THE IMPACT OF NUTRIENT LOADING ON THE SOIL AND ROOT RESPIRATION RATES OF FLORIDA MANGROVES

by

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ABSTRACT

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Coastal nutrient loading is a growing concern in urbanized communities and has led to alterations in above- and belowground processes throughout estuarine systems. Mangrove forests are highly productive coastal habitats that exhibit large carbon stocks contained mostly to the deep soils. Since nutrient enrichment has been found to increase mangrove aboveground growth, it's presumed that nutrient enrichment will also increase belowground respiration rates. Disturbances in soil nutrient content may alter the mangrove carbon cycle by increasing the amount of CO_2 lost to the atmosphere from enhanced microbial and root respiration. In this study, soil respiration responded greatest to nitrogen enrichment, but pneumatophore root respiration responded greatest to phosphorus enrichment. Nutrient limitation can shift between different ecological processes and responses to nutrient enrichment tend to be system specific in tidally

influenced ecosystems. Understanding the implications of coastal nutrient loading will improve ecosystem models of carbon exchange and belowground processes.

DEDICATION

To the Colorado Rockies, for instilling in me a curiosity of the natural world and a drive to travel more, learn more, ask more, and do more.

THE IMPACT OF NUTRIENT LOADING ON THE SOIL AND ROOT RESPIRATION RATES OF FLORIDA MANGROVES

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CHAPTER 1 – INTRODUCTION: THE MANGROVE CARBON BUDGET AND NUTRIENT ADDITIONS

The flow of high-nutrient water into near shore habitats is a growing ecological and economic concern for coastal ecosystems and communities. Coastal waters receive nutrient input and surface runoff from industrial, agricultural, commercial, and residential processes (Paerl et al. 2006). Increased nutrient loading into coastal waters has accompanied the urban development of coastal watersheds (Boesch et al. 2001) and has been a primary cause for increased algal blooms (Paerl 1988), decreases in water quality (Cloern 2001), and hypoxic events (Rabalais et al. 2002). Eutrophic outflows can produce accelerated algae growth on the water surface (Cloern 2001; Sellner et al. 2003) and cause harmful algal blooms that release toxins into the water and atmosphere, destroy fish habitat by shading the submerged vegetation, and degrade seagrass beds and coral reefs (Anderson et al. 2002; National Research Council 2000; Paerl et al. 2006). In estuaries, susceptibility to nutrient enrichment is controlled by a wide variety of factors that include the physical setting, biological processes, and hydrological regime (National Research Council 2000) and the response of estuaries to nutrient loading is system specific (Painting et al. 2007). Eutrophication of coastal waters is a growing concern and human activities can accelerate nutrient loading by increasing the natural movement of nutrients from inland watersheds to coastal environments.

Mangrove forests, found throughout South Florida and tropical regions globally, are critical components of coastal estuaries. Mangrove forests cover over 135,000 km^2 of

the Earth's surface and occupy about one quarter of the Earth's coastlines (Giri et al. 2011; Spalding et al. 2010; Upadhyay et al. 2002). However, the current extent of mangrove forests has declined by 30-50% over the past several decades (Alongi 2002; Burke et al. 2001) and much of what remains is in a degraded condition (Polidoro et al. 2010; Spalding et al. 2010). Degradation of mangrove habitat can result from a combination of abiotic factors, such as changing hydrology and salinity, shifts in nutrient cycles and biological processes, and from human exploitation for coastal development and timber harvesting (Kathiresan 2002; Polidoro et al. 2010).

The destruction of mangrove habitat is concerning because these forests are some of the most productive and biologically important ecosystems in the world due to the significant ecological value and diverse ecosystem services that they provide. The importance of wetlands, specifically mangrove forests, as carbon sinks is widely recognized. Mangroves contribute to the global carbon budget by sequestering disproportionate amounts of carbon compared to their total global extent and that of other ecosystems (Donato et al. 2011; Spalding et al. 2010). Mangroves are responsible for 15 % of the global accumulated carbon each year and 11 % of the organic carbon present in marine sediments (Alongi 2009; Jennerjahn and Ittekkot 2002). Carbon concentrations tend to be lower in mangrove soils, since tidal systems receive more allochthonous inputs of inorganic materials that dilute the organic material from the above and belowground sediments (Chmura et al. 2003). However, rates of soil carbon accumulation and sediment deposition tend to be higher in mangrove soils, due to the roots' contribution to belowground carbon and their ability to trap large amounts of organic material at soil surface (Connor et al. 2001; Krauss et al. 2014).

Furthermore, mangrove forests protect nearby inland human communities by mitigating the devastating impact of tropical storms, provide critical habitat for organisms occupying the land-sea interface and act as nursing grounds for many aquatic animals (Das and Vincent 2009; Getzner and Islam 2020). As a result, seafood production throughout the world relies directly or indirectly on mangrove habitat (Ronnback 1999). On a global scale, mangrove forests have the potential to counteract the effects of sea level rise by building sediments and stabilizing shorelines. A complex underground root system combined with aerial roots anchors the mangrove tree and traps organic material into the sediment surface (Krauss et al. 2014). Additionally, the anaerobic conditions of mangrove soils slow the decomposition of the belowground roots and therefore increases the amount of root matter accumulating in the soil (Krauss et al. 2014; Middleton and McKee 2001). Mangrove forests also contribute largely to the cycling of matter, which is driven by the physical events of tides, runoff, and rainfall and the biological processes of leaf fall, decomposition, and uptake rates (Feller et al. 2010; Lugo and Snedaker 1974). The increased threats from eutrophication and land development could have negative consequences on mangroves' ability to process organic material and the influence these forests have on CO_2 emissions into the atmosphere.

