1} removed from Jenkins et al. (2010) (Schmidt et al., 2014).

Another study of the economic value of denitrification was conducted by Piehler and Smyth (2011) in five estuarine habitat types. This study looked at denitrification in submerged aquatic vegetation, salt marshes, oyster reefs, intertidal flats, and subtidal flats. The researchers based their calculations for the value of denitrification on a value of \$13·kg N^{-1} removed. This value was obtained from the North Carolina nutrient offset program, a state program where farmers can voluntarily install N removal wetlands on their agricultural land, and industrial or municipal facilities pay \$13·kg N^{-1} removed to the farmers to help meet standards that cannot be met at their facilities. The mean cost to replace N removal in the five habitats from this study varied for each habitat type, showing that these values can vary between location and habitat type (Table 2) (Piehler and Smyth 2011).

While denitrification studies have been done in other areas, a study quantifying and monetizing N mitigation services for both natural and restored wetlands have not been done in the Texas Coastal Bend. A big concern with the degradation of ecosystems, is the need for making decisions about conservation and restoration projects, as well as justifying the cost of this type of project (Chen et al., 2009). Valuing the N mitigation services provided by the wetland ecosystems can aid in the decision-making process, to inform policymakers and stakeholders of the services and their worth.

This work assessed N mitigation in restored and natural wetlands of the Texas Coastal Bend under the influence of wastewater discharge and provides an economic valuation of the N mitigation ecosystem service of two restored wetlands. This is done by meeting the following objectives; 1) identify potential denitrification in wetland sediments; 2) quantify the concentration of nitrogen species and identify nitrogen sources in WWTP effluent and surrounding wetland water columns; 3) quantify denitrification rates for each site and establish seasonal cycles for denitrification; 4) convert denitrification rates to a rate per area per time unit to quantify the N mitigation ecosystem service for use in the monetization of N mitigation; 5) provide a case study to apply economic valuation of N mitigation to two restored wetland sites in the Texas Coastal Bend. This work also addressed the following specific hypotheses; 1) *denitrification rates will increase as temperature increases*; and 2) *denitrification positively correlates with wetland age; rates will be lower in restored wetlands than in natural wetlands*.

Investments are being made along the Texas Coastal Bend for wetland restoration projects including two locations being studied in this project, Egery Flats and Nueces Bay. Wastewater treatment plants have high manufacturing, and energy costs. Using wetlands as a system for treating wastewater reduces energy costs, is economically efficient, and decreases environmental pollution (Shahi et al., 2013). Assessing the N mitigation services in wetlands along the Texas Coast will improve the understanding of N cycling and N mitigation in restored and natural wetlands in the region, and valuation of N mitigation ecosystem services will aid in cost justification of current and future restoration projects to stakeholders and decision-makers.

Table 2. List of the values of nitrogen removal for five habitat types from a study by Piehler and Smyth 2011.

Habitat Type	Annual Value ($\$\cdot acre^{-2}\cdot yr^{-1}$)
Salt Marsh	2,480
Oyster Reef	2,969
Submerged Aquatic Vegetation	2,999
Intertidal Flats	1,552
Subtidal Flats	414

2. Methods

2.1 Study Area Overview

Sampling for this project took place at eight sites, three in Corpus Christi, TX, three in Bayside, TX, and two in Portland, TX. Sampling sites included three wastewater treatment plants, and five wetland sites (two restored and three natural wetlands) adjacent to WWTPs along the Texas Coast (Figure 1). Each wetland location is adjacent to at least one of the following large bodies of water: Oso Bay, Corpus Christi Bay, Nueces Bay, and Copano Bay.

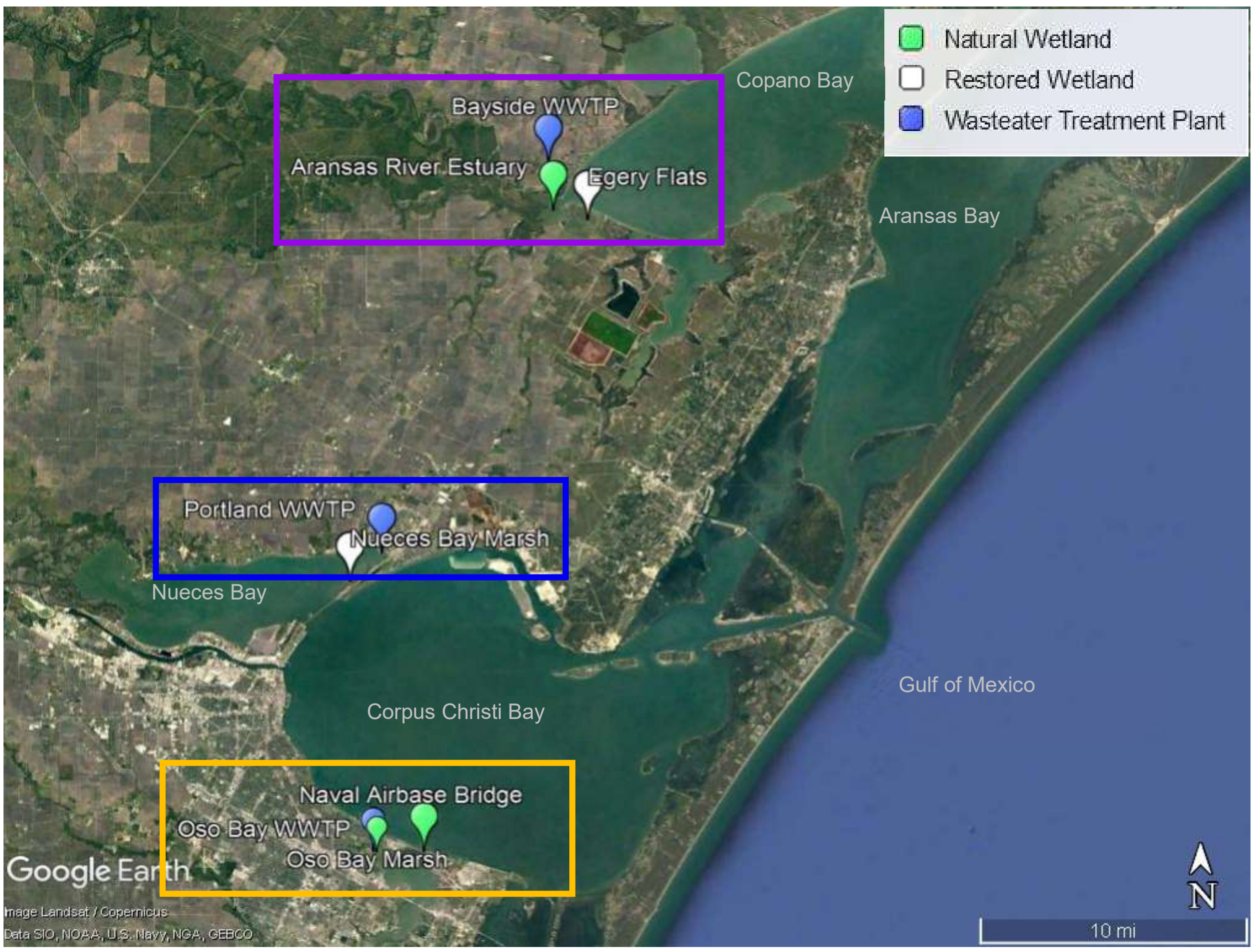


Figure 1. Map of all sampling sites along the Texas Coastal Bend. Corpus Christi, TX sites are outlined in yellow. Portland, TX sites are outlined in blue. Bayside, TX sites are outlined in purple. Map developed with Google Earth.

2.1.1 Site Description: Corpus Christi, TX, USA

Corpus Christi, TX, located in Nueces County, is surrounded by agricultural land to the west and northwest, and three large bodies of water: Corpus Christi Bay, Oso Bay, and the Gulf of Mexico (Figure 1). One sampling site in Corpus Christi, TX was the Oso Bay WWTP effluent that was discharged into the Oso Bay, which connects to the Corpus Christi Bay (Figure 2). There are also two natural wetland sites located in the Oso Bay, adjacent to the Oso Bay WWTP. The first, the Oso Bay marsh site found at the mouth of the stream where the WWTP effluent flows into the Oso Bay (Figure 2, Figure 3a). At this site, the sediment is primarily clay with a thin layer of sand (less than 1cm) covering the surface. The vegetation was *Tamarix ramosissima* (Salt cedar shrubland), *Borrichia frutescens* (sea ox-eye daisy), *Prosopis* sp. (mesquite), and *Spartina* sp. (cordgrass). The second, the Naval Airbase Bridge wetland site was located at the connection point of Oso and Corpus Christi Bays, giving this site influence from two bay systems (Figure 3b). There was no vegetation at this site, and the sediment is coarse sand and shells.

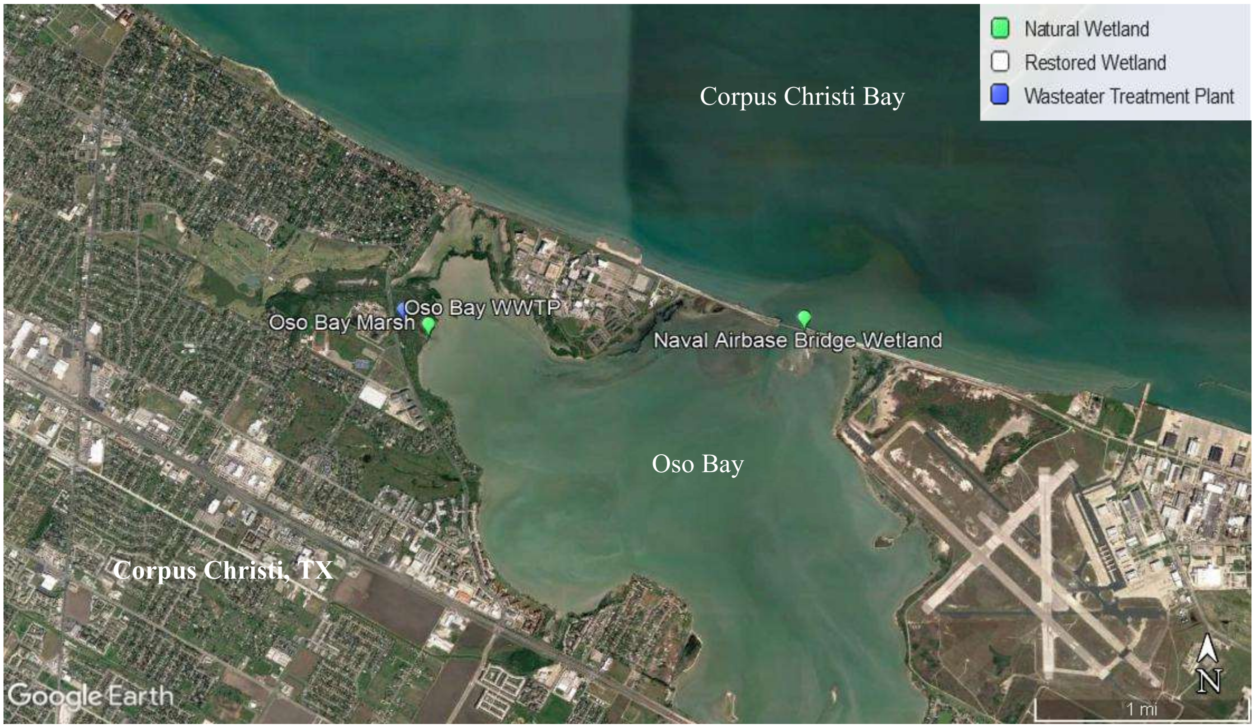


Figure 2. Map of Corpus Christi, TX sampling sites, including the Oso Bay WWTP, and two adjacent natural wetland sites in the Oso Bay. Map developed using Google Earth.

Figure 3. Photos of sampling sites in Corpus Christi, TX in the Oso Bay. **a-** photos of the Oso Bay marsh site, **b-** photos of the Naval Airbase Bridge site.

2.1.2 Site Description: Portland, TX, USA

Portland, TX, located within Nueces and San Patricio counties, was surrounded by agricultural land to the north, west, and east, and the Nueces and Corpus Christi Bays to the south (Figure 1, Figure 4). There are two sampling sites in Portland, TX, the first is the Portland WWTP, which released effluent into the Nueces Bay (Figure 4, Figure 5a). The second is the Nueces Bay restored wetland site, which is adjacent to the Portland WWTP, and near the connection point of the Nueces and Corpus Christi Bays (Figure 4, Figure 5b). The restored wetland was part of a 70 ha, $5,326,820 restoration project completed by the Coastal Bend Bays and Estuaries Program (CBBEP) beginning in 2010 and completed in 2015 (Figure 6). Sediments were brought in to form sediment mounds surrounded by larger rocks to prevent further erosion. The sediments contain pebbles, sand, and shell particles. Created sediment mounds were planted with native marsh plants including *Spartina alterniflora* and *Spartina patens.* Other saltwater friendly plants had landed and planted themselves here including Pickleweed (*Salicornia spp.*), *Batis maritima,* and *Lycium carolinianum* (CBBEP, 2014). The area of Nueces Bay restored marsh was established using the polygon feature in Google Earth Pro to outline the perimeter of the site based on maps of the restored area provided by CBBEP and calculate the total area in hectares.

Figure 4. Map of Portland, TX sampling sites, including Portland WWTP, and adjacent restored wetland site in the Nueces Bay. Map developed using Google Earth.

Figure 5. Sampling sites located in Portland, TX. **a-** Portland WWTP effluent outfall, **b-** Nueces Bay restored marsh site.

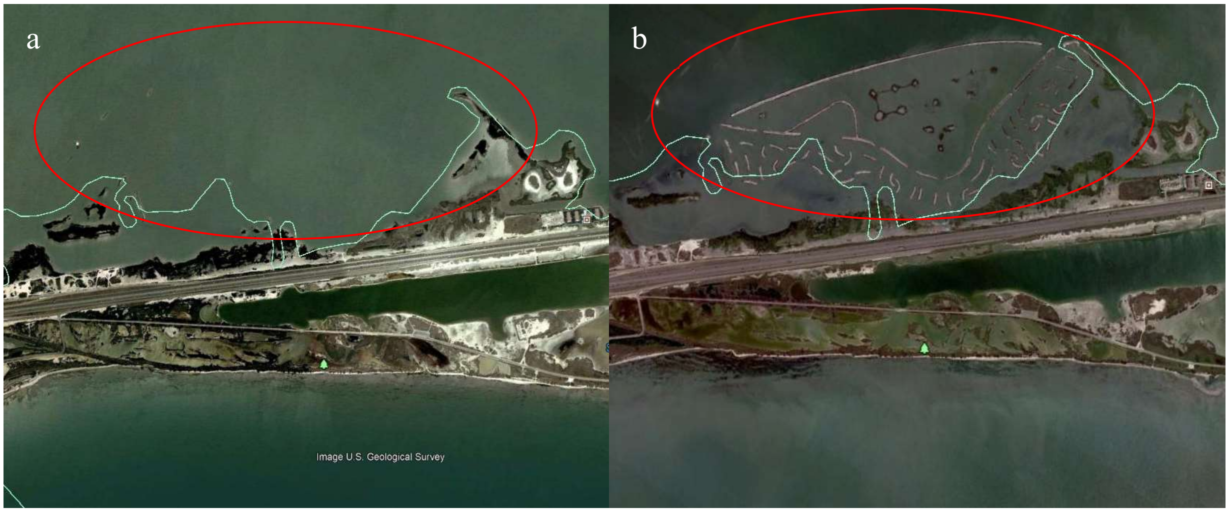


Figure 6. Location of the Nueces Bay restored marsh site. **a-** Nueces Bay site pre-restoration, 2006, **b-** Nueces Bay site post-restoration, 2017. Image made using Google Earth.

2.1.3 Site Description: Bayside, TX, USA

The town of Bayside, TX, located in Refugio County, is surrounded by agricultural land to the north, south, and west, and two bodies of water, the Aransas River, and Copano Bay to the east (Figure 1). One sampling site was the Bayside WWTP effluent, the effluent flows into two wetland sites, the Aransas River Estuary, and Egery Flats, then out into Copano Bay (Figure 7, Figure 9). The Bayside WWTP is a constructed wetland planted with *Schoenoplectus californicus*, *Typha domingensis, Sagittaria graminea,* and *Pontederia cordata.* This treatment plant was designed to reduce nitrate through denitrification before the effluent is released into the environment. This process is efficient due to the low flow rate of the Bayside WWTP (O'Malley Engineers 2004). The natural wetland site located in the Bayside, TX study area is the Aransas River Estuary to the west of FM136 (Figure 1, Figure 7). The restored wetland site located in the Bayside, TX study area is to the east of FM136, in Egery Flats (Figure 1, Figure 7). Egery Flats is part of a 270 ha marsh restoration project that began in 2018 and was completed in early 2019, where $1,587,000 was invested to expand the culverts connecting the Aransas Bay to Egery Flats passing under the

FM136 highway near the northwest edge of Copano Bay (Figure 8). This restoration project aims to restore hydrology and reduce the salinity in the Egery Flats marsh (NFWF, 2014). This site contains emergent marsh, submerged aquatic vegetation and is home to a plethora of marine life, and many waterfowl. The sampling sites in both the Aransas River Estuary and Egery Flats have clay sediment, with rocks and shells (Figure 10). The area of Egery Flats was established using the polygon feature in Google Earth Pro to outline the perimeter of the site and calculate the total area in hectares.