The productivity and stability of coastal wetland ecosystems is largely influenced by the tidal exchange of nutrients. In most plant communities, nutrient availability is an important factor in determining forest structure. Generally, mangrove systems operate under low nutrient conditions due to limited input from terrestrial soils (Lovelock et al. 2005). Furthermore, many mangrove habitats show patterns of zonation, where frequency of inundation, soil salinity, and species dominance creates three generic zones within a

mangrove forest (Lugo and Snedaker 1974; Spalding et al. 2010). Physical factors, such as wave energy and tidal flushing, influence patterns of salinity and nutrient delivery to different zones of the forest (Feller et al. 2003a, b) and influence the dominant species present in each zone (Snedaker 1982). The red mangrove, *Rhizophora mangle*, dominates the most seaward fringe zone, which is a low nutrient environment exhibiting a large tidal influence (Lugo and Snedaker 1974; McKee 1993). The black mangrove, *Avicennia Germinans*, dominates the higher elevation basin zone, which has greater nutrient availability and is less influenced by tides, compared to the fringe (Lugo and Snedaker 1974; McKee 1993). These forest zones present divergent patterns of leaf litter accumulation, decomposition, and export, due to difference in tidal flushing (Lugo and Snedaker 1974). Basin forests are less frequently flushed by tides and have higher rates of litter accumulation compared to the fringe forest, due to the frequent removal of aboveground litter by tides at the seaward zone (Middleton and McKee 2001). Hydrology, soil type, and mineral input all influence the soil sedimentation and decomposition rates within a single mangrove forest (Keuskamp et al. 2015; Lovelock et al. 2007), which can affect the availability of specific nutrients in the soil.

Changes in geomorphology, tidal influence, and site-specific characteristics explain why soil nutrient content can vary across a single mangrove island forest and switch from nitrogen (N) to phosphorus (P) limited in small spatial gradients (Feller et al. 2003a). Experimental evidence from a mangrove forest in Belize has shown increased growth of trees in the basin zone of an island as a response to P fertilization, but not N, suggesting P-limitation in the interior of the island (Feller 1995; Feller et al. 2003a). Trees in the fringe zone responded to the addition of N, but not P, suggesting N-

limitation at the seaward portion of the island (Feller et al. 2003a). Furthermore, the transition zone between the fringe and basin showed a response to N and P fertilization, indicating that a shift in nutrients was occurring in that area. Mangrove forests display an uneven distribution of nutrients within the soil and demonstrate that nutrient limitation in these ecosystems is complex and similar area can respond differently to the same nutrient. Understanding mangrove nutrient limitation is a focus of this study and is an important factor in determining the health of coastal mangrove forests faced with increased anthropogenic nutrient enrichment.

Mangrove aboveground biomass has been observed to increase when fertilized with both N and P (Feller et al. 2003a, b; Lovelock et al. 2007), but less is understood about belowground activity and nutrient additions (Lovelock et al. 2006). Data on mangrove litter fall and aboveground activity is abundant but estimates on belowground allocation is scarce due to methodological constraints and environmental variability (Bouillon et al. 2008). Factors such as geomorphology, tidal influence, climate, surrounding vegetation, and biotic interactions within the soil all affect the use and export of organic matter (McIvor and Smith 1995; Nordhaus et al. 2006; Twilley et al. 1986). Microbial activity also contributes to organic matter retention and nutrient susceptibility in mangrove soils. Microbes in the soil and around the roots decompose most of the carbon and nutrients found in organic material and heavily influence the availability of nutrients in the soil (Keuskamp et al. 2015). Generally, decomposition increases with nutrient availability in terrestrial and wetland ecosystems (Cebrian et al. 1998). Nutrient enrichment may increase the decomposition rate in a variety of ways, including changing the litter composition, increasing the abundance of and activity of decomposers, and

decreasing the abundance of carbon-rich secondary compounds (Keuskamp et al. 2015). Therefore, nutrient additions may negatively affect the carbon storage ability of mangroves by increasing the soil decomposition rate to surpass the primary production rate (Hessen et al. 2004), which would limit mangroves' ability to build enough sediments to offset the effect of sea level rise.

Soil respiration is an important component of the mangrove carbon cycle because it represents the release of soil, root, and microbial carbon to the atmosphere, mostly in the form of CO_2 (McKee 1996). Large carbon stocks occur in mangrove forests due to the high deposition of carbon from allochthonous and autochthonous inputs and low carbon oxidation rates due to anaerobic soil conditions (Twilley et al. 1992). Soil respiration may have a strong influence on the carbon stocks of these tropical forests and should be accounted for in the global carbon budget. It was demonstrated that soil temperature, leaf area index (i.e. aboveground biomass), and litterfall all strongly influence CO_2 production in mangrove forests (Lovelock 2008). These variables may also be strongly influenced by nutrient availability, which can vary extensively within and among mangrove forests (Feller et al. 2003a, b; Lovelock et al. 2007). Overall, the carbon loss from mangrove soils is low and is affected by the microbial communities, photosynthetic microorganisms, canopy development, increased temperatures, and nutrient enrichment (Lovelock 2008; Lovelock et al. 2007).

Some mangrove species develop aerial pneumatophore roots that extend upward from the underground root system and allow for more efficient gas exchange to the otherwise submerged roots (Purvaja et al. 2004). Pneumatophores allow atmospheric O_2 to diffuse into the anerobic soils and contribute to belowground soil respiration while also

acting as vectors for CO_2 emissions into the atmosphere (Kitaya et al. 2002). Mangrove aerial roots are valuable to tidal ecosystems because they allow direct connection to the submerged anerobic muddy root system. Due to high primary productivity and a complex system of above and belowground roots, it has been proposed that mangroves allocate a large portion of their fixed carbon to the roots (Donato et al. 2011; Krauss et al. 2014; Lovelock 2008; Lugo and Snedaker 1974). In mangrove forests, root activity and soil nutrient availability are important factors influencing belowground CO_2 emissions (Lovelock et al. 2006).

More research is needed to better understand the relationship between nutrient enrichment and root versus soil respiration in belowground mangrove soils. By manipulating the soil nutrient content, we can observe the respiration response from the soil and the isolated pneumatophore roots. Since eutrophication is a growing concern and mangroves protect our coasts while largely contributing to the global carbon cycle, we must be able to measure how the carbon dynamics of mangrove soils change in response to elevated nutrients. Quantifying this response will promote a better understanding of mangrove health and their capacity to build organic soils and sequester carbon while faced with increased anthropogenic nutrients in the water.

In this thesis, we use whole ecosystem fertilization in a basin mangrove forest to measure soil and pneumatophore CO_2 flux from nitrogen, phosphorus, and unfertilized treatment plots. My first objective is to assess the effect of fertilization and seasonality on bulk soil respiration. I investigated the CO_2 flux from mangrove soils when exposed to elevated levels of nitrogen and phosphorus and compared those responses across two seasons (wet and dry). I hypothesize that soil respiration will be greatest in the

phosphorus fertilized plot during the wet season. Basin mangrove forests experience less tidal flushing, which promote higher rates of litter accumulation and nitrogen availability in the soil, and reduced phosphorus input from seawater Also, higher soil temperatures during the wet season will stimulate greater flux responses compared to the dry season. My second objective is to assess the effect of fertilization and seasonality on pneumatophore root respiration. I investigated the CO_2 flux response from isolated mangrove aerial roots when exposed to elevated levels of nitrogen and phosphorus and compared those responses across both seasons. I hypothesize that pneumatophore respiration will follow the same patterns as soil respiration and be greatest in the phosphorus fertilized basin plot during the wet season. However, pneumatophore flux values will tend to be greater than soil flux values, due to their direct connection to the belowground root system and various belowground respiration pathways. By quantifying differences in the soil and root respiration rates of Florida mangroves between three fertilization treatments (nitrogen, phosphorus, and unfertilized) and two seasons (wet and dry), we can identify potential disturbances in the carbon cycling of mangrove forests and discuss the implications of eutrophic outflows on the health of coastal ecosystems.

CHAPTER 2 – DIFFERENCES IN SOIL AND ROOT RESPIRATION RATES BETWEEN THREE TREATMENT LEVELS AND TWO SEASONS

Introduction

Coastal mangrove ecosystems

Mangrove forests and their associated aquatic habitats are dominant ecosystems in the lower latitudes across the world. Mangroves cover over 135,000 km^2 of the Earth's surface and occupy about one quarter of the Earth's coastlines (Giri et al. 2011; Spalding et al. 2010). These tidal forests have adapted to saltwater conditions and provide significant ecological value and diverse ecosystem services. They act as critical habitat for a variety of organisms occupying the land-sea interface (Getzner and Islam 2020), as ecosystem engineers building sediments and stabilizing shorelines (Krauss et al. 2014; Lovelock et al. 2015), and as coastal buffers to tropical storms and anthropogenic nutrients that are sent into the ocean (Das and Vincent 2009; Lugo and Snedaker 1974).

Mangroves are some of the most productive ecosystems and contribute largely to the global carbon cycle. Mangrove soils contain a thick, tidally submerged suboxic layer supporting anaerobic decomposition and high carbon concentration (Kristensen et al. 2008; Chmura et al. 2003). As a result, these forests can maintain large biomass and counteract the effects of sea level rise, due to the slow belowground decomposition rate promoting an increased amount of root matter accumulating in the soil (Alongi 2009; Krauss et al. 2014; Middleton and McKee 2001). Mangroves are well known for high carbon sequestration and sediment deposition rates (Bouillon et al. 2008; Kristensen et al

2008; Twilley et al. 1992), but data on whole ecosystem scale carbon storage, especially belowground storage, is difficult to measure and lacking for mangrove forests (Donato et al. 2011). Several authors have indicated that marine organic matter derived from mangrove forests is of global significance, estimating that mangroves are responsible for ~ 15 percent of the annual carbon accumulated into marine sediments (Jennerjahn and Ittekkot 2002), and that much of this carbon storage is contained to the deep belowground soils (Donato et al. 2011).

The current extent of mangrove forests has declined by 30-50% over the past several decades due to a combination of abiotic and biotic factors (Alongi 2002; Polidoro et al. 2010). Rapid sea level rise, land clearing and development, aquaculture expansion, increased atmospheric CO_2 concentration, and surface runoff have all been cited as notable threats to the health of mangrove forests globally (Gilman et al. 2008; Giri et al. 2011; Polidoro et al. 2010). Consequently, reduced mangrove area will increase the threat to human safety from coastal hazards such as erosion, flooding, and storm surges (Dahdouh-Guebas et al. 2005; Das and Vincent 2009; Kathiresan and Rajendran 2005). Mangrove loss will also eliminate fish nursery habitats, reduce costal water quality, reduce biodiversity, and adversely affect neighboring coastal habitats (Ewel et al. 2008; Nagelkerken et al. 2008). Destroying mangrove habitats may also release large amounts of stored carbon from the belowground soils and contribute to global warming and increased atmospheric CO_2 concentration (Kristensen et al. 2008).