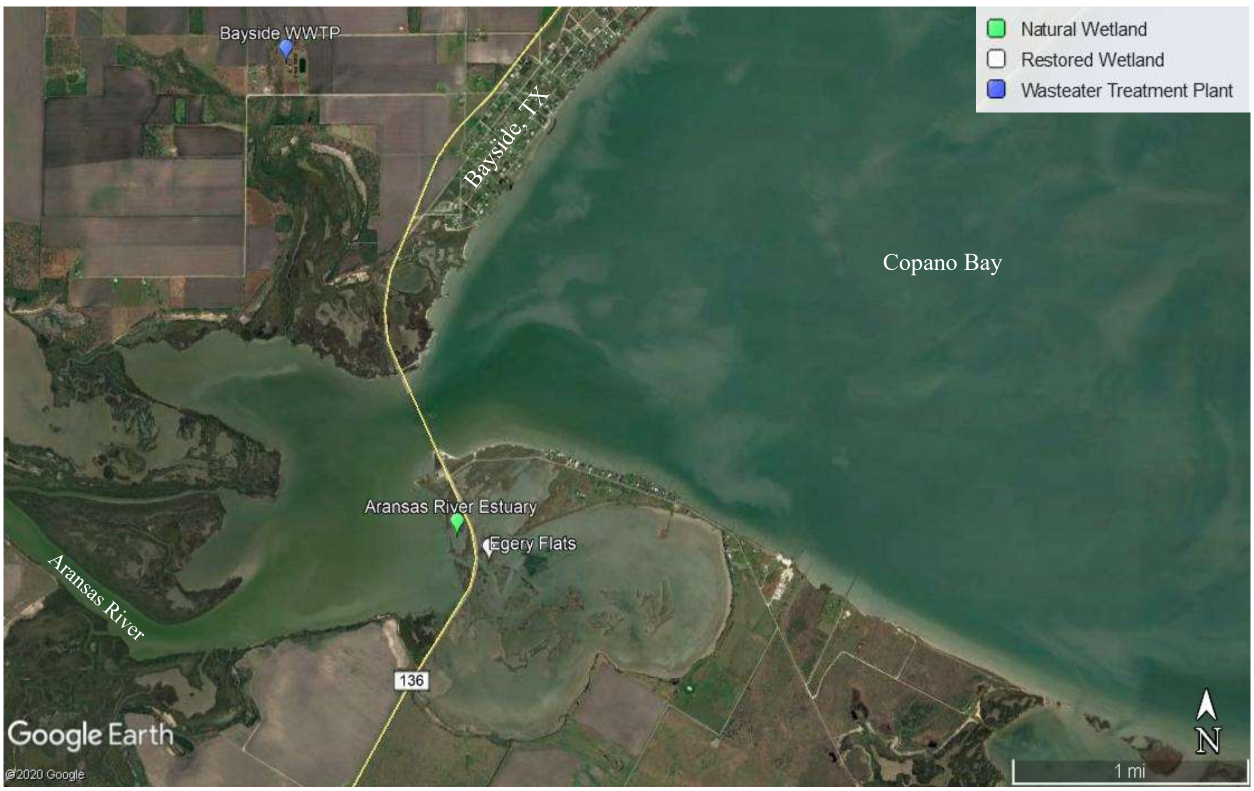


Figure 7. Map including Bayside WWTP, and adjacent natural and restored wetland sampling sites in the Aransas Bay complex and Egery Flats. Map developed with Google Earth.

Figure 8. Images of the culvert restoration project in Egery Flats. **a-** map of Egery Flats showing the two locations of culvert replacement, **b-** photo of 30" pipe culverts, pre-reconstruction in 2018, **c-** photo of 3'x6' box culverts, post-reconstruction, 2019. Map created using Google Earth.

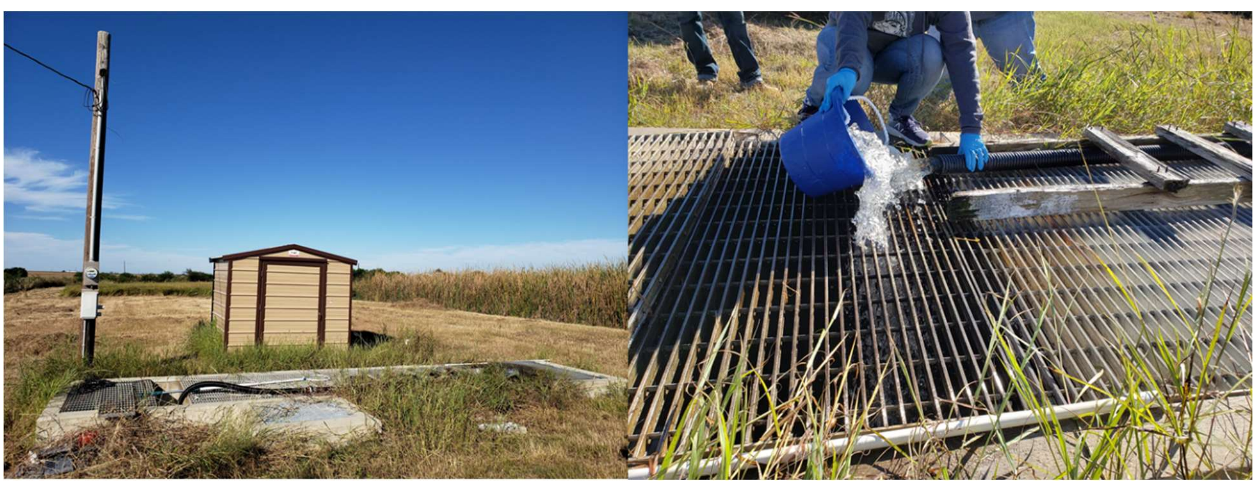


Figure 9. Photos of the Bayside WWTP effluent outfall.

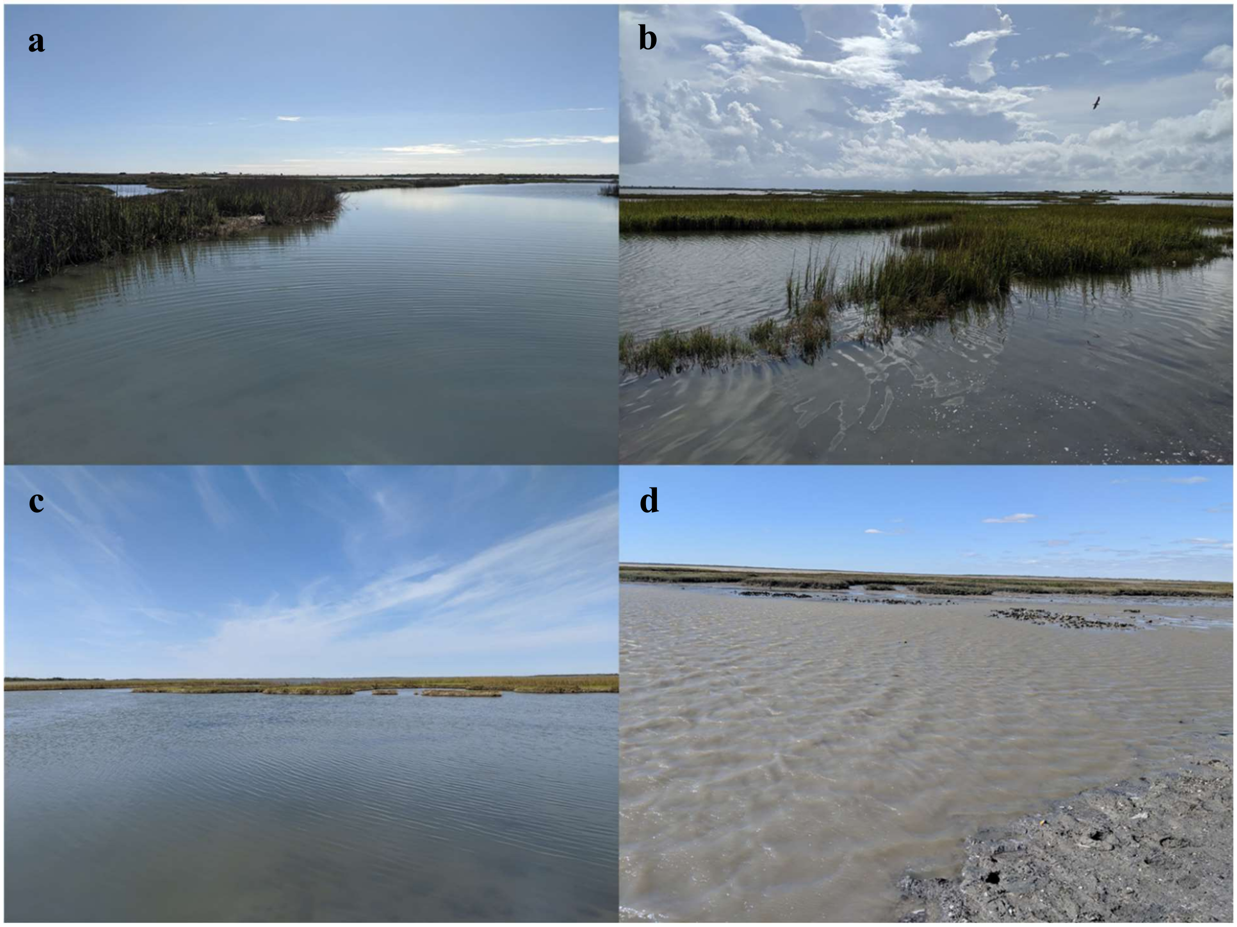


Figure 10. a, b- Photos of Egery Flats. **c, d-** Photos of the Aransas River Estuary.

2.2 Field Sampling

Each of the eight sampling sites were sampled monthly from October 2018 through December 2019. For each site, environmental parameters were measured including pH, water temperature, salinity, and dissolved oxygen (DO). Water temperature and pH were measured using a YSI Meter 63. Salinity was measured using a Thermo Orion model 135A conductivity meter, and dissolved oxygen was measured using a Thermo Orion model 835A advanced DO meter. Each instrument was calibrated monthly.

Water samples were collected monthly for nutrient analysis of nitrate, nitrite, and ammonium; 10mL of surface water were filter sterilized with 0.22µm PES syringe filters and collected in triplicate. Water samples for nitrate, nitrite, and ammonium nitrogen stable isotopes were collected in triplicate quantities of 15mL for each nitrogen species. Water was filtered sterilized using 0.22µm PES syringe filters into 15mL centrifuge tubes, and nitrate and nitrite samples were chemically preserved using 2.5mM sulfamic acid in 25% HCl, and 6M NaOH, respectively, using nitrogen isotope chemical preservation techniques outlined in Bourbonnais et al. (2017).

In situ dissolved gas samples were collected using 12mL serum bottles, pre-flushed with N_2 gas, and vacuum evacuated. 10mL of surface water was collected with a 10mL Hamilton GASTIGHT® syringe and injected into the vacuum evacuated vials, in triplicate for laboratory analysis, using the Gas Chromatograph (GC) headspace equilibrium technique (Hudson 2004; Osburn et al., 2014; Helton et al., 2014; Brazelton et al., 2017).

At each wetland site, sediment samples were collected monthly to quantity denitrification using the acetylene blocking method. These samples were collected from the top 5 cm of submerged soil using a shovel and placed in a 32 oz Mason Jar and sealed. All samples were held on ice for transport back to the laboratory, where water and gas samples were stored at -20°C for future analysis. Sediment samples were stored at 4°C to slow down microbial activity until analysis.

2.3 Study Area Seasonal Climate Variation

Seasons were designated as winter: December, January, February; spring: March, April, May; summer: June, July, August; and fall: September, October, November. To understand seasonal climate variation for the study area over, data from NOAA National Centers for Environmental Information was collected. This data included daily measurements of total precipitation, and maximum and minimum air temperatures collected from January 2010 through December 2019. The data was measured in Corpus Christi NAS, TX station USW00012926. Statistical analyses including ANOVA and student's t-test were performed using this data to determine significant differences between seasons, between the study year and the previous decade, and between El Niño, La Niña, and ENSO-neutral years to determine if the measurements in this study are typical, or if the conditions of this year are anomalous.

2.4 Nutrient Analysis

To identify and quantify the presence of different N species in water from all sampling locations, nutrient concentrations for NO_3^-, NO_2^-, and NH_4^+ were measured using a SEAL AQ300 Discrete Analyzer. For NH_4^+ analysis, AQ300 method EPA-148-D Rev 0 was used with a range of 0.21-71 µM. Samples with greater than 71 µM concentration of NH_4^+ were diluted into the detection range. In this method 400 µL of sample is used, which reacts with hypochlorite from 40µL of dichloroisocyanurate. The chloramine that is formed reacts with 90 µL of salicylate reagent at alkaline pH is the presence of nitroferricyanide. A blue-green indophenol dye forms and is then measured spectrophotometrically at 660 nm. Concentration is then calculated using the absorbance unit compared to an 8 point calibration curve (R^2> 0.9990).

Nitrite samples were analyzed using AQ300 method EPA-115-D Rev A with a range of 0.05 to 107 µM. Samples with concentrations greater than 107 µM of NO_2^- were diluted into the range. This method mixes 200 µL of the sample with 200 µL of sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride and 100 µL of a pH buffer solution to form a red-purple dye that is measured spectrophotometrically at 520 nm. Concentration is calculated using an 8 point calibration curve (R^2> 0.9998).

Nitrate samples are analyzed using cadmium reduction according to the AQ300 method EPA-126-D Rev yielding concentrations of NO_3^- + NO_2^-, with a range of 0.57-357 µM. This method first mixes the sample with 290 µL of a pH buffer and pulls the 430 µL of sample through a copper treated cadmium coil, where NO_3^- is reduced to NO_2^-. The reduced sample then reacts with 350 µL of sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride which turns the mixture red-purple, which is measured using a spectrophotometer at 520 nm. This test uses an 8 point calibration curve (R^2> 0.9990). Once values are obtained, NO_2^- concentrations are subtracted from the total NO_3^- + NO_2^- value to get the final NO_3^- concentration. Each sample for each chemistry is measured in triplicate. Nutrient samples are collected monthly from each site and analyzed in triplicate for NO_3^-, NO_2^-, and NH_4^+ reported in µM concentrations.

2.5 Dissolved Gas Concentrations

Gas samples collected in vacuum evacuated serum bottles were injected with helium to fill the headspace and set to equilibrate before measurement using a Thermo Scientific Trace 1310 Gas Chromatograph fitted with a Flame Ionization Detector (FID), Thermal Conductivity Detector (TCD), and an Electron Capture Detector (ECD) to measure the concentration of greenhouse gasses methane (CH_4), carbon dioxide (CO_2), and nitrous oxide (N_2O), respectively. Once balanced, 2 mL of headspace was removed from the bottle for injection into the Gas Chromatograph (Hudson 2004; Brazelton et al., 2017). Dissolved gas concentrations were calculated according to their solubility constant, at the analytical temperature and pressure, using standard gas mixture for calibration (Hudson 2004; Osburn et al., 2014; Helton et al., 2014; Brazelton et al., 2017). The detection of N_2O in these samples is a prospective indicator of *in situ* denitrification occurring in the surface water and can give insight to possible biogeochemical reactions. These gas samples were collected monthly from each of the eight sampling sites and analyzed in triplicate replications.

2.6 Stable Isotope Analysis

Stable isotopes in NO_3^-, NO_2^-, and NH_4^+ were used to identify nitrogen sources in water samples. Different nitrogen sources have different ^{15}N:^{14}N ratios (‰), allowing for the source identification (Freyer and Republic 1978; Felix et al., 2013). Nitrate $\delta^{15}N$ and $\delta^{18}O$ can be used to differentiate between synthetic fertilizer, sewage, atmospheric, denitrification and nitrification origins (Bottcher et al., 1990; Kendall 1998; BryantMason et al., 2013). Stable isotope samples for NH_4^+ are analyzed following an established method (Zhang et al., 2007). Briefly, water samples are treated with sulfamic acid and 10% HCl to remove pre-existing NO_2^-. Once this reaction has taken place, NH_4^+ was oxidized to NO_2^- using hypobromite (BrO^-). Sodium arsenite was then added to remove any additional BrO^- and NO_2^-, yield was then measured on the SEAL AQ300 Discrete Analyzer. The nitrite was then sent to an isotope lab at the University of Massachusetts Dartmouth, School for Marine Science and Technology, where it was further reduced to N_2O for isotope analysis. Nitrate samples were reduced to NO_2^- using cadmium (Cd). The produced NO_2^- samples were reduced to N_2O following previously published procedures (McIlvin and Altabet 2005). Along with the samples that were analyzed, blanks were also analyzed to account for any nitrogen in the water that was used for reagents. Analysis of samples were performed in triplicate replications. The equation used to calculate the $\delta^{15}N$ ratio in the samples is seen below:

$$\delta^{15}\mathrm{N}(‰) = \frac{((^{15}\mathrm{N}/^{14}\mathrm{N})\ \mathrm{sample}) - ((^{15}\mathrm{N}/^{14}\mathrm{N})\ \mathrm{standard})}{((^{15}\mathrm{N}/^{14}\mathrm{N})\ \mathrm{standard})}\ \mathrm{X}\ 1000$$

2.7 Quantification of Denitrification

Potential rates of denitrification were determined through gas chromatography using the acetylene blocking method (Groffman and Tiedje 1989; Groffman et al., 2006). Sediment from the top 5cm of soil were well mixed into a slurry and funneled into three 160mL serum bottles. The 160mL serum bottles were each filled with 70mL of the slurry and were injected with 10mL of Milli-Q water. The bottles were then closed with butyl rubber stoppers and flushed with N_2 gas for 10 minutes to create anaerobic conditions. The sediments are then incubated at sampling temperatures overnight to stabilize the surface water-sediment interface. The serum bottles were then injected with 20mL of wastewater from the adjacent WWTP, which is high in NO_3^-, and then injected with 30mL of acetylene gas (C_2H_2) (Richardson et al., 2004; Schipper et al., 2005). The sample was then well-mixed and balanced back to atmospheric pressure. A 2mL sample was then injected into a Thermo Scientific Trace 1310 Gas Chromatograph, and N_2O production was measured once per hour for six hours. Denitrification rates were calculated using the rate of N_2O accumulation over time (Groffman and Tiedje, 1989). Samples were collected from each wetland site monthly and analyzed for denitrification using the method listed above in triplicate replications to see monthly and seasonal variations in potential denitrification rates.

The addition of wastewater into the serum bottles allows for the measurement of denitrification potential if the sediments were not nitrate- and diffusion-limited. The C_2H_2 addition was used to block nitrification of NO_3^- to NH_4^+, and further reduction of N_2O to N_2 gas. This allows for the isolation of the reduction of NO_3^- to N_2O, which is easier to measure compared to N_2 gas with its high ambient concentration (Figure 11). The rates of denitrification are then corrected using the Henry's Law Constant for N_2O for dissolved N_2O in the aqueous portion of the incubation layer (Sander 1999; Lindau et al., 2008). Once the total concentration of N_2O produced has been obtained N_2O flux can be calculated as kg $N \cdot ha^{-2} \cdot yr^{-1}$ using the equation below (Rolston 1986; Lindau et al., 2008):

$$\text{Flux} = \left(\frac{\text{Headspace Volume}}{\text{Sediment Area}}\right) * \left(\frac{273}{\text{Absolute Temperature}}\right) * \left(\frac{\Delta\text{Concentration}}{\Delta\text{time}}\right)$$

The use of this equation converts the N_2O flux of denitrification into a unit that can be used to value nitrogen mitigation ecosystem services.

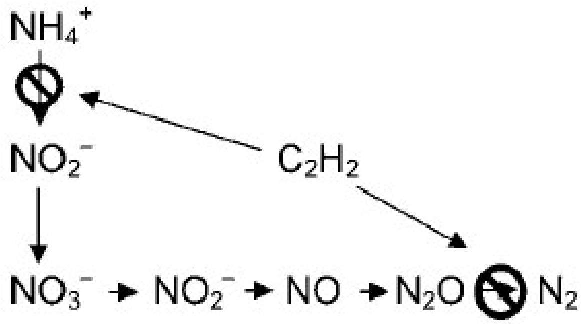


Figure 11. The effects of acetylene gas on the nitrification and denitrification pathways. From Groffman et al., 2006.

2.8 Statistical Analysis

Mean denitrification rates were compared using one- and two-way analysis of variance (ANOVA) to determine significance between different sampling sites, as well as different seasons. Linear regressions were used to determine relationships between temperature, dissolved oxygen, salinity, pH, and denitrification rates (Piehler and Smyth 2011). Excel was used for all statistical calculations.

2.9 Economic Evaluation

Nitrogen mitigation in restored wetlands was valued by assessing the cost equivalent value of nitrogen removal through denitrification using the replacement cost of building and maintaining a manmade nitrogen removal system in connection with a wastewater treatment plant. This valuation was done using a biological nitrogen removal process that removes total nitrogen by utilizing microbial metabolisms under specific environmental parameters (Pollack et al., 2013). The replacement cost method has three requirements that must be met 1) the service provided by the manmade system must be the same as the natural system; 2) the service must be necessary for society; and 3) the alternative must be the lowest cost alternative (Salem and Mercer 2012; Pollack et al., 2013).

To prepare for this analysis, an extensive literature review was performed (Appendix 2). This literature review was done to assess studies that valued the nitrogen mitigation ecosystem service in different habitats, to better understand the monetary value of this service. The method used for calculating the monetary value of the wetland nitrogen mitigation ecosystem service was first outlined in the study by Pollack et al. (2013), evaluating nitrogen mitigation in oyster reefs.

The two restored wetlands in this study, Egery Flats and Nueces Bay restored marsh, will be used as case studies using the replacement cost method. For these case studies, the Back Water River Wastewater Treatment Plant in Maryland will be used as an example of the manmade nitrogen removal alternative to the wetland nitrogen mitigation. This WWTP was chosen because all relevant data for this approach to the replacement cost method was readily available, including the volume of water processed by the plant, the amount of nitrogen removed from the water by the nitrogen removal system, and the capital costs for building the nitrogen removal system (EPA 2007, Pollack et al., 2013). The Back Water River WWTP utilizes a Modified Ludzack-Ettinger biological nitrogen removal (BNR) system, processes 180 mgd, and removes approximately 22.4 mg $N \cdot L^{-1}$ (EPA 2007; Pollack et al., 2013). The capital cost for building the BNR system was $138,305,987 US$2006 (EPA 2007).

The first step to calculate the value of nitrogen mitigation was to calculate the total nitrogen removed by the manmade alternative per year, in the case of the Back Water River WWTP. This is done using the following equation:

$$Total\ N\ removed = \frac{kg\ N \cdot L^{-1}\ removed}{0.264172\ gal \cdot L^{-1}} \times \frac{gal\ processed}{day} \times \frac{365\ day}{yr}$$

The second step was to calculate the amount of nitrogen removed from the wetland per season, using measured seasonal denitrification values. According to the seasons designated previously, winter consists of 90 days, spring consists of 92 days, summer consists of 92 days, and fall consists of 91 days. Nitrogen removed per season can be calculated using the following equation:

$$kg\ N\ removed \cdot ha^{-2} = (seasonal\ kg\ N \cdot ha^{-2} \cdot yr^{-1}) \times 0.25 \cdot yr$$

The sum of the nitrogen removed for each season is then multiplied by the total area of the wetland:

$$annual\ total\ kg\ N\ removed = \ kg\ N\ removed \cdot ha^{-2} \times area\ in\ ha$$

Next, calculate the percent of the nitrogen removed by the wetland based on the total nitrogen removed by the manmade alternative:

$$\%\ N\ removed = \frac{annual\ total\ kg\ N\ removed\ by\ wetland}{annual\ total\ N\ removed\ by\ WWTP} \times 100$$

The equivalent processing capacity of the wetland compared to the manmade alternative can be calculated by the following equation:

$$\begin{aligned} &wetland\ processing\ capacity\ (mgd) \\ &\quad = WWTP\ processing\ capacity\ (mgd) \times \%\ N\ removed \end{aligned}$$

Next the capital cost of the Back Water River WWTP must be converted from US\$2006 to US\$2019 to base the monetary value of the nitrogen mitigation ecosystem service on a more recent dollar value. This is done using the inflation value from January 2006 to January 2019 from the U.S. Bureau of Labor Statistics, where 1 US\$2006 is equivalent to 1.269 US\$2019:

$$\$175{,}510{,}298\ US\$2019 = \$138{,}305{,}987\ US\$2006 \times 1.269$$

Next the total capital, and annual operation and maintenance (O&M) costs need to be calculated based on the converted US\$2019 capital costs. This study used a 10% annual O&M cost based on the average O&M costs of WWTP with MLE systems from the biological nutrient removal processes and costs publication from the Environmental Protection Agency (2007). This study also used an estimated 15-year life span for the MLE system, where 15 years in the typical WWTP life span for upgrades (Foley et al., 2007; Pollack et al., 2013):

$$Capital\ cost\ US\$2019 + (Capital\ cost\ US\$2019 \times 0.10\ O\&M \times 15yrs)$$
$$= total\ 15\ year\ unit\ cost$$

This total unit cost is then divided by the processing capacity, to provide a monetary value for the WWTP in terms of US$2019 per mgd:

$$\frac{total\ 15\ year\ unit\ cost}{WWTP\ processing\ capacity\ (mgd)} = \$ \cdot mgd^{-1}$$

Using this US$2019 per mgd monetary value for the Back Water River WWTP, the value for the equivalent processing capacity for the wetland can be calculated using the following equation:

$$wetland\ processing\ capacity\ (mgd) \times WWTP\ \$ \cdot mgd^{-1}$$
$$= total\ 15\ year\ wetland\ unit\ cost$$

The annualized wetland unit cost can be calculated using the following equation:

$$\frac{total\ 15\ year\ wetland\ unit\ cost}{15\ years} = annualized\ wetland\ unit\ cost$$

The annualized unit cost is then multiplied by the 10% annual O&M cost to achieve the potential annual engineered equivalent cost for the nitrogen mitigation ecosystem service provided by the restored wetland:

$$annualized\ wetland\ unit\ cost + (total\ 15\ year\ wetland\ unit\ cost \times 0.10)$$
$$= total\ annualized\ wetland\ unit\ cost$$

This final value is the annualized capital, O&M cost of a BNR system to replace the denitrification capabilities of the restored wetlands.

3. Results

3.1 Study Area Seasonal Climate Variation

Using the climate data collected from NOAA for Corpus Christi, TX, it was found that for the 10 year period spring and fall had significantly higher total precipitation compared to winter and fall had significantly higher precipitation compared to summer (ANOVA, P<0.05, Figure 12). Spring had higher precipitation, though not significantly (P=0.06), and there was no significant difference in precipitation between winter and summer, or spring and fall, respectively (P>0.05). There was no difference in total annual precipitation between the years from 2010 to 2019 or between El Niño, La Niña, and ENSO-neutral years (P>0.05). There was no significant difference between seasons in El Niño, La Niña, and ENSO-neutral years (ANOVA, P>0.05).

Seasonal mean maximum air temperatures differed significantly between all seasons for the ten year period between 2010 and 2019, with winter being the coolest, followed by spring, fall, and then summer (ANOVA, P<0.05, Figure 13). There were no significant differences between seasons for La Niña and ENSO-neutral years, or La Niña and El Niño years (P>0.05). There were no significant differences in mean maximum seasonal temperatures between years from 2010 to 2019, or between El Niño, La Niña, and ENSO-neutral years (P>0.05). There were significant differences in seasonal mean minimum air temperature between all seasons, from 2010 to 2019 except between spring and fall, with spring being the coolest, then spring, fall, and summer (P<0.05, Figure 14).

There were no significant differences in seasonal minimum air temperature between all seasons for El Niño, compared to La Niña years from 2010 to 2019 (P<0.05). ENSO-neutral winters had higher minimum air temperatures compared to El Niño winters, El Niño summers had higher minimum air temperatures compared to ENSO-neutral summers (P<0.05). La Niña summers had significantly higher minimum air temperatures compared to ENSO-neutral summers, and there was no significant difference between El Niño and ENSO-neutral spring and fall, or between La Niña and ENSO-neutral winter, spring, and fall.

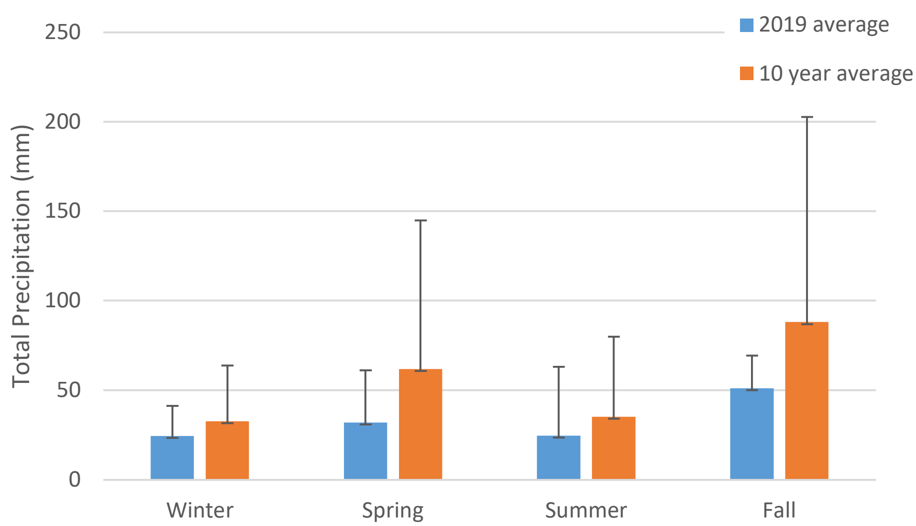


Figure 12. Total precipitation (mm) averaged seasonally for 2019 (blue), compared to the 10 year seasonal average from 2010 to 2019 (orange).

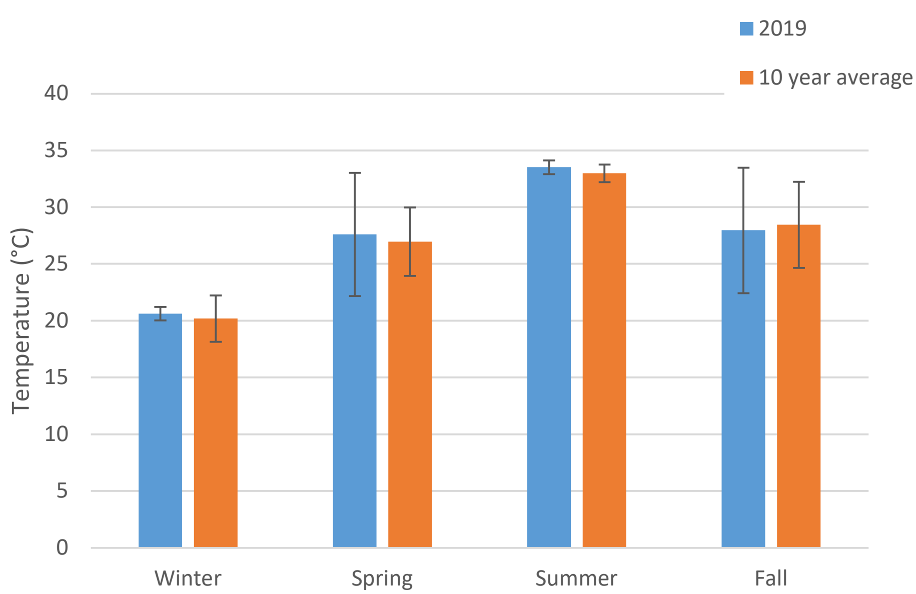


Figure 13. Seasonal mean maximum air temperature in 2019 (blue) and averaged for 10 year period from 2010 to 2019 (orange).

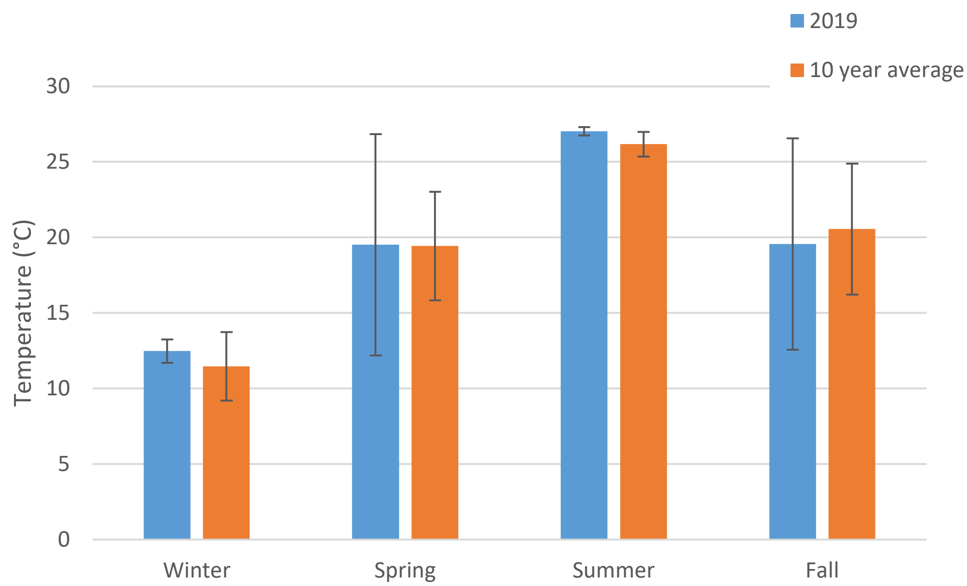


Figure 14. Seasonal mean minimum air temperature in 2019 (blue) and averaged for 10 year period from 2010 to 2019 (orange).