Another notable threat to mangrove forests is the eutrophication of coastal waters. Anthropogenic nutrient enrichment, also referred to as eutrophication, describes nutrient pollution in freshwater and coastal areas from sources such as agriculture, septic tanks,

urban wastewater, stormwater runoff, industry, and fossil fuel combustion (Paerl et al. 2006). The flow of high-nutrient water into near shore habitats is a growing ecological and economic concern for coastal ecosystems and communities. Eutrophic outflows can produce accelerated growth of phytoplankton and macroalgae on the water surface (Cloern 2001; Sellner et al. 2003), which affects nutrient cycling, water quality, and overall ecosystem health (Paerl et al. 2006). Increased nutrient loading into coastal waters has been the primary factor causing increased harmful algal blooms (HABs) that release toxins into the water and atmosphere (Anderson et al. 2002; Paerl et al. 2006) and can create hypoxic 'dead' zones by shading the submerged vegetation (Rabalais et al. 2002). A 2002 study estimated approximately US $50 million is lost to HAB-related impacts in the United States annually (Hoagland et al. 2002). This includes monetary loss from shellfish and fish poisoning, commercial fishing, recreation and tourism, and management costs. Little doubt exists that the economic impact of specific HAB events can be serious and significant, especially at the local level and in smaller coastal communities. Algal blooms do occur naturally, but human activities can accelerate eutrophication by increasing the natural movement of nutrients from inland watersheds to coastal water bodies.

Nutrient cycling in mangrove forests

Mangrove habitats present a paradox as they are highly productive carbon sinks while also operating under nutrient-poor conditions (Lovelock et al. 2005). It's also important to note that mangrove forests protect the very coastlines experiencing heightened anthropogenic nutrient runoff. Consequently, research on the role of nutrient limitation and nutrient cycles has become a more focused concern for mangrove

ecologists over the past several years. Direct and indirect ecological impacts of nutrient enrichment include increased primary productivity and phytoplankton biomass, reduced water quality, more frequent hypoxia events, and changes in the trophic structure and interactions (National Research Council 2000). In estuaries, susceptibility to nutrient enrichment is controlled by a variety of physical and biological processes including the geomorphic setting, types of primary producers, typical nutrient load, water residence time, and tidal flushing (National Research Council 2000). Mangroves are not generally limited by sulfur, potassium, magnesium, and sodium that are present in large quantities in seawater but are limited by nitrogen and phosphorus due to limited input from terrestrial soils and the lack of nutrients in upland tropical soils (Alongi 2018; Feller et al. 2003a). Nutrient availability has repeatedly been found to be an important factor limiting primary production in mangrove forests and most of the nutrient pool is stored in the belowground soils (Alongi et al. 2003; Reef et al. 2010).

Furthermore, the effect of nitrogen (N) and phosphorus (P) enrichment can vary among mangrove species in the same island forest (Feller et al. 2003b). This phenomenon can be explained partly by differences in tidal flushing and leaf litter decomposition between the seaward and landward zones of the forest (Feller et al. 2003a). Mangroves have a large reservoir of dead roots and abundant soil microbes that immobilize inorganic nitrogen into organic forms, which allow these trees to retain phosphorus and nitrogen, respectively (Alongi 2009). However, these nutrient pools are kept small to stay consistent with the rapid rate of nutrient cycling in these forests (Kristensen et al. 2000). As a result, when fertilized with both N and P, mangrove aboveground biomass has been observed to increase (Feller et al. 2003a; Lovelock et al. 2007) but less is understood

about belowground activity and nutrient additions (Lovelock et al. 2006) due to methodological constraints and environmental variability (Bouillon et al. 2008).

Soil microbial communities decompose most of the carbon and nutrients found in the belowground organic material and microbial metabolic processes heavily influence the availability of nutrients in the soil (Alongi 2018; Keuskamp et al. 2015). Denitrifying bacteria deplete the nitrate pools and produce ammonia, making ammonium the most common form of nitrogen in mangrove soils (Kristensen et al. 2008; Twilley et al. 1986). Alongi (2013) created a nitrogen budget for the world's mangrove ecosystems. This budget showed that nitrogen burial equates almost 25% of the total nitrogen input where nitrogen fixation represents less than 5% of the total nitrogen input. Nitrogen fixing bacteria have been found in microbial communities living on trees, roots, decaying leaves, aerial pneumatophore roots, and benthic microbials mats which all contribute to the mangrove nitrogen cycle (Reef et al. 2010). Furthermore, a large portion of belowground soil respiration is dedicated to microbial uptake of nitrogen, especially ammonium which requires the least energy investment (Alongi 2018). Lastly, about 40% of the mangrove nitrogen net primary production budget is allocated to belowground root production and over 50% is allocated to aboveground litterfall (Alongi 2013). The large belowground root biomass may overcome the immobility of soil ammonium since a large volume of belowground soil is covered by roots (Reef et al. 2010). In mangrove systems, nitrogen burial is an important conservation strategy heavily controlled by soil microbes and fluctuations in belowground root production and aboveground litterfall will likely have the greatest effect on nitrogen net primary productivity.

The phosphorus cycle is much less understood in mangrove soils. Phosphate is immobile in mangrove soils and unavailable for plant use. Consequently, organisms that solubilize phosphate have important implications for plant growth, especially in nutrient poor conditions (Reef et al. 2010). In many estuarine and coastal systems, primary production appears to be limited by phosphorus availability (Ruttenberg 2003). Symbiotic relationships between roots and arbuscular mycorrhizal (AM) fungi can be found in nearly all terrestrial soil types and are important for the uptake of immobile nutrients like phosphate (Treseder and Cross 2006). However, AM fungi have only been found in low salinity mangrove soils (Sengupta and Chaudhuri 2002) and very few studies have studied the occurrence of AM fungi in mangrove soils (Kothamasi et al. 2006).