3.2 Environmental Parameters

Measured environmental parameters varied between seasons at each site (Figure 15). There was no significant difference between annual mean pH levels between all sampling sites. Mean annual DO concentrations varied between sampling sites (ANOVA, P<0.05). DO concentrations were lowest at the Oso Bay WWTP; all wetland sites, as well as Bayside WWTP had significantly higher DO levels than the Oso Bay WWTP and the Portland WWTP (t-test, P<0.05). The mean annual temperature at the Bayside WWTP was significantly lower than all other sites. There were no other significant differences between annual mean temperatures. The annual mean salinity varied significantly between different sites. Salinity was significantly higher in all wetland sites compared to all WWTPs. There is a salinity gradient, with higher mean annual salinities in the Nueces Bay restored marsh site and the Naval Airbase Bridge site compared to the other sites (t-test, P<0.05).

Some of the environmental conditions varied by season (Figure 16). Summer showed the highest temperatures for all sites, and winter showed the lowest temperatures for all sites. Fall showed warmer temperatures than spring and fall, and spring had no significant differences in temperatures for all sites (t-test, $P>0.05$). DO was significantly lower during the summer season at the Naval Airbase Bridge site and was also lower in the fall than in spring or winter. This was the only site with a significant change in DO between seasons. The highest DO concentrations were found at WWTP sites. Only the Aransas River Estuary showed seasonal variation in salinity, with fall and winter salinities being significantly lower than spring or summer (ANOVA, T-test, $P<0.05$). Three sites showed significant differences in pH levels between seasons. The Bayside WWTP and Egery Flats had higher pH levels in the winter and spring compared to summer and fall, and the Naval Airbase Bridge site had lower levels in the fall compared to other seasons. All sites had very low mean annual nitrous oxide concentrations, as well as low methane concentrations, with higher mean annual carbon dioxide concentrations compared to nitrous oxide and methane concentrations (Table 3).

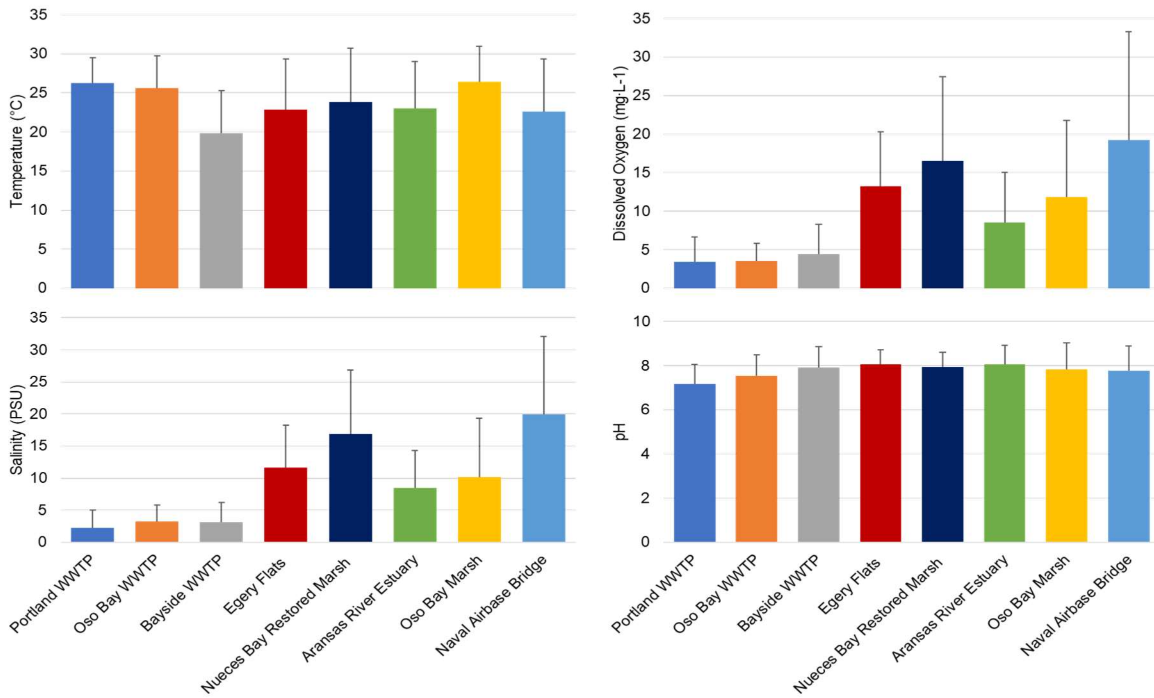


Figure 15. Bar graph depicting annual means of environmental variables at each sampling site including water temperature (upper left), DO concentration (upper right), salinity (lower left), and pH (lower right) for surface water.

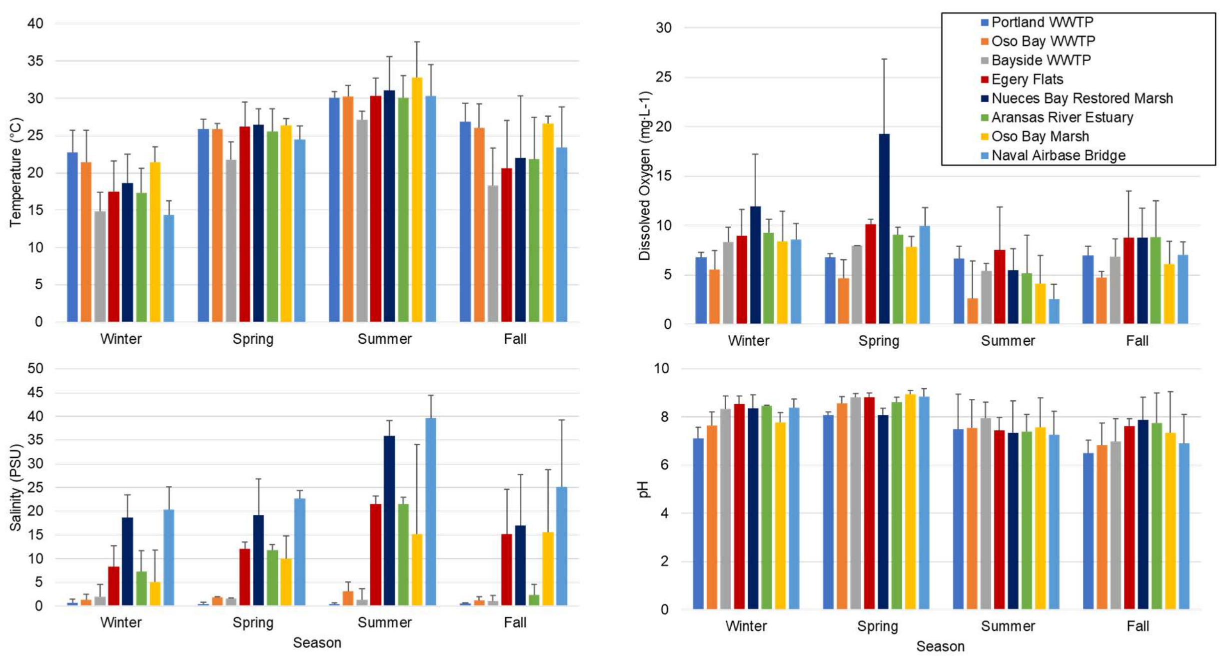


Figure 16. Bar graph depicting seasonal means of environmental variables for each sampling site including water temperature (upper left), dissolved oxygen (upper right), salinity (lower left), and pH (lower right).

Table 3. Annual mean dissolved gas concentrations for nitrous oxide, carbon dioxide, and methane for all sampling sites (mean ± standard deviation).

Site	N_2O (µM)	CO_2 (µM)	CH_4 (µM)
Portland WWTP	1.21 ± 1.49	154.22 ± 119.00	0.24 ± 0.27
Oso Bay WWTP	2.39 ± 1.72	185.65 ± 137.57	0.09 ± 0.07
Bayside WWTP	0.02 ± 0.05	114.42 ± 73.45	2.00 ± 4.00
Egery Flats	0.00 ± 0.01	56.54 ± 67.47	0.14 ± 0.15
Nueces Bay Restored Marsh	0.02 ± 0.07	36.76 ± 49.47	0.10 ± 0.16
Aransas River Estuary	0.01 ± 0.03	49.90 ± 59.02	0.20 ± 0.38
Oso Bay Marsh	0.49 ± 0.78	65.72 ± 74.30	0.13 ± 0.09
Naval Airbase Bridge	0.01 ± 0.01	24.85 ± 17.77	0.06 ± 0.08

3.3 Nutrient Concentrations

Nutrient concentration varied at each site. Mean annual NH_4^+ concentrations at the Portland WWTP, 339 µM, was significantly higher than at the Bayside WWTP, 34 µM, and all of the wetland sites, but was not significantly different from the Oso Bay WWTP, 180 µM ($P<0.05$, Figure 17). The mean annual NO_3^- concentration at the Portland WWTP, 511 µM, was significantly higher than all other sites ($P<0.05$, Figure 18). Mean annual NO_2^- concentration at the Portland WWTP, 30 µM, was significantly higher than all other sites except the Bayside WWTP, 10 µM, where there was no significant difference ($P<0.05$, Figure 19).

Mean annual NH_4^+ concentration at the Oso Bay WWTP was significantly higher than the Bayside WWTP concentrations, as well as all wetland sites except for the Oso Bay marsh site, 49 µM ($P<0.05$, Figure 17). The annual mean NO_3^- concentration measured at the Oso Bay WWTP was significantly higher than all other sites, except the Portland WWTP (Figure 18). The Oso Bay WWTP mean annual NO_2^- concentration was not significantly different from any sites, aside from the Portland WWTP, mentioned above ($P>0.05$). The NH_4^+ and NO_3^- annual means for the Bayside WWTP were higher than all wetland sites, except for the Oso Bay marsh site (Figure 17, Figure 18). There were no significant differences in mean annual NO_2^- concentrations between the Bayside WWTP and any of the wetland sites ($P>0.05$, Figure 19).

Mean annual NH_4^+ concentration at the Oso Bay marsh site was significantly higher than all wetland sites but was not significantly different from concentrations at the Bayside or Oso Bay WWTPs (Figure 17). The Oso Bay marsh had a significantly higher mean annual NO_3^- concentration, 81 μM, than all wetland sites and the Bayside WWTP ($P<0.005$, Figure 18). There was significantly higher mean annual NO_2^- concentrations at the Oso Bay marsh, 5 μM, compared to Egery Flats, Aransas River Estuary, and Nueces Bay restored marsh (Figure 19). There was no difference between the mean annual NO_2^- concentrations at the Oso Bay marsh, and the Bayside WWTP, or the Naval Airbase Bridge site ($P>0.05$, Figure 19). There were no significant differences in mean annual NH_4^+, NO_3^-, and NO_2^- concentrations between Egery Flats, Aransas River Estuary, Nueces Bay restored marsh, and the Naval Airbase Bridge ($P>0.05$, Figure 19).

Nutrient concentrations also varied between sites for different seasons. The NH_4^+ concentrations for the Portland WWTP were significantly higher than all other sites for both spring and winter and were higher in the fall compared to all sites except for the Oso Bay WWTP ($P<0.05$, Figure 20). There were no significant differences between the NH_4^+ concentration at the Portland WWTP and the other sites during the summer season. The NO_3^- concentrations for the Portland WWTP were significantly higher than all other sites for the winter season, and for the fall were significantly higher than all other sites except for the Oso Bay WWTP and the Oso Bay marsh site ($P<0.05$, Figure 21). There was no significant difference between NO_3^- concentrations for the Portland WWTP and all other sites during the spring and summer months. There was no significant difference in NO_2^- concentrations at the Portland WWTP compared to all other sites in winter, spring, and fall, but was significantly higher than the Oso Bay WWTP, Egery Flats, the Aransas River Estuary, and the Naval Airbase Bridge site during the summer (Figure 22).

The Oso Bay WWTP mean NH_4^+ concentrations were significantly higher than the Aransas River Estuary, Nueces Bay restored marsh, and the Naval Airbase Bridge site during winter ($P<0.05$, Figure 20). There was no significant difference in NH_4^+ concentration at the Oso Bay WWTP and all other sites in the spring, summer, or fall. The mean NO_3^- concentrations in the Oso Bay WWTP were significantly higher than all sites in the fall, and higher than all sites except for the Portland WWTP and Oso Bay marsh sites in the winter and spring. The mean NO_3^- concentrations in the Oso Bay WWTP were also significantly higher than Egery Flats, the Aransas River Estuary, Nueces Bay restored marsh, and the Naval Airbase Bridge during the summer ($P<0.05$ Figure 21). There was no significant difference in NO_2^- concentrations between the Oso Bay WWTP and the other sites for winter, spring, fall, and the Oso Bay WWTP NO_2^- concentrations were significantly higher than Egery Flats, the Aransas River Estuary, and the Nueces Bay restored marsh during the summer ($P<0.05$, Figure 22).

The Bayside WWTP, a constructed wetland used to treat wastewater, showed no significant differences in seasonal mean NH_4^+, NO_3^-, and NO_2^- concentrations for fall, and winter. There was no significant difference between NH_4^+, NO_3^-, and NO_2^- concentrations in the Bayside WWTP and all wetland sites in the spring, except for NO_2^- concentrations were significantly higher than the Naval Airbase Bridge ($P<0.05$, Figure 21). The Oso Bay marsh site had the highest nutrient concentrations for NH_4^+, NO_3^-, and NO_2^- of all wetland sites during all seasons likely due to its close proximity to the outfall of the Oso Bay WWTP outfall (Figure 20; Figure 21; Figure 22). The Bayside WWTP and the other wetland sites had low nutrient concentrations.

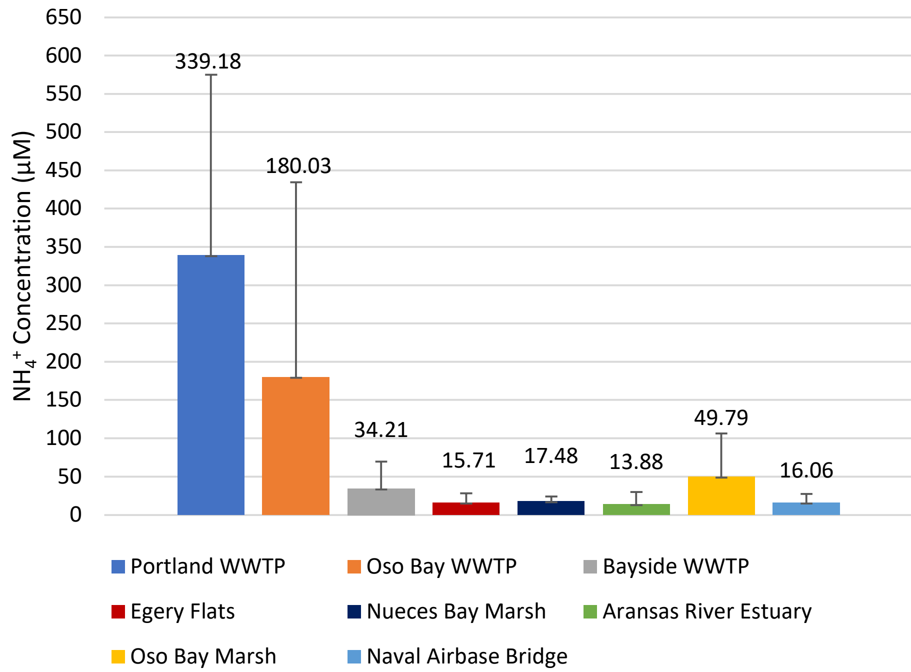


Figure 17. Mean annual ammonium concentrations for all sampling sites.