The absence of these fungi species is countered by the phosphate-solubilizing bacteria associated with the oxygenated zone surrounding mangrove roots and therefore serve an important role in phosphorus uptake by mangroves (Kothamasi et al. 2006). Furthermore, phosphorus dynamics are also closely linked to the activity of sulfate-reducing bacteria, the primary decomposer in anoxic mangrove soils (Sherman et al. 1998). In the presence of oxygen, phosphorus will bind to iron making it unavailable for plant use. Under anoxic conditions, sulphate-reducing bacteria reduce iron to a form that is unfavorable for binding, which releases phosphorus to the porewater potentially for plant uptake (Holmer et al. 1994). Singh et al. (2015) outlines the components of the mangrove phosphorus cycle. The major phosphorus pools include above and belowground biomass; phosphorus input includes atmospheric deposition, canopy and litterfall, and anthropogenic sources; and phosphorus output involves microbial uptake,

tidal exchange, and soil immobilization (Singh et al. 2015). More research is needed to better understand how nutrient enrichment affects the mangrove carbon budget, specifically the effect of excess nitrogen and phosphorus on the various belowground processes occurring within a mangrove forest

Gas exchange

Mangrove forests provide large ecosystem carbon (C) stocks and sequester atmospheric CO_2 through plant primary production and bury it as organic matter, referred to as 'blue carbon', or store it in plant biomass and soil (Alongi 2012; Shiau and Chiu 2020). Coastal wetlands can transfer carbon to the ocean, process and sequester carbon in sediments, or exchange CO_2 with the atmosphere. The presence of sulfates in seawater can help soil microbes yield more energy than methanogens, combined with anoxic soil conditions, may inhibit mangrove ability to reduce atmospheric CO_2 concentrations (Shiau and Chiu 2020) and result in a CO_2 efflux from mangrove soils (Shiau et al. 2016; Weston et al. 2006). As a result, respiration rates in tidally influenced wetlands are typically greater than respiration observed in freshwater wetlands (Lu et al. 2017). Furthermore, recent estimates suggest that coastal ecosystems sequester approximately 50% of the delivered carbon into sediments, export 20% to the ocean, and 30% is emitted to the atmosphere as CO_2 (Cai 2011; Regnier et al. 2013). The average CO_2 emission from mangrove forests was calculated to be 0.7-3 g C m^{-2} d^{-1} (Hien et al. 2018; Rosentreter et al. 2018), which is comparable to coastal marshes (0.3-2 g C m^{-2} d^{-1}) (Shiau and Chiu 2020; Shiau et al. 2016), but slightly higher than freshwater wetlands (0.8-1.6 g C m^{-2} d^{-1}) (Lu et al. 2017). However, because of the strong spatial and

temporal heterogeneity of tidally influenced ecosystems, the magnitude and implications of mangrove carbon fluxes on the surrounding environment are still uncertain.

Mangrove roots must cope with short periods of anoxia that accompany the tidal cycle, since plant survival and growth depend on maintaining root oxygen levels (Alongi 2009). Some mangrove species, specifically the *Avicennia and Sonneraita* genera, develop aerial pneumatophore roots that arise vertically from the belowground radially running cable roots. As originally described by Scholander et al. (1955), a single *Avicennia* tree can produce several thousand aerial roots, usually 20-30 cm tall and a centimeter thick, soft and spongy, and covered with small white lenticels that are hydrophobic and prevent water from passing into the root. Pneumatophore roots are covered with lenticels and have extensive aerenchyma (Kitaya et al. 2002; Scholander et al. 1995) that provide belowground root ventilation via atmospheric exposure (McKee 1993; Srikanth et al. 2016). These features promote more efficient gas exchange by allowing atmospheric O_2 to diffuse into the anerobic soils and contribute to belowground soil respiration while also acting as vectors for CO_2 emissions into the atmosphere (Kitaya et al. 2002; Purvaja et al. 2004).

One of the first direct studies on pneumatophore gas exchange measured movement between the lenticels and the horizontal root system below. During a rising tide, the air pressure in the aerial root increases and root oxygen concentration is greatest just before the roots are submerged (Kitaya et al. 2002). When the lenticels are submerged, both pressure and oxygen concentration continue to decrease until the roots are exposed during the falling tide. At this point, air enters the lenticels so the atmospheric pressure within the root system is rapidly restored (Scholander et al. 1955)

and oxygen concentration begins to rise (Curran et al. 1986; Kitaya et al. 2002). The CO_2 root concentration is opposite to that of oxygen and increases as the lenticels are submerged and decreases as the roots become exposed to the atmosphere on a falling tide (Curran et al. 1986). Furthermore, when the pneumatophore roots are eliminated, the oxygen in the belowground root system can reach one percent or less within 48 hours (compared to 10-15 percent during low tide; Scholander et al. 1955), proving that the aerial pneumatophore roots serve as ventilators to the belowground anaerobic root system.

In this study, I investigated how elevated levels of nitrogen and phosphorus affect the respiration rates of soils and aerial pneumatophore roots in a South Florida mangrove forest to examine changes in the mangrove carbon cycle when exposed to coastal nutrient loading. I measured soil and root CO_2 fluxes across three fertilization treatments (control, nitrogen, phosphorus) and two seasons (wet and dry) from a basin mangrove forest located within J.N. Darling National Wildlife Refuge on Sanibel Island, Florida. The study site is within the forest basin and experiences less tidal flushing and more litter accumulation, making the site believed to be nitrogen rich and phosphorus limited. I hypothesize an increase in CO_2 emissions from both the nitrogen and phosphorus treatments for both objectives, with the greatest flux occurring in the phosphorus plot during the wet season.