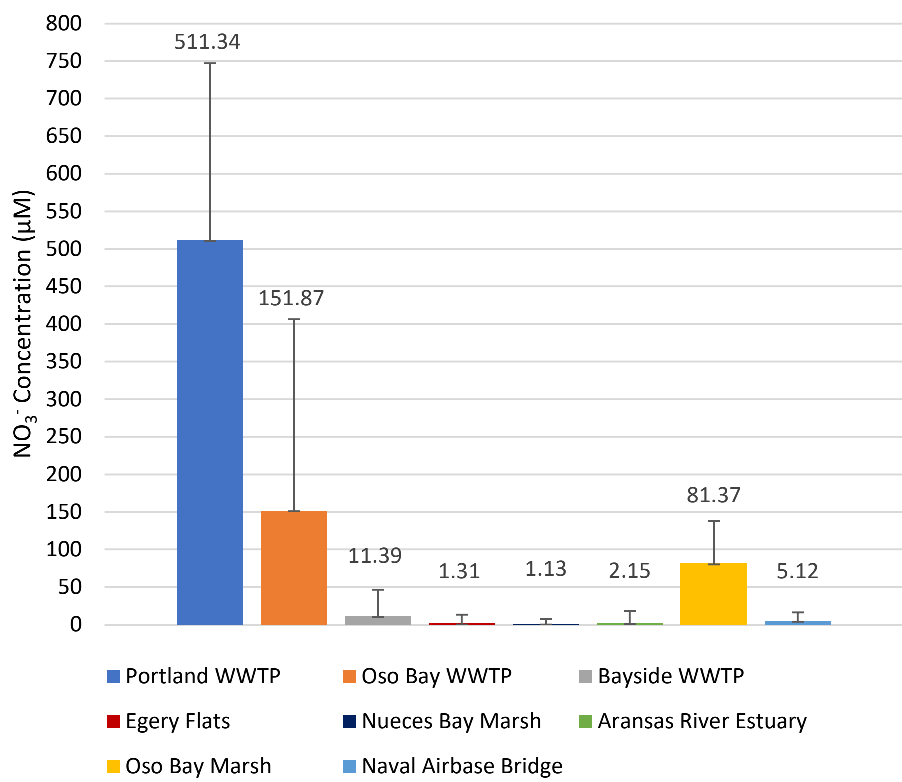


Figure 18. Mean annual nitrate concentrations for all sampling sites.

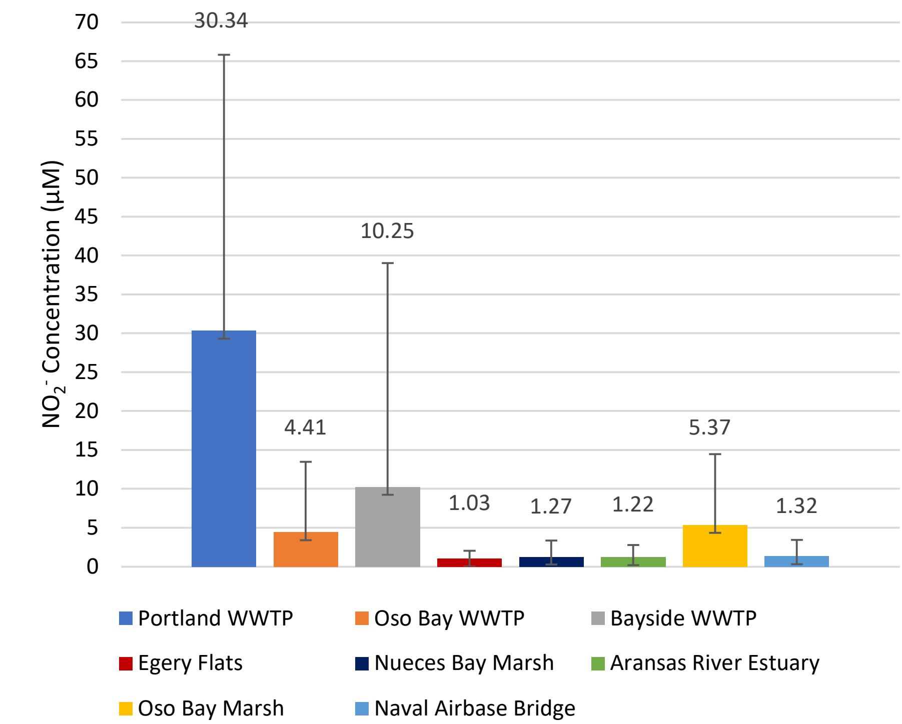
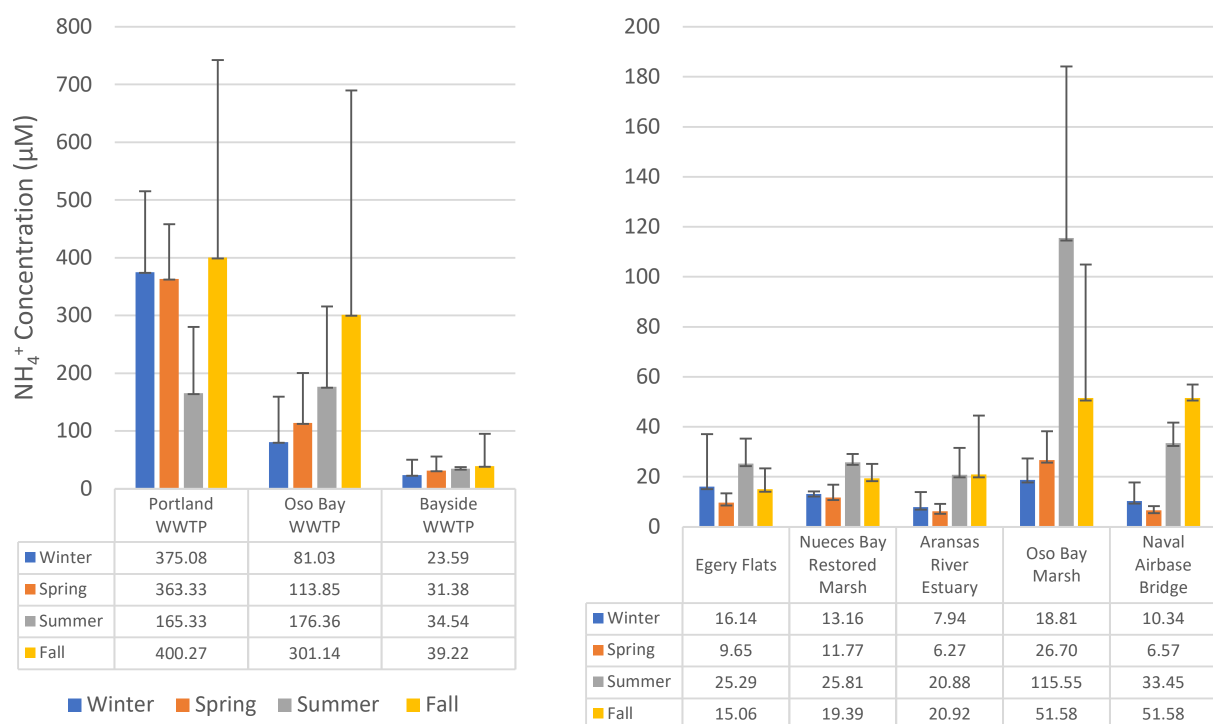


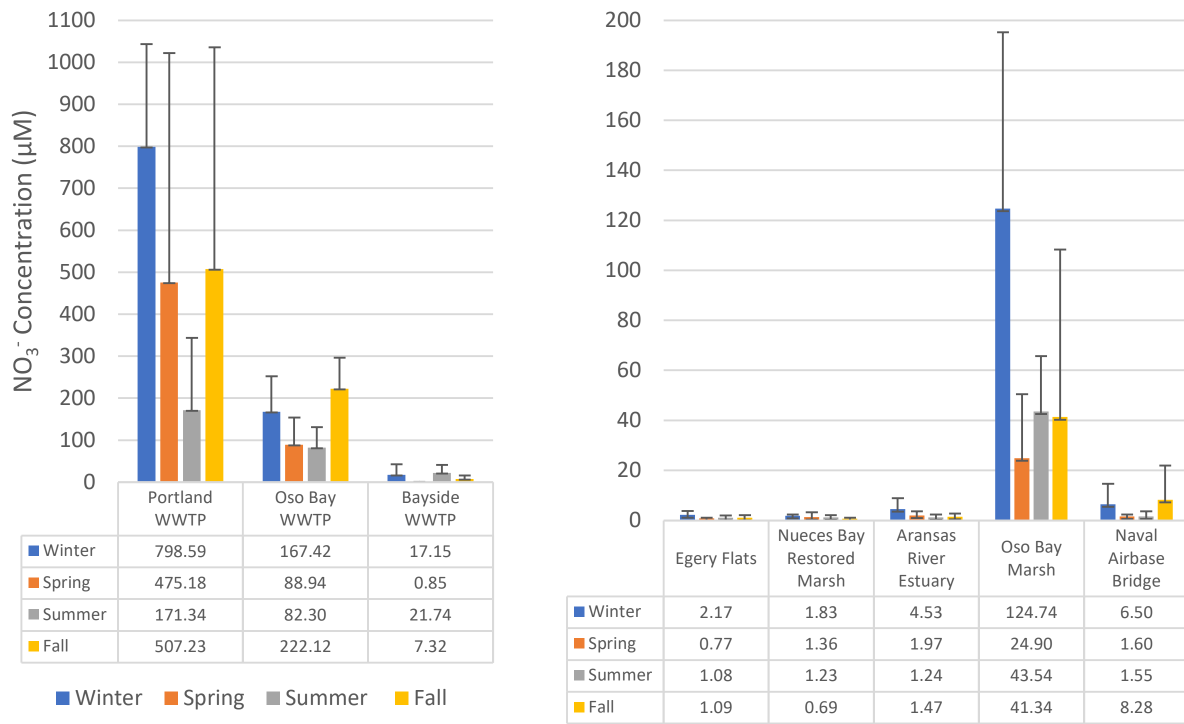
Figure 19. Mean annual nitrite concentrations for all sampling sites.



	Portland WWTP	Oso Bay WWTP	Bayside WWTP
Winter	375.08	81.03	23.59
Spring	363.33	113.85	31.38
Summer	165.33	176.36	34.54
Fall	400.27	301.14	39.22

	Egery Flats	Nueces Bay Restored Marsh	Aransas River Estuary	Oso Bay Marsh	Naval Airbase Bridge
Winter	16.14	13.16	7.94	18.81	10.34
Spring	9.65	11.77	6.27	26.70	6.57
Summer	25.29	25.81	20.88	115.55	33.45
Fall	15.06	19.39	20.92	51.58	51.58

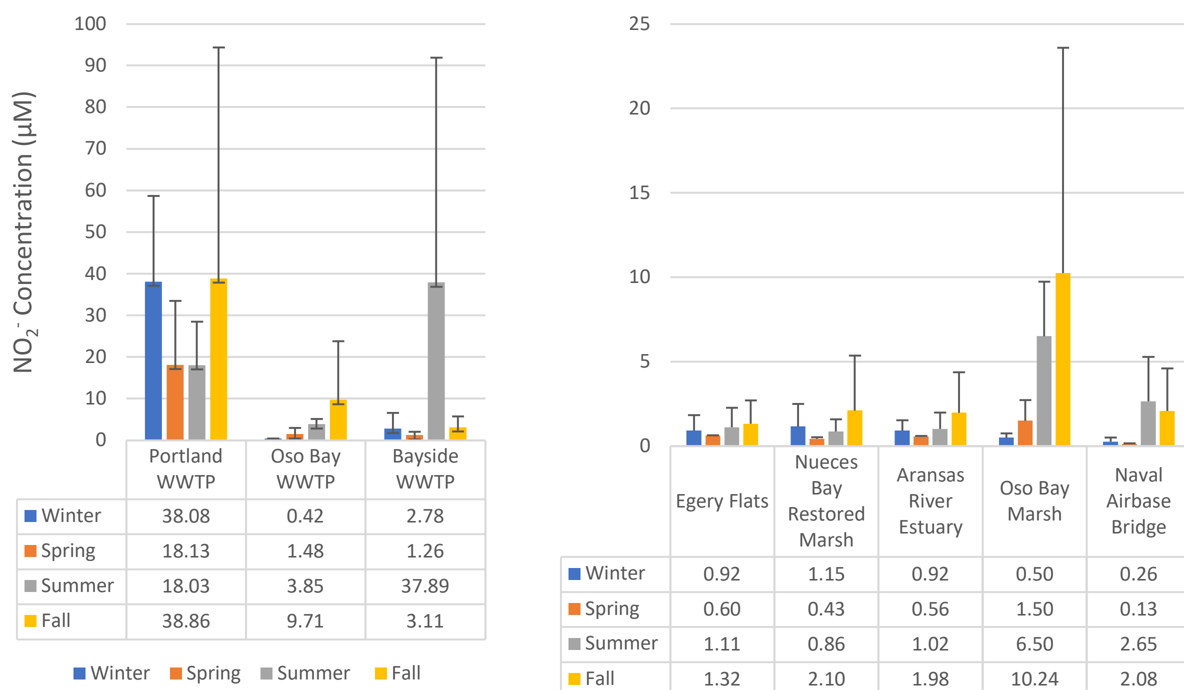
Figure 20. Mean ammonium concentrations by season for all sites, WWTPs (left), wetlands (right). Seasonal means for each site are listed in the table beneath each site.



	Portland WWTP	Oso Bay WWTP	Bayside WWTP
Winter	798.59	167.42	17.15
Spring	475.18	88.94	0.85
Summer	171.34	82.30	21.74
Fall	507.23	222.12	7.32

	Egery Flats	Nueces Bay Restored Marsh	Aransas River Estuary	Oso Bay Marsh	Naval Airbase Bridge
Winter	2.17	1.83	4.53	124.74	6.50
Spring	0.77	1.36	1.97	24.90	1.60
Summer	1.08	1.23	1.24	43.54	1.55
Fall	1.09	0.69	1.47	41.34	8.28

Figure 21. Mean nitrate concentrations for all sites by season, WWTPs (left), wetlands (right). Seasonal means for each site are listed in the table beneath each site.



	Portland WWTP	Oso Bay WWTP	Bayside WWTP
Winter	38.08	0.42	2.78
Spring	18.13	1.48	1.26
Summer	18.03	3.85	37.89
Fall	38.86	9.71	3.11

	Egery Flats	Nueces Bay Restored Marsh	Aransas River Estuary	Oso Bay Marsh	Naval Airbase Bridge
Winter	0.92	1.15	0.92	0.50	0.26
Spring	0.60	0.43	0.56	1.50	0.13
Summer	1.11	0.86	1.02	6.50	2.65
Fall	1.32	2.10	1.98	10.24	2.08

Figure 22. Mean nitrite concentrations for all sites by season, WWTPs (left), wetlands (right). Seasonal means for each site are listed in the table beneath each site.

3.4 *Stable Isotope Analysis*

The $\delta^{15}N- NO_3^-$ in the Portland WWTP ranged from 1.0 to 3.1‰, and the $\delta^{15}N- NH_4^+$ in the Portland WWTP ranged from -27.91 to 10.22‰ (Figure 23). The Oso Bay WWTP had slightly higher $\delta^{15}N- NO_3^-$ values which ranged from 5.6 to 16.1‰, and the $\delta^{15}N- NH_4^+$ values ranged from -10 to 6.6‰ of ammonium fertilizers, ammonium volatilization, and soil ammonium (Figure 23). The $\delta^{15}N- NO_3^-$ in the Bayside WWTP were higher than both Portland and Oso Bay WWTP, ranging from 23.9 to 25.9‰, and the $\delta^{15}N- NH_4^+$ values ranged from 26.57 to 38.86‰, which were significantly higher than both the Portland WWTP and the Oso Bay WWTP values.

Egery Flats had a wide range of $\delta^{15}N-NO_3^-$ from -2.7 to 39.1‰. The Nueces Bay restored marsh had very light $\delta^{15}N-NO_3^-$ and $\delta^{15}N- NH_4^+$ values, ranging from -32.6 to -16.6‰, and -37.08 and -34.05‰, respectively (Figure 23). The Aransas River Estuary had very light $\delta^{15}N-NO_3^-$ values ranging from -8.9 to 0.0‰. Oso Bay marsh and the Naval Airbase Bridge sites had very similar $\delta^{15}N-NO_3^-$ values ranging from -0.5 to 2.8‰, and -3.1 to 1.2‰, respectively (Figure 23). The Oso Bay marsh $\delta^{15}N- NH_4^+$ values range from 3.03 to -5.04‰. Naval Airbase Bridge $\delta^{15}N-NH_4^+$ ranged from -5.11 to -0.32‰ (Figure 23). Due to constraints with the method and interference with dissolved organic nitrogen in the sample the $\delta^{15}N- NH_4^+$ values for Egery Flats and Aransas River Estuary were not able to be reported.

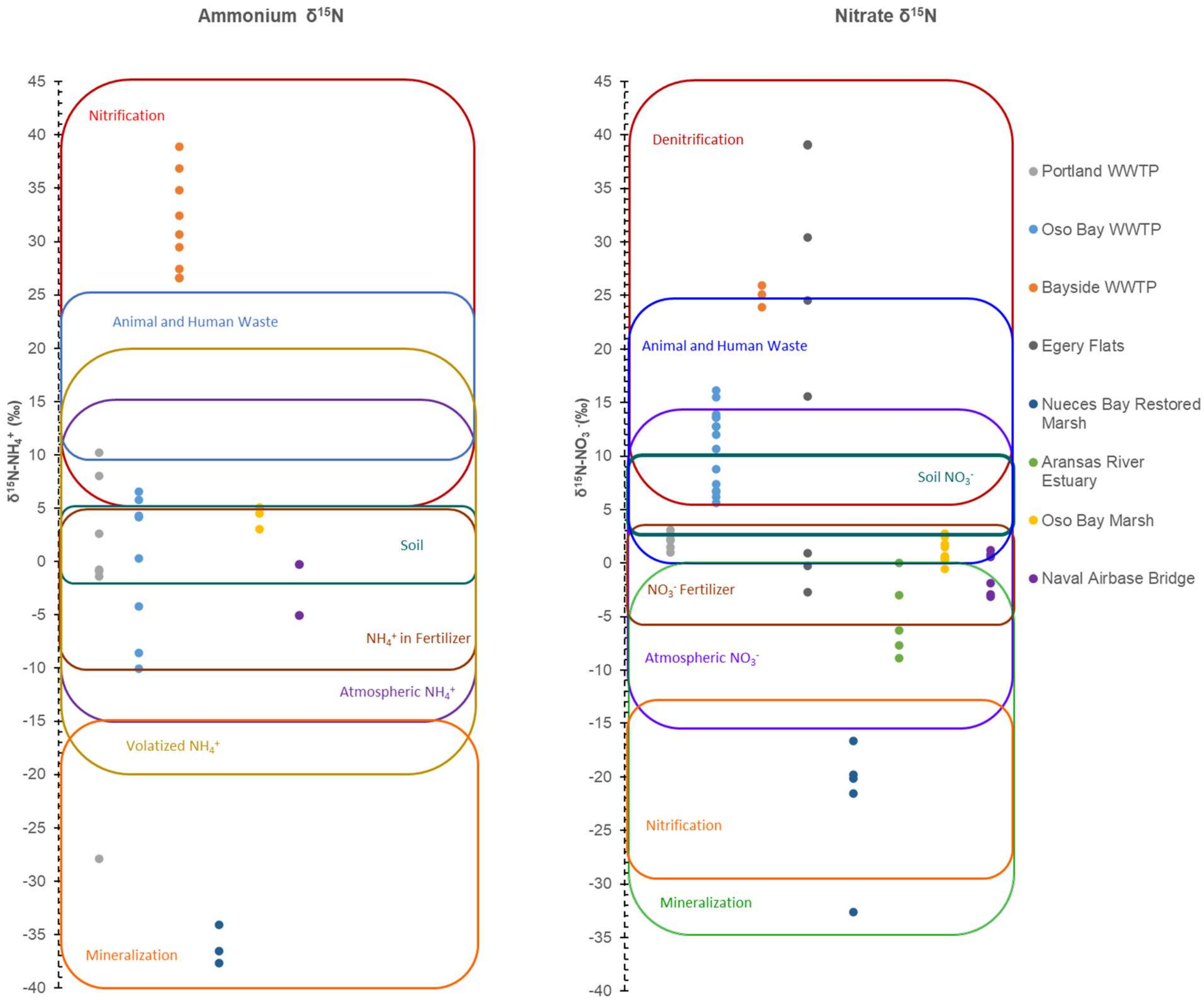


Figure 23. $\delta^{15}N\text{-}NH_4^+$ source plot with ranges reported for various NH_4^+ sources based on Kendall et al., (2007) for all sampling sites (left). $\delta^{15}N\text{-}NO_3^-$ source plot with ranges reported for various NO_3^- sources based on Kendall et al., (2007) for all sampling sites (right). Isotope source and process values adapted from Kendall et al. (2007).

3.5 Denitrification in Restored and Natural Wetlands

Mean annual denitrification rates between the five wetland sites were significantly varied (ANOVA, $P<0.05$, Figure 24). Egery Flats, Nueces Bay restored marsh, and Aransas River Estuary had the lowest mean annual denitrification rates, 11.47, 10.85, 22.51 kg $N \cdot ha^{-2} \cdot yr^{-1}$, respectively, with no significant difference among the three sites. The mean annual denitrification rates of these three sites were also significantly lower than Oso Bay marsh and Naval Airbase Bridge sites, 29.39 and 39.27 kg $N \cdot ha^{-2} \cdot yr^{-1}$, respectively ($P<0.05$).

Mean seasonal denitrification rates varied among sites, but the combined seasonal means for all sites showed that rates in the summer were significantly higher than all other seasons (ANOVA, $P<0.05$). At Egery Flats, denitrification ranged from 0.40 to 18.04 kg $N \cdot ha^{-2} \cdot yr^{-1}$. Winter had significantly lower denitrification rates than all other seasons, with a denitrification rate of 0.40 kg $N \cdot ha^{-2} \cdot yr^{-1}$ ($P<0.05$). There were no other significant differences between denitrification rates for other seasons with rates of 6.23, 15.28, and 18.04 kg $N \cdot ha^{-2} \cdot yr^{-1}$ for spring, summer, and fall, respectively ($P>0.05$, Figure 25).

Denitrification rates at the Nueces Bay restored marsh site ranged from 4.97 to 24.82 kg $N \cdot ha^{-2} \cdot yr^{-1}$. Denitrification rates were significantly higher in the summer compared to all other seasons, 24.82 kg $N \cdot ha^{-2} \cdot yr^{-1}$ ($P<0.05$). Denitrification rates at the Aransas River Estuary site ranged from 5.59 to 40.69 kg $N \cdot ha^{-2} \cdot yr^{-1}$ (Figure 24). Summer and fall denitrification rates were significantly higher than winter and spring (ANOVA, $P<0.05$). The Aransas River Estuary showed the highest levels in the summer, 40.69 kg $N \cdot ha^{-2} \cdot yr^{-1}$, compared to 5.95 kg $N \cdot ha^{-2} \cdot yr^{-1}$, the lowest in the spring.

Denitrification rates at the Oso Bay marsh site ranged from 24.17 to 33.87 kg $N \cdot ha^{-2} \cdot yr^{-1}$. There are no significant differences in denitrification between seasons at the Oso Bay marsh site. Denitrification rates at the Naval Airbase Bridge site varied from 15.04 to 76.19 kg $N \cdot ha^{-2} \cdot yr^{-1}$, where summer denitrification was significantly higher than all other seasons ($P<0.05$).

There is no significant linear correlation between denitrification rates and pH, temperature, salinity, or dissolved oxygen (Table 4). While the seasonal trends may not be consistent across sites, there are clear age-based trends, where restored wetlands have significantly lower mean annual denitrification rates compared to natural wetlands.

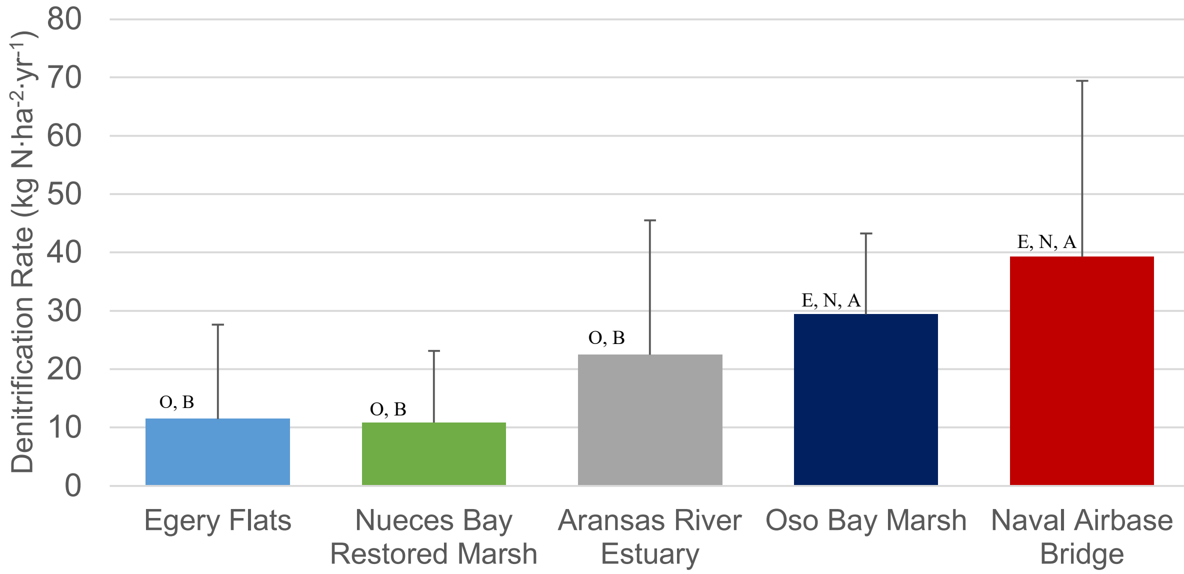


Figure 24. Annual mean denitrification rates for all wetland sites. Significant differences (P<0.05): **E-** significant difference from Egery Flats, **N-** significant difference from Nueces Bay restored marsh, **A-** significant difference from Aransas River Estuary, **O-** significant difference from Oso Bay marsh, **B-** significant difference from Naval Airbase Bridge.

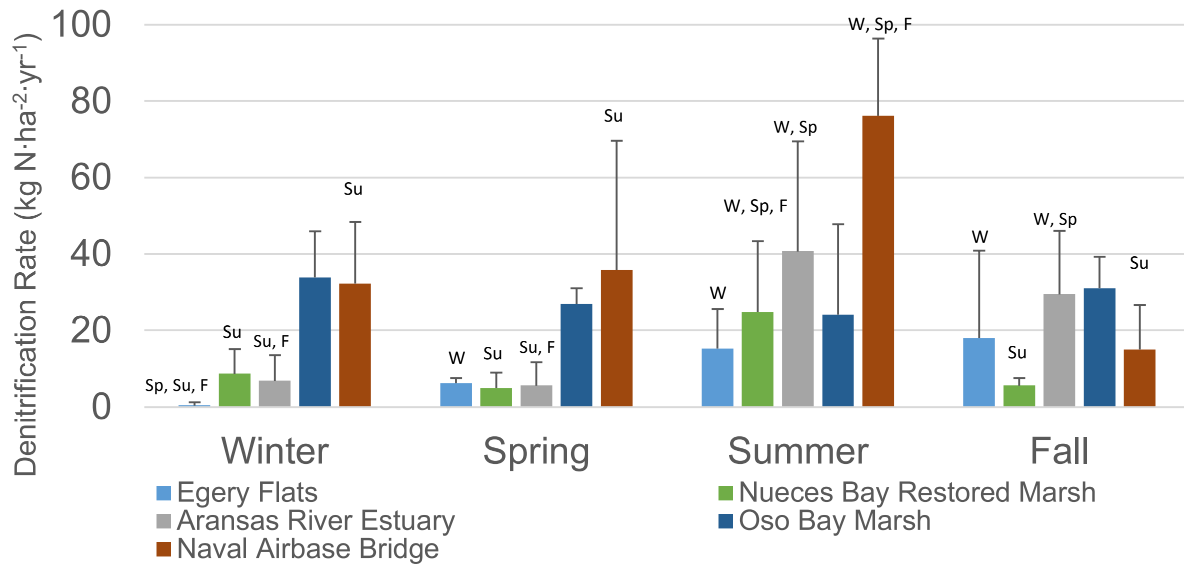


Figure 25. Mean seasonal denitrification rates for all wetland sites. Significant differences between seasons of respective sites (P<0.05): **W-** significant difference from winter, **Sp-** significant difference from spring, **Su-** significant difference from summer, **F-** significant difference from fall.

Table 4. The slopes of the regression analyses comparing environmental parameters to denitrification rates for each site. There was no strong linear relationship for any of the environmental parameters or sampling sites.

Site	Environmental Parameter	Slope	R^2
Egery Flats	pH	-10.009	0.1518
	Temperature (°C)	1.0330	0.2325
	Salinity (PSU)	0.9361	0.2188
	Dissolved Oxygen ($mg \cdot L^{-1}$)	-3.4437	0.3613
Nueces Bay Restored Marsh	pH	1.4374	0.0108
	Temperature (°C)	0.0444	0.0016
	Salinity (PSU)	0.2265	0.0779
	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.0381	0.0003
Aransas River Estuary	pH	-7.3600	0.0654
	Temperature (°C)	1.0071	0.0845
	Salinity (PSU)	-0.4692	0.0767
	Dissolved Oxygen ($mg \cdot L^{-1}$)	-3.4098	0.5055
Oso Bay Marsh	pH	4.0442	0.1321
	Temperature (°C)	-1.3298	0.2420
	Salinity (PSU)	-0.3114	0.0923
	Dissolved Oxygen ($mg \cdot L^{-1}$)	1.6223	0.1554
Naval Airbase Bridge	pH	-6.3633	0.0619
	Temperature (°C)	1.5724	0.1636
	Salinity (PSU)	0.9702	0.2664
	Dissolved Oxygen ($mg \cdot L^{-1}$)	-2.7648	0.1471

3.6 Economic Evaluation of Nitrogen Mitigation at Egery Flats

Egery Flats is a wetland than underwent a restoration project from 2018 to 2019 to replace the culverts, allowing more fresh water to flow into the wetland area. It is a 270-ha wetland area. This restored wetland was used as a case study for determining the monetary value of nitrogen mitigation in restored wetlands by comparing the nitrogen removal capacity to that of a manmade alternative. The annualized capital, O&M cost of a BNR system to replace the denitrification capabilities of the Egery Flats restored wetland were calculated to be equivalent to $36,565 US$2019·yr^{-1} and was calculated below (Table 7).

The Back Water River WWTP processes 180 mgd, the influent into the biological N removal system contains 30 mg·L^{-1} total N and the Modified Ludzack-Ettinger (MLE) process removes approximately 75% of the N, or 22.4 mg·L^{-1} N (EPA 2007; Pollack et al., 2013). The total annual N removal at the Back Water River WWTP is 5,570,915 kg N removed·yr^{-1}, according to the following equation: $Total\ N\ removed = \frac{0.0000225\ kg\ N \cdot L^{-1}}{0.264172\ gal \cdot L^{-1}} \times \frac{180{,}000{,}000\ gal}{day} \times \frac{365\ day}{yr}$

According to data measured in this study, the wetlands at Egery Flats remove an average of 0.40 kg N·ha^{-2}·yr^{-1} in the winter, 6.23 kg N·ha^{-2}·yr^{-1} in the spring, 15.29 kg N·ha^{-2}·yr^{-1} in the summer, and 18.04 kg N·ha^{-2}·yr^{-1} in the fall (Figure 25; Table 5). Using the seasonal nitrogen removal equation, and the denitrification rates measured for Egery Flats, it was calculated that 0.1, 1.56, 3.86, and 4.54 kg N denitrified per hectare, for winter, spring, summer, and fall, respectively (Table 5). The sum of nitrogen removed through denitrification for each season at Egery Flats is 9.99 kg N·ha^{-2}. Therefore, based on the whole 270-ha area of Egery Flats, through denitrification, the restored wetland can remove 2,697 kg N·yr^{-1}:

$$annual\ total\ kg\ N\ removed = 9.99\ kg\ N \cdot ha^{-2} \times 270ha$$

$$= 2{,}697\ kg\ N\ removed \cdot yr^{-1}$$

The Egery Flats restored wetland can remove ~0.05% of the nitrogen that can be removed by the Back Water River WWTP. This 0.05% is equivalent to a WWTP that processes 0.09 mgd. The capital cost for the installation of the MLE system at the Back Water River WWTP was $138,305,987 US$2006. After adjusting for inflation, the capital cost was $175,510,298 US$2019. A 10% O&M cost over an estimated 15-year life span is added to the inflation-adjusted capital cost:

$$\$175{,}510{,}298 + (\$175{,}510{,}298 \cdot 0.10 \cdot 15) = \$438{,}775{,}745$$

$$\frac{\$438{,}775{,}745}{180mgd} = \$2{,}437{,}643 \cdot mgd^{-1}$$

A total unit cost of \$438,775,745 US\$2019 for the Back Water River WWTP, which is equivalent to \$2,437,643·mgd^{-1}.

The annualized capital, and O&M cost of the equivalent 0.09 mgd WWTP for Egery Flats would be calculated as:

$$0.09\, mgd \times \$2{,}437{,}643 \cdot mgd^{-1} = \$219{,}388$$

$$\frac{\$219{,}388}{15\, yrs} = \$14{,}626 \cdot yr^{-1}$$

$$\$14{,}626 + (\$219{,}388 \times 0.10) = \$36{,}565 \cdot yr^{-1}$$

Table 5. Seasonal denitrification rates, and the amount of nitrogen removed per hectare of wetlands per season in Egery Flats.

Season	Seasonal Denitrification rate (kg N·ha^{-2}·yr^{-1})	kg N denitrified per hectare
Winter	0.4	0.10
Spring	6.23	1.56
Summer	15.29	3.82
Fall	18.04	4.54
Annual Total		9.99

3.7 Economic Evaluation of Nitrogen Mitigation at Nueces Bay restored marsh

The Nueces Bay marsh site is a 70 ha wetland area. Based on the following analysis the annualized capital, operation, and maintenance cost of a biological N removal system to replace the denitrification capabilities of the Nueces Bay restored marsh would be equivalent to \$8,125 US\$2019·yr^{-1} (Table 7).