Methods

Site description

Research was conducted at J.N. "Ding" Darling National Wildlife Refuge (hereafter, the Refuge) on Sanibel Island near Fort Meyers, FL (26º 26'43.77" N, 82º 06'44.32" W). Sanibel Island is considered a subtropical barrier island in the Gulf of Mexico with an average elevation of 2 meters above sea level and mixed tidal cycle with a mean amplitude of 1 meter. The island has a subtropical climate with a mean annual temperature of 23.0 ºC and two distinct seasons. The rainy season (May – September) provides an average of 107 cm of rainfall per season while the dry season (October – April) provides an average of 36 cm of rainfall per season. The Refuge is part of the largest undeveloped mangrove ecosystem in the United States and contains over 6,000 acres of mangrove forest, freshwater marsh, tidal mudflats, seagrass beds, and hardwood hammocks (USFWS 2017). Mangrove forests throughout the Refuge consist of three distinct geomorphic zones: fringe, basin, and interior (Lugo and Snedaker 1974). The fringe zone is the most seaward zone, most influenced by tidal cycles, and is dominated by the red mangrove, *Rhizophora mangle.* The basin zone is located landward of the fringe zone at slightly higher elevation, irregularly flooded, and is dominated by the black mangrove, *Avicennia germinans*. The interior zone is located landward of the basin zone at the highest forest elevation and is dominated by *Avicennia germinans* and the white mangrove, *Laguncularia racemosa*. The study sites are in the basin zone and dominated by the black mangrove species *Avicennia germinans.*

The Refuge is approximately 6 miles southwest of the mouth of the Caloosahatchee River, which serves as the drainage system from Lake Okeechobee (Lake

O) and the Northern Everglades into the Gulf of Mexico (Figure 2.1). Lake O experiences agricultural runoff from the Everglades Agricultural Area which increases the nutrient content of the lake and an increased threat of algal blooms. Freshwater discharge from Lake O carries nutrients West through the Caloosahatchee River watershed while also encountering increased surface runoff as the water travels through urbanized communities. Approximately 32% of the nutrients found in the Caloosahatchee watershed come from Lake O, 48% of the nutrients come from the non-tidal basin area, and 20% of the nutrients come from the tidal basin area (Lee County 2019). These eutrophic outflows can adversely affect the natural resources in the Caloosahatchee estuary system, including the many habitats represented in the Refuge.

Fertilization technique

This research is part of a larger ecosystem-scale fertilization study conducted by the United States Geological Survey and the United States Fish and Wildlife Service. Eighteen 8 m^2 fringe and basin treatment plots were fertilized with either nitrogen, phosphorus, or unfertilized (control). The treatment plots were fertilized with granular nitrogen (urea NH4 - 45:0:0) or phosphorus (superphosphate P2O5 - 0:45:0) bi-annually starting in 2018 and ending in 2020. Fertilization treatments have an effective fertilization radius of 1m (Feller et al. 2003a, b) and therefore were distributed at 2m regular grid intervals totaling 16 fertilization cores per treatment plot. Fertilization cores were extracted using an auger device to a depth of 30 cm. Once the cavity was excavated, 150 g of fertilizer was inserted within the active root zone and the excavated soil returned to the cavity on top of the fertilizer. The fertilization core locations within the plot remained the same for the duration of the experiment. Control plots were cored, but no

fertilizer was inserted. This fertilization technique follows the procedures described in Feller (1995), McKee et al. (2002), and Feller et al. (2003a, b). Three subplots are created in the basin zone, one subplot within each type of treatment plot, to measure soil and pneumatophore CO_2 emissions when exposed to elevated levels of nitrogen and phosphorus.

Measuring in situ CO_2 flux

Within each subplot, I measured the CO_2 flux from the entire soil surface and the CO_2 flux from individual pneumatophore roots. I measured both soil and pneumatophore flux at the mangrove sites during the wet and dry seasons: June 2020 and November 2020, respectively. Flux measurements occurred over a 4-day consecutive period during both seasons. The control subplot required one full day to measure, and the two fertilized subplots were both measured on the second day. This procedure was repeated once more to complete two rounds of sampling for each subplot within each season. Soil gas fluxes were measured using the LI-8100A Automated Soil Gas Flux System (LI-COR Biosciences, Lincoln NE) which includes a closed respiration chamber attached to an infrared gas analyzer and a 20 cm diameter PVC soil collar. The flux output from the LI-8100A represents the change in gas concentration over time measured in umol CO_2 m^{-2} s^{-1}. Each soil collar was sampled for 90 seconds and repeated three times for a total of 4.5 minutes of flux measurements per collar. Soil temperature and soil moisture were measured simultaneously with soil respiration using the LI-COR Omega soil temperature probe and the LI-COR Delta-T Theta soil moisture probe (Delta-T Devices Ltd 1998; LI-COR Biosciences 2015).

A total of 18 soil collars were distributed across the three subplots: 10 collars in the control, 4 in the nitrogen treatment, and 4 in the phosphorus treatment. Due to this project being part of a much larger ongoing study, available undisturbed soil space within the fertilized subplots was limited, but the control subplot was oversampled to provide more robust representation of unmanipulated mangrove belowground emissions. Collars were spaced 2-3 feet apart from surrounding collars and the subplot perimeter. The collars were placed approximately 0.5 cm into the soil surface to create a tight seal and ensure no gas leakage or sunlight exposure occurs during sampling (Lovelock 2008). To perform flux measurements, the soil collars were installed 30 minutes prior to sampling to minimize the effect of soil disturbance on the flux measurements. Special care was taken to avoid saplings and remove fresh leaves and litter from the interior area of the chambers.

Pneumatophore gas fluxes were measured using the EGM-4 Environmental CO_2 Gas Monitor System (PP Systems, Amesbury MA) attached to a custom 1.3 cm diameter PVC chamber via plastic tubing and two gas ports. A thin, flexible latex skirt (excised from the finger of an examination glove) is secured to the bottom of the chamber. This rubber covering slips over the pneumatophore and the chamber is twisted slightly to isolate the pneumatophore from the soil surface before data collection and provide an air-tight chamber seal. Three individual pneumatophores per soil collar were sampled, each pneumatophore measured for a period of 3 minutes. Representative pneumatophores were chosen based on average height and size of all the aerial roots in the collar.

Statistical analyses

To assess the effects of treatment and season (hereafter, TRT and SEA, respectively) on CO_2 flux, I used a nested ANOVA design model for both the soil and pneumatophore analyses. Because sampling occurred over a two-day period for each TRT and SEA combination (6 total), I nested DAY within SEA to control for variability in both models (significant at alpha=0.05 in both models for each term with DAY in it). SEA was checked for a repeated measures correlation but was not significant ($p = 0.41$). I did an AIC comparison and found that the model without the repeated measures factor performed better. I performed a two-way ANOVA with DAY nested within SEA. Model residuals were checked for normality and homogeneity, indicating a square root transformation was necessary for both variables (TRT and SEA) for both models. To obtain mean comparisons on TRT x SEA for the pneumatophore results, I removed the SEA x DAY x TRT error term due to missing treatment combinations for "wet/day2/phosphorus." I included the SEA x DAY x TRT error term in the overall pneumatophore model but had to remove it in order to compare means between different pneumatophore TRT x SEA combinations. This was not an issue for the soil flux model. Tukey's comparison tests were performed to identify differences between seasons and fertilizer treatments. To further understand the relationship between seasonality and belowground CO_2 emissions, I performed three multiple regressions to assess how soil temperature and soil moisture affect the flux response within each fertilizer treatment. The regressions were performed separately for both the soil flux and pneumatophore flux. Pearson's correlation test was used to determine the correlation between temperature and

moisture within each fertilizer treatment. Analyses was performed using the R version 4.0.5 software.

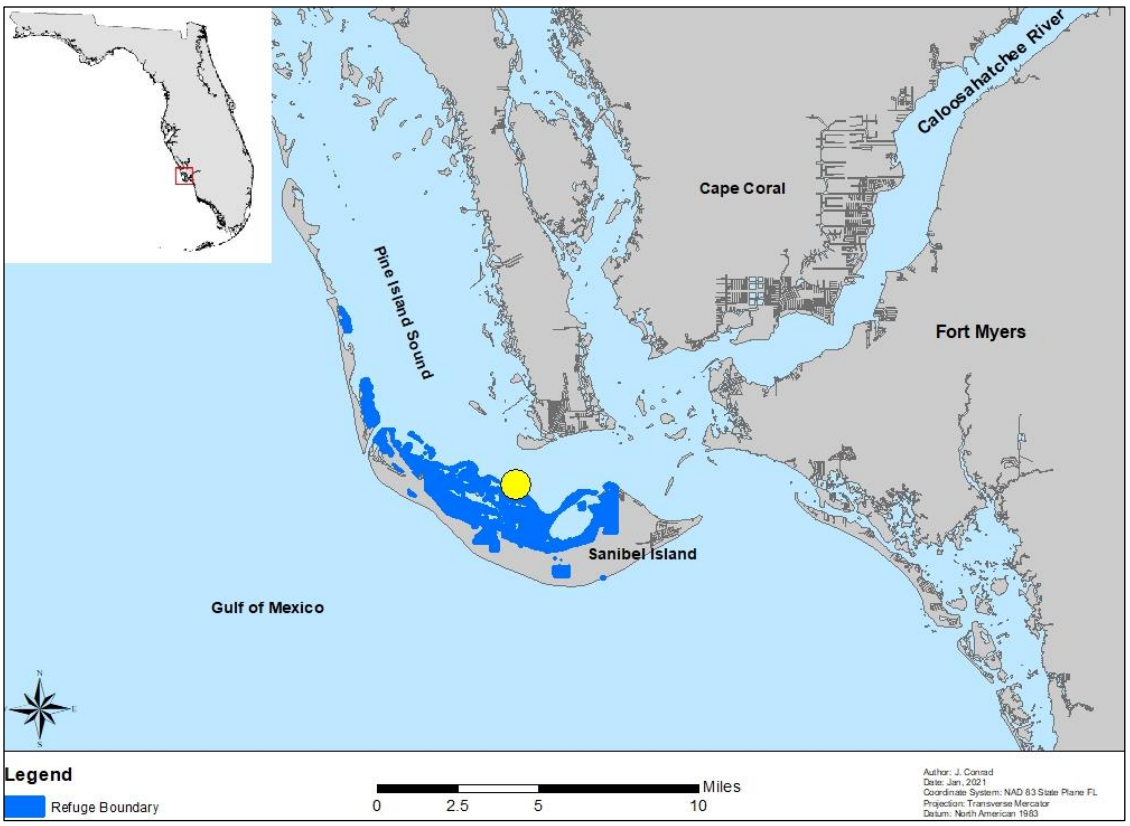

Figure 2.1: Map of J.N. Darling National Wildlife Refuge. The Refuge boundaries are outlined in blue and the yellow circle indicates the location of this fertilization study. This map was provided by Jeremy Conrad, senior wildlife biologist at the Refuge.