From denitrification measurements in this study, it was found to remove an average of 8.7 kg N·ha^{-2}·yr^{-1} in the winter, 4.97 kg N·ha^{-2}·yr^{-1} in the spring, 24.82 kg N·ha^{-2}·yr^{-1} in the summer, and

5.62 kg N·ha^{-2}·yr^{-1} in the fall (Figure 25, Table 6). Using the seasonal nitrogen removal equation, and the denitrification rates measured for the Nueces Bay restored marsh, it was calculated that 2.18, 1.24, 6.21, and 1.41 kg N denitrified per hectare, for winter, spring, summer, and fall, respectively (Table 6). The sum of nitrogen removed through denitrification for each season at the Nueces Bay restored marsh was 11.03 kg N·ha^{-2}. Therefore, based on the whole 70-ha area of the Nueces Bay restored marsh, through denitrification the restored wetland can remove 772 kg N·yr^{-1}, according to the following equation:

$$annual\ total\ kg\ N\ removed = 11.03\ kg\ N \cdot ha^{-2} \times 70ha$$

$$= 772\ kg\ N\ removed \cdot yr^{-1}$$

The Nueces Bay restored marsh can remove approximately 0.01% of the N removed by the Back Water River WWTP. This 0.01% is equivalent to a WWTP that processes 0.02 mgd. The annualized capital and O&M costs of the equivalent 0.02 mgd WWTP for the Nueces Bay restored marsh site is \$8,125·yr^{-1}, based on the following equations:

$$0.02\ mgd \times \$2{,}437{,}643 \cdot mgd^{-1} = \$48{,}753$$

$$\frac{\$48{,}753}{15\ yrs} = \$3{,}250 \cdot yr^{-1}$$

$$\$3{,}250 + (\$48{,}753 \times 0.10) = \$8{,}125 \cdot yr^{-1}$$

Table 6. Seasonal denitrification rates, and the amount of nitrogen removed per hectare of wetlands per season in the Nueces Bay restored marsh.

Season	Seasonal Denitrification rate (kg N·ha^{-2}·yr^{-1})	kg N denitrified per hectare
Winter	8.7	2.18
Spring	4.97	1.24
Summer	24.82	6.21
Fall	5.62	1.41
Annual Total		11.03

Table 7. Nitrogen removal through denitrification and replacement cost equivalent provided by 270 hectares of restored estuarine habitat at Egery Flats and 70 hectares of restored marsh in the Nueces Bay.

	Egery Flats	Nueces Bay
Annual N removed (kg)	2697	772
Replacement cost equivalent (yr^{-1})	$36,565	$8,125

4. Discussion

4.1 Overview

Eutrophication is a global issue that is exacerbated by excessive nitrogen loading into coastal environments from anthropogenic activities (DeLaune et al., 2005; Rabalais et al., 2014). As of 2012, 55% of states have an active nutrient offset program, while 22% of states are in the process of developing one (ACWA 2012). A nutrient offset program is a common method for controlling point-source nutrient outputs to make up for non-point source outputs as a way of limiting nutrient outputs into aquatic ecosystems. Restoring the denitrification ability of natural habitats has been proposed to help reduce nutrient loads into many different important aquatic ecosystems (Lindau et al., 2008; Pollack et al., 2013). Wetlands can effectively remove excess nitrogen through denitrification (Lindau et al., 2008; Piehler and Smyth, 2011).

This study occurred during a weak El Niño year. However, after analyzing 10 years of climate data for the study area, it was determined that this study period did not differ significantly from La Niña and ENSO-neutral years. Therefore, seasonal trends from this study may be representative for seasons of other years in this study area.

4.2 Study Area Seasonal Climate Variation

When discussing seasonality, El Niño-Southern Oscillation (ENSO) must be considered. There were significant seasonal trends measured for the 10 year period from 2010 to 2019, which showed that measuring trends by season in this study is an accurate way of representing the data. There were no differences between the years from 2010 to 2019, which shows that 2019 was representative of a typical year in this study area. Also, because there were no significant differences between El Niño, La Niña, and ENSO-neutral years, it can be said that though 2019 was a weak El Niño, seasonal trends seen this year may be a good representation of trends in other years.

4.3 Nutrient Concentrations and $\delta^{15}N$ Stable Isotopes

Stable isotopes and nutrient concentrations can help to shed light on the sources and processes occurring in these systems. Portland WWTP $\delta^{15}N$- NH_4^+ values show the mineralization of organic matter of human waste, and $\delta^{15}N$-NO_3^- confirm that NO_3^- sources are human waste. The decrease in all three nitrogen species concentrations in the summer may show an increase of water column denitrification as there is a net loss of N in the system.

Oso Bay WWTP $\delta^{15}N$-NO_3^- values indicate human waste sources and are slightly higher than the values of the Portland WWTP; this is indicative of water column denitrification. Since it is not expected that the Oso Bay WWTP effluent would contain soil NH_4^+, or fertilizer NH_4^+, the $\delta^{15}N$-NH_4^+ values most likely indicate a mixture of organic matter mineralization to ammonium, and nitrification, and human waste (Heaton 1986; Kendall et al., 2007). The increase of NH_4^+ concentration during the summer months and decrease of NO_3^- may indicate some dissimilatory nitrate reduction to ammonium (DNRA) is occurring in the water (Domangue and Mortazavi 2018).

The Bayside WWTP had nutrient concentrations that were more like the wetland sites and had consistently low NH_4^+ and NO_3^- concentrations, which is consistent with the efficiency of wetland WWTPs to remove excess nitrogen from influent as it passes through the created wetland cells (Coban et al., 2015). The high $\delta^{15}N$- NH_4^+ values are indicative of nitrification of NH_4^+ to NO_2^-, the $\delta^{15}N$-NO_3^- values show denitrification (Kendall et al., 2007). The increase of NO_2^- concentrations may indicate NH_4^+ being nitrified or NO_3^- being denitrified as NO_2^- is an intermediate of both processes.

The wetlands tended to have more mixed sources and processes noticeable in the isotope signatures. Egery Flats had higher NH_4^+ concentrations in all seasons compared to both NO_3^- or NO_2^-. The NH_4^+ and NO_2^- concentrations increase in the summer months which may indicate mineralization of organic matter to ammonium, and the nitrification of ammonium to nitrite. Influences from agricultural runoff and WWTP effluent were seen in the $\delta^{15}N$ values (Heaton 1986; Kendall et al., 2007). This area is surrounded by agricultural land and has inflow from the Bayside WWTP. The $\delta^{15}N\text{-}NO_3^-$ values show a mixture of NO_3^- fertilizer, denitrification, and human and animal waste.

The Nueces Bay restored marsh site isotope sources were mostly indicative of nitrogen cycling processes, including mineralization of organic matter to ammonium, and nitrification of NO_2^- to NO_3^-. The increase of both NH_4^+ and NO_3^- in the summer months provides more evidence of mineralization of organic matter to ammonium and denitrification reducing the NO_3^- concentration to N_2 gas. This site shows very little influence from anthropogenic nitrogen sources, and mostly shows influence of N cycling processes. This indicates that the water from the Portland WWTP does not flow directly to the restored wetland system, or the concentration of nitrogen in the effluent is diluted before it reaches the site. The low isotopic ratios at this site show mineralization of organic matter to ammonium, and nitrification of NO_2^- to NO_3^-, but do not show a strong indication of denitrification occurring, although our study shows that the potential for denitrification is there. This low occurrence of denitrification is one reason that the monetary value of nitrogen mitigation at this site is relatively low, $\$8{,}125 \cdot yr^{-1}$. The low occurrence of denitrification may also be impacted by the age of the wetland and is likely to increase as the wetlands age and soil structure increases in complexity.

The Aransas River Estuary $\delta^{15}N\text{-}NO_3^-$ values indicate mixing of N sources, including nitrate fertilizers, nitrification processes and atmospheric nitrate (Kendall et al., 2007). The increase in Aransas River Estuary NH_4^+ and NO_2^- concentrations in the summer months may indicate mineralization of organic matter to ammonium.

The Oso Bay marsh site NO_3^- concentrations were highest in the winter and lower during the other seasons, potentially showing nitrification activity in the warmer temperatures. NO_2^- concentrations increase in the summer and fall which can be indicative of both nitrification and denitrification processes. The $\delta^{15}N\text{-}NO_3^-$ values show a mixture of NO_3^- fertilizers, soil NO_3^-, and human and animal waste, where the $\delta^{15}N\text{-}NH_4^+$ values were in line with soil NH_4^+, NH_4^+ fertilizers, mineralization of organic matter to NH_4^+ and human waste (Kendall et al., 2007). This site is surrounded by agricultural land, a golf course, and a wastewater treatment plant; therefore, this mixing of sources is expected and shows that this site is influenced heavily by anthropogenic N sources. The DO concentrations upstream of the Oso Bay marsh site near the Oso Bay WWTP outfall were lower than many of our other sites, with low averages during the summer of 2.6 $mg \cdot L^{-1}$, and the DO concentrations at the Oso Bay marsh had the lowest average of 4.1 $mg \cdot L^{-1}$ during the summer months. These low DO concentrations are a possible indicator of eutrophication occurring near this site, causing near hypoxic conditions. The Oso Bay marsh site NH_4^+ concentrations increased in the summer months which may indicate mineralization of organic matter, or influence of fertilizers.

The Naval Airbase Bridge had higher NH_4^+ concentrations than either NO_3^- or NO_2^-. The increase of NH_4^+ seen in the summer and fall months are most likely due to either fertilizer influence or mineralization by microbes. The NO_3^- concentrations decreasing in the spring and summer months indicate denitrification occurring in the sediments as a means of NO_3^- reduction.

The increase of NO_2^- in the summer and fall months could potentially be due to increased nitrification or denitrification by microbes during the warmer seasons, as NO_2^- is an intermediate oxidation state for both reactions. The $\delta^{15}N\text{-}NO_3^-$ values show NO_3^- fertilizers, human and animal waste, and mixing of nitrification and denitrification processes. The $\delta^{15}N\text{-}NH_4^+$ values were like NH_4^+ fertilizers, and a mixture of nitrification and mineralization of organic matter to ammonium (Heaton 1986; Kendall et al., 2007). This site is influenced by two different large bodies of water, the Oso Bay, and the Corpus Christi Bay, which are both surrounded by agricultural land, this site is also influenced by the Oso Bay WWTP, because the flow of the effluent travels directly around Ward Island and out the Corpus Christi Bay. This site is heavily influenced by anthropogenic nitrogen sources.

4.4 Denitrification in Restored and Natural Wetlands

Denitrification is the major process of direct nitrogen removal from a system into the atmosphere (An and Gardner, 2002; Koop-Jakobsen and Giblin, 2009). Many studies that show that denitrification is strongly affected by temperature changes, where denitrification increases as temperatures increase (Byström et al., 2000; Richardson et al., 2004; Lindau et al., 2008; Vymazal 2011; Song et al., 2014). It was hypothesized that the temperature would be the main driving factor for denitrification. However, the correlation between water temperature and denitrification rates were not significant, though there were visible seasonal trends. The seasonal variation showed that Egery Flats had the lowest denitrification rates in the winter. The Nueces Bay restored marsh saw the highest rates in the summer. The Aransas River Estuary had the highest rates in the summer and fall. The Naval Airbase bridge showed the highest rates in the summer, and the Oso Bay marsh showed no significant seasonal trends. This means there may be other factors other than temperature that are affecting denitrification. For example, the wastewater used as a nitrate addition, ~200μM, for the Oso Bay marsh site may have been limiting during the summer at this location, as the microbial community becomes more active, and uses the nitrate at a higher rate (DeLaune et al., 2005; Wolf et al., 2011; Song et al., 2014). This means that the microbial community grows faster during the warmer summer months, so they require more energy in a shorter amount of time. Therefore, they use the nitrate in the water at a faster rate than in other months, and if the nitrate is not replenished fast enough than it becomes a limiting agent for the denitrification reaction.

Soil characteristics may have been another factor affecting the rates of denitrification. The Oso Bay site has clay sediment, where the Naval Airbase Bridge is sand and shell fragments. The sand and shell-based sediment allows for more nitrate to diffuse into the sediments through larger pore sizes. This diffusion allows more contact of nitrate with the microbial communities in the sediments for use as an electron acceptor under anaerobic conditions (Groffman and Tiedje 1989; DeLaune et al., 2005; Wolf et al., 2011; Theriot et al., 2013). Organic matter can also affect denitrification. Complex soil structure leads to more complex microbial communities. With higher organic carbon availability, denitrification rates may increase (Richardson et al., 2004; Lindau et al., 2008; Jenkins et al., 2011; Vymazal 2011; Wolf et al., 2011). Studies have also suggested that retention time in a wetland system increases the amount of nitrogen removal by the sediments. The velocity of the water through the wetland greatly affects the degree of nitrogen removal (Jenkins et al., 2010; Itoh et al., 2011). Other biogeochemical reactions may also affect denitrification rates. Studies have shown that high concentrations of chlorides and the production of sulfides (HS^-) may inhibit the reduction of NO_3^- through denitrification (Joye and Hollibaugh 1995; Kendall et al., 2007; Marks et al., 2016).

The age of a wetland is a major contributing factor to its ability to remove nitrate through denitrification. Many studies have investigated restored and natural wetlands and their respective denitrification ability (Jenkins et al., 2010; Wolf et al., 2011; Theriot et al., 2013; Song et al., 2014). A study by Song et al. (2014) determined that NO_3-N removal in wetlands increased from 27% to greater than 50% over the 15 years from when the study wetlands were created to when the study was completed. The nitrogen removal rate also became more stable from year nine to year 15.

In this study the two restored wetland sites, Egery Flats and the Nueces Bay restored marsh, showed significantly lower mean denitrification rates compared to the three natural wetland sites, Aransas River Estuary, Oso Bay marsh, and the Naval Airbase Bridge site. This indicates a possible age-based trend in denitrification at these sites. Given more time, the denitrification rates of the restored wetlands will likely increase. Denitrification rates increase with the accumulation of organic matter; restored wetlands may require more time to become favorable to denitrifiers (Jenkins et al., 2010; Wolf et al., 2011; Mitsch et al., 2012; Song et al., 2014). It is expected that in the next five to ten years the restored wetlands will have denitrification rates that are like those of the natural wetlands.

The acetylene blocking method for measuring denitrification, which was used in this study, is a conservative measurement of denitrification. As acetylene blocks the production of NO_3^- through nitrification, this can underestimate the amount of NO_3^- that can be removed by sediments (Groffman et al., 2006). Sulfide can also interfere with C_2H_2 which can block the inhibition of C_2H_2 on N_2O reductase in the microbial community. This can cause more of the N_2O to denitrify all the way to N_2 gas, providing an underestimation of the NO_3^- that is denitrified by measuring N_2O in the headspace (Groffman et al., 2006). The limitations of this method show that potential denitrification rates may be higher than measured in this study, but the trends and accumulation rates should be like those measured in this study.

4.5 Valuing Nitrogen Mitigation Ecosystem Services

Wetlands can remove a substantial amount of nitrogen per year, which significantly helps to reduce the amount of anthropogenic nitrogen pollution and provides better water quality for different ecosystems. The restored wetlands in Egery Flats and the Nueces Bay restored marsh removed 2,697 kg N and 772 kg N per year, respectively. These values should increase as the wetlands age and soil structure and microbial communities become more complex. The total nitrogen removed by these sites equates to a replacement cost (the cost of replacing the system with a manmade alternative) equivalent value of $36,565 per year for Egery Flats and $8,125 per year for the Nueces Bay restored marsh, which is equivalent to $13.55·kg N^{-1} removed. This dollar amount is similar to those seen in other studies, adjusted for inflation to US$2019, including $9.11·kg N^{-1} removed in Pollack et al. (2013), $14.86·kg N^{-1} removed seen in Piehler and Smyth (2011), $30.13·kg N^{-1} removed in Jenkins et al. (2010), and $31.77·kg N^{-1} removed in Newell et al. (2005). The value calculated in this study is relatively conservative compared to other similar studies (Appendix 4).

This study exemplifies the use of the replacement cost method for valuing the nitrogen mitigation ecosystem service. There are a few ways this study could be improved. The economic value calculated for nitrogen mitigation is dependent on the alternative option that is chosen for the analysis. The capital costs for the Back Water River WWTP used in this study, are not site-specific. The WWTP is in Maryland but the study site is in south Texas. The WWTP used was chosen based on the available information relevant for this type of economic evaluation, including processing capacity, capital costs, and the amount of nitrogen removed by the BNR system. A value derived from a more locally-relevant site might provide a better estimate of the replacement cost value of nitrogen mitigation in the study sites.

The nitrogen mitigation ecosystem service provided by the wetland sediments can have a huge impact on human health and well being, as well as ecosystem health, yet there is no direct cost to the public for this service. Along with having no direct cost to the public, the nitrogen mitigation provided by the wetlands may save taxpayers from having to pay additional taxes for the implementation of more advanced treatment systems to remove excess nitrogen from wastewater effluent. In addition to saving taxpayers money, an increase in the understanding of the importance of healthy ecosystems to the community can aid in the development of ecosystem service markets, increasing the interest in the health of our environment by providing opportunities for economic growth. Also, the taxpayers can enjoy the social benefits of improved water quality in these wetland ecosystems through recreational use and increased wildlife diversity. Restoration costs are a one time cost that will continue to provide benefits and an increase in benefits as time progresses. Whereas the cost of the manmade alternative methods of reducing nitrogen loading from anthropogenic sources require continued operation and maintenance costs, and over time will become more expensive.

The greater Corpus Christi, TX area has recently been undergoing development and is expected to increase its development with an increased population over the next 50 years (Texas Shores 2013). This increased development will lead to an increase in pollution and land degradation, which will increase the importance of wetland restoration projects, and studies like this one to show the value of these ecosystems. While denitrification is a valuable ecosystem service that effectively removes nitrogen from water systems that are nitrogen polluted, wetland restoration should be attempted alongside conservation efforts for existing ecosystems, as the natural wetlands have a higher ability to remove nitrogen, and it will take some time for the restored wetlands to have the same rates of nitrogen mitigation (Jenkins et al., 2010; Wolf et al., 2011; Mitsch et al., 2012).

For the restoration of Egery Flats, $1,587,000 was invested by the National Fish and Wildlife Foundation through the Gulf Environmental Benefit Fund, established from the settlement from the Deep Water Horizon oil spill in 2010. These funds were allocated to replace the culverts with the goal to reduce salinity and restore hydrology to the wetland. The restoration will provide a more habitable environment for important fish species, and to help protect endangered waterfowl that inhabit the area. Using the replacement value of nitrogen mitigation in this system, the value of the restoration will be regained in approximately 44 years or less as this system becomes more developed and denitrification increases with the age of the restoration.

For the restoration of the Nueces Bay marsh, $5,326,820 was invested by several partners: Coastal Management Program, U.S. Fish and Wildlife Service, Texas Commission on Environmental Quality, Coastal Impact Assistance Program, Centre for Environmental Research and Policy, Environmental Protection Agency, Coastal Conservation Association, and the Hollomon Price Foundation to rebuild the entire habitat. This project was funded to restore habitat and increase the flora and fauna diversity that had been lost due to dredging and change in hydrology (Smee 2016). It would take 656 years, or less as the wetland ages and denitrification increases, to make up the cost of the investment using the replacement value of nitrogen mitigation alone.

This study is not an attempt to construct a full cost-benefit analysis of restored wetlands. This study is looking at only one ecosystem service of many offered by these habitats that provide value in these systems that, if evaluated, would help to exemplify the economic benefit of these ecosystems immensely, including the market price for many important fish species, replacement cost for carbon sequestration, pollution mitigation, water quality improvement, climate regulation, and ecotourism.

5. Conclusion

Nutrient loading of coastal systems is a continuing global issue, and wetland restoration is one method for increasing nitrogen removal. By studying nutrient concentration and stable isotopes in these systems we can better understand the sources of nitrogen, the amount of nitrogen, and the cycling processes that the nitrogen undergoes in the system. Using stable isotopes can shed light on the sources of nitrogen in the system to determine if the nitrogen is from point- or nonpoint-source pollution, or from naturally occurring nitrogen cycling.

Denitrification is an important service offered by wetlands that helps to reduce nitrogen pollution in aquatic ecosystems. This study found that Egery Flats can remove 10 kg $N{\cdot}ha{\cdot}yr^{-1}$, the Nueces Bay restored marsh site can remove 11 kg $N{\cdot}ha{\cdot}yr^{-1}$, Aransas River Estuary can remove 20 kg $N{\cdot}ha{\cdot}yr^{-1}$, Oso Bay marsh can remove 29 kg $N{\cdot}ha{\cdot}yr^{-1}$, and the Naval Airbase Bridge site can remove kg $N{\cdot}ha{\cdot}yr^{-1}$. The increase of population in coastal cities over that last few decades has caused extra strain on natural wetlands and coastal ecosystems, through increased activity, development, agriculture, and wastewater production that has degraded wetland ecosystems. Wetland restoration is a valuable and effective way to regain these lost ecosystem services, and to increase the removal of excess nitrogen pollution caused by anthropogenic activities. As the restored wetlands age, the nitrogen mitigation ability will only get more valuable, as the amount of nitrogen removed from the system will increase with increased soil development and microbial community complexity.

Ecosystem management requires tools that can be utilized by managers and stakeholders to make decisions about ecosystems and the surrounding communities. Economic evaluation is a tangible method of showing the dollar value of different services offered by ecosystems, that do not have a conventional market that can help justify the cost of restoration and conservation projects in the future. This study provides an example of a multidisciplinary approach to assessing ecosystem services, which is necessary for future effective ecosystem management, and will aid in education about the importance and value of nitrogen mitigation and other ecosystem services in restored and natural wetlands.

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LIST OF APPENDICES

Appendix 1

Reference	Location	Ecosystem Type	Natural/ Restored/ Constructed	Mean Rates	Converted Units (kg N/ha/yr.)	Factors Affecting Denitrification	Method
Groffman and Tiedje 1989	Michigan USA	Freshwater Wetland	Natural	199 g N/ha/d	72.64	soil texture and drainage	Static Core, acetylene blocking, nitrate amendment
Groffman and Tiedje 1989	Michigan USA	Freshwater Wetland	Natural	1251 g N/ha/d	456.62	soil texture and drainage	Static Core, acetylene blocking, nitrate amendment
Bruesewitz et al 2017	Aransas River, TX, USA	Saltwater Wetland	Natural	3.25 mg N/m2/h	284.70	during drought may decrease anthropogenic N input to coastal systems	sediment slurry, river water as NO3 source, 10uM NO3, MIMS
Bruesewitz et al 2017	Aransas River, TX, USA	Saltwater Wetland	Natural	1.5 mg N/m2/h	131.40	during drought may decrease anthropogenic N input to coastal systems	
DeLaune et al 2005	Louisiana, USA	Freshwater Wetland	Restored	31 mg N/m2/d	113.15	NO_3^- concentration, diffusion rate of NO_3^- to anaerobic soil layer	5 cm soil slurry Acetylene Blockage
Lindau et al 2008	Louisiana, USA	Freshwater Wetland	Restored	386.6 g N/ha/d	141.11	at 22C, temperature dependent	amended 100mg/l NO3, acetylene blockage, sediment slurry
Richardson et al 2004	Wisconsin, USA	Freshwater Wetland	Natural	1.97 ug N/cm2/h	1725.72	temperature dependent, nutrient enrichment, and C availability. High or low flow rates, wetland surface area	slurry, 14mg/L NO3 acetylene blockage
Pollack et al 2013	Mission-Aransas Estuary, TX, USA	Oyster Reef	Natural	502.5 kg N/km2/yr.	5.03		based on a 20% denitrification efficiency from previous laboratory studies
Jenkins et al 2010	MAV, USA	Freshwater Wetland	Restored	28.8 kg N/ha/yr.	28.80	Age	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created- 3yr old	35 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created- 4yr old	30 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created- 7yr old	85 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Created-10yr old	60 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Wolf et al 2011	Virginia, USA	Freshwater Wetland	Natural	75 umol N2O-N kg/dw/day		Age, soil composition, total N, Organic C concentration, NO_3^- concentration	DEA potential denitrification
Song et al 2011	Ohio, USA	Freshwater Wetland	Created-15yr old	316 ug N/m2/hr	27.68	Temperature, NO_3^- concentration, vegetation uptake competition	In situ acetylene blocking denitrification in shallow wetlands
Breaux et al 1995	Louisiana, USA	Freshwater Wetland	Natural	14.3 g N/m2/yr.	143.00		Based on 72% denitrification efficiency calculated through biochemical balance analysis
Mitsch et al 2012	Ohio, USA	Freshwater Wetland	Created-15yr old	2.1 g N/m2/yr.	21.00	Age, expected to increase over time with organic matter accumulation	In situ acetylene blocking denitrification
Dehnhardt 2002	Elbe River, Germany	Freshwater Wetland	Natural	200 kg N/ha/yr.	200.00		statistical modeling

Appendix 1. Literature review tables of denitrification studies, including results, methods, and factors affecting denitrification.

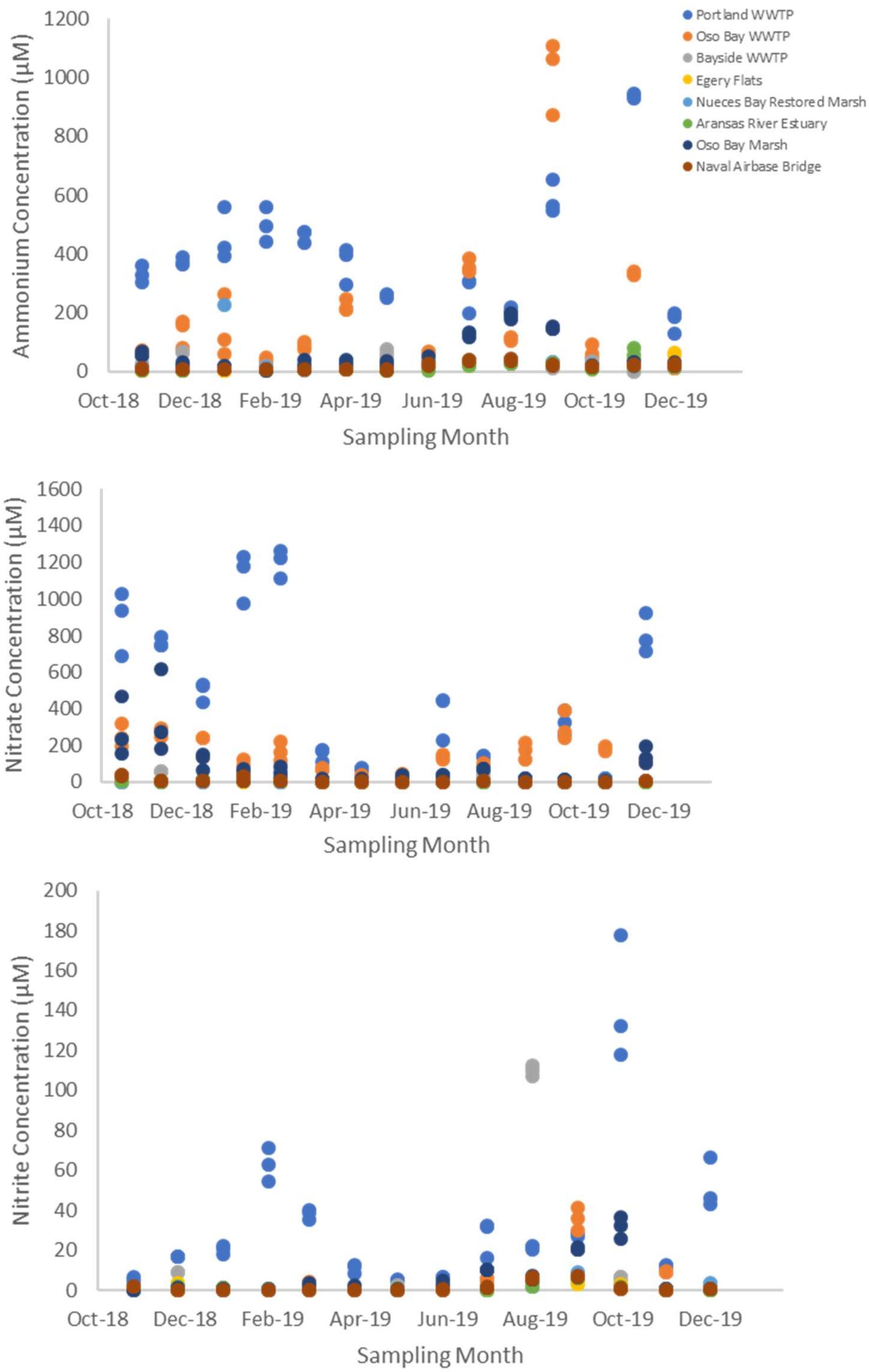
Appendix 2

Location	Type of Ecosystem	author	Type of study	date	US$ Value	Unit	Converted Value US$ 2019	Converted Unit	Comments
Thibodaux, Louisiana, USA	Freshwater Wetland	Breaux et al., 1995	RCM	1995	$ 64.61	per ha per yr	$ 108.39	per ha per yr	
Sweden	Saltwater Wetland	Byström, O. 2000	RCM	2000	$ 3,913.51	per ha per yr	$ 7,131.28	per ha per yr	used low range converted from 1992 SEK to 1992 US$ from Jan 2, 1992
Elbe River, Germany	Freshwater Wetland	Dehnhardt 2002	RCM	2002	$ 308.45	per ha per yr	$ 438.34	per ha per yr	Converted from 2002€ to 2002 USD from Jan 2, 2002
Stockholm, Sweden	Freshwater Wetland	Gren et al 1995	RCM	1995	$ 1,275.19	per ha per yr	$ 2,139.19	per ha per yr	converted from 1995 ECU to 1995 $US using Hanley and Owen, 2004
Mississippi Alluvial Valley, USA	Freshwater Wetland	Jenkins et al., 2010	Benefit transfer	2010	$ 1,248.04	per ha per yr	$ 1,481.96	per ha per yr	from Ribaudo et al 2005 converted to kg N from lb N, and inflated to $2008
Fiji	Mangroves	Lal, P.N., 1990	RCM	1990	$ 2,125.00	per ha per yr	$ 4,156.63	per ha per yr	
Denver, Colorado, USA	Freshwater Wetland	Loomis et al., 2000	CV	2000	$ 6,180.00	per ha per yr	$ 9,175.16	per ha per yr	Based on if only 26% of households pay for N mitigation
Bogue Sound, North Carolina	Oyster Reef	Piehler and Smyth, 2011	PM	2011	$ 7,330.86	per ha per yr	$ 8,331.99	per ha per yr	
Bogue Sound, North Carolina	Submerged Aquatic Vegetation	Piehler and Smyth, 2011	PM	2011	$ 7,404.94	per ha per yr	$ 8,416.19	per ha per yr	
Bogue Sound, North Carolina	Salt Marsh	Piehler and Smyth, 2011	PM	2011	$ 6,123.46	per ha per yr	$ 6,959.70	per ha per yr	
Bogue Sound, North Carolina	Intertidal Flat	Piehler and Smyth, 2011	PM	2011	$ 3,832.10	per ha per yr	$ 4,355.43	per ha per yr	
Bogue Sound, North Carolina	Subtidal Flat	Piehler and Smyth, 2011	PM	2011	$ 1,022.22	per ha per yr	$ 1,161.82	per ha per yr	
Mission-Aransas Estuary, TX, USA	Oyster Reef	Pollack et al., 2013	RCM	2013	$ 41.29	per ha per yr	$ 45.31	per ha per yr	
Zazari-Cheimaditida, Greece	Freshwater Wetland	Ragkos et al 2006	CV	2006	$ 0.01	per ha per yr	$ 0.01	per ha per yr	converted from 2006€ per person to 2006$/ha/yr from Jan 3, 2006
Mississippi River Delta, USA	Freshwater Wetland	Ribaudo et al., 2005	PM	2005	$ 10.50	per lb N removed	$ 13.75	per lb N removed	
World	Mangroves	Salem and Mercer, 2012	MRA	2012	$ 44.00	per ha per yr	$ 48.99	per ha per yr	
McIntosh County, Georgia, USA	Forested Wetland	Schmidt et al., 2014	BT	2014	$ 1,248.00	per ha per yr	$ 1,347.75	per ha per yr	BT from Jenkins et al 2010, therefore from Ribaudo et al 2005

McIntosh County, Georgia, USA	Freshwater Wetland	Schmidt et al., 2014	BT	2014	$ 19.00	per ha per yr	$ 20.52	per ha per yr	BT from Jenkins et al 2010, therefore from Ribaudo et al 2005
McIntosh County, Georgia, USA	Brackish Wetland	Schmidt et al., 2014	BT	2014	$ 27.00	per ha per yr	$ 29.16	per ha per yr	BT from Jenkins et al 2010, therefore from Ribaudo et al 2005

Appendix 2. Literature review table of economic evaluation studies for ecosystem services.

Appendix 3



Appendix 3. Figure of nutrient concentrations for ammonium, nitrate, and nitrite monthly from October 2018 through December 2019.

Appendix 4

Study	Original Value $\$\cdot kg\ N^{-1}$	US\$2019 Value $\$\cdot kg\ N^{-1}$
Pollack et al. 2013	8.33	9.11
Piehler and Smyth 2011	13.00	14.86
Jenkins et al. 2010	25.27	30.13
Newell et al. 2005	24.07	31.77
This Study		13.55

Appendix 4. Table showing the values of nitrogen mitigation from this study and from previous studies adjusted for inflation to the year of this study.