Results

Soil flux

Soil flux measurements were compared across two seasons and three fertilizer treatments and the response to treatment depended on the season. The soil flux differed between seasons ($F_{1,60} = 29.235$, $p < 0.001$), between the two sampling days within each season ($F_{2,60} = 10.607$, $p < 0.001$), and between treatments within each season ($F_{2,60} = 7.568$, $p = 0.001$). The overall model (SEA x DAY x TRT) was significant ($F_{4,60} = 10.321$, $p < 0.001$) and suggests a strong interaction between the three variables (i.e., the interaction between SEA and TRT changes slightly across DAY).

The soil flux from the control and nitrogen (+N) treatments were both significantly greater in the wet season compared to the dry season based on results from the Tukey's comparison test at alpha = 0.05 ($p = 0.0012$ and $p < 0.001$, respectively). Soil flux increased in the wet season compared to the dry season for the control (23%) and +N treatments (53%; Table 2.1), suggesting a higher sensitivity of soil microbes to +N fertilizer, compared to phosphorus (+P) fertilizer, during the wet season. However, during the dry season, the soil flux from the +P treatment was significantly greater than the control ($p = 0.045$) and promoted the greatest soil flux rate across all treatments in the dry season (Figure 2.2). This differs from the wet season results where the +N treatment produced the greatest soil CO_2 flux, demonstrating a shift in nutrient limitation between seasons for soil microbes in this system. Furthermore, the soil flux in the +P treatment did not differ between seasons and increased by only approximately 3% during the dry season (Table 2.1). The +N treatment produced a soil flux response more than 15-fold

that of the +P treatment between seasons, further explaining a microbial sensitivity to nitrogen in this system (Figure 2.2).

	Control	Nitrogen	Phosphorus
Dry	0.154 ± 0.01	0.147 ± 0.008	0.188 ± 0.013
Wet	0.189 ± 0.007	0.225 ± 0.023	0.182 ± 0.01

Table 2.1: Mean soil flux rates ± 1 SE for each treatment x season combination. Flux rate units are consistent throughout and are reported in gC-CO_2 m^{-2} h^{-1}.

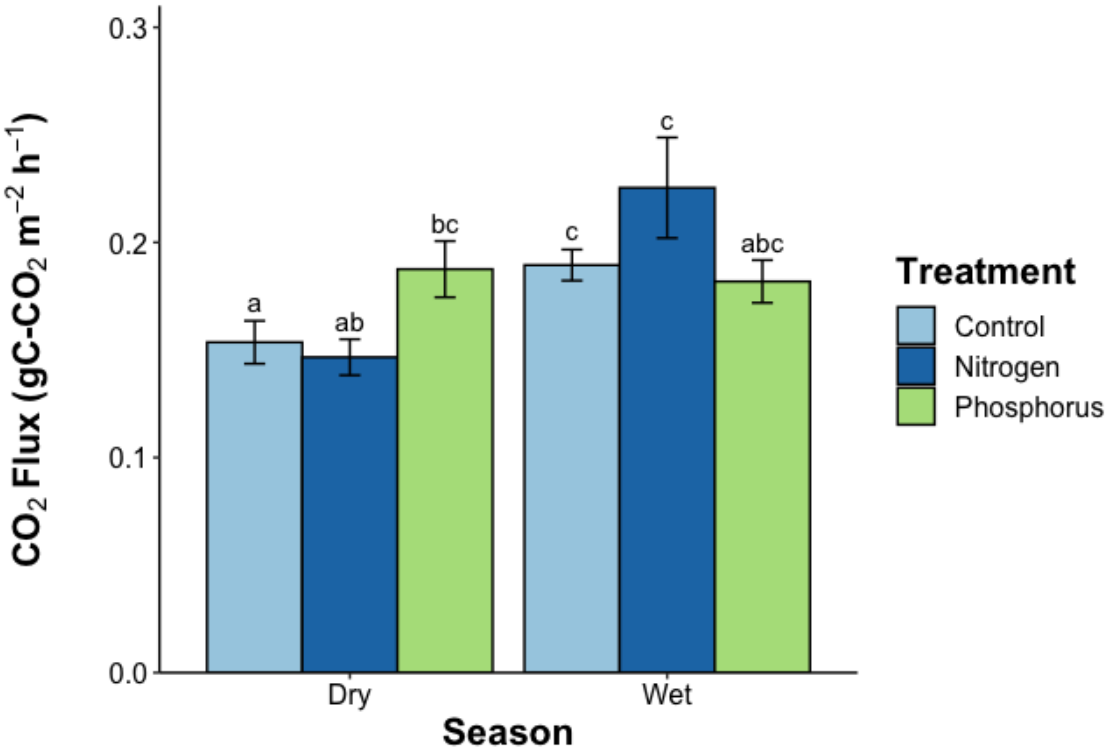

Figure 2.2: Soil flux response to fertilizer treatment and season. Error bars represent ± 1 SE and the standard error was computed separately for each cell. Bars with the same letter above are not significantly different at alpha = 0.05.

Pneumatophore flux

Pneumatophore flux measurements were compared across two seasons and three fertilizer treatments and the response to treatment also depended on the season. The pneumatophore flux differed between seasons ($F_{1,67} = 54.736$, $p < 0.001$), between the two sampling days within each season ($F_{2,67} = 8.8345$, $p < 0.001$), and between treatments within each season ($F_{2,67} = 14.001$, $p < 0.001$). The overall model (SEA x DAY x TRT) was significant ($F_{3,67} = 7.027$, $p < 0.001$) and mirrors the soil flux main effects described above.

The pneumatophore flux from the control and +P treatments were both significantly greater in the wet season compared to the dry season based on results from the Tukey's comparison test at alpha = 0.05 ($p < 0.001$ and $p < 0.001$, respectively). Pneumatophore flux increased in the wet season compared to the dry season for the control (103%) and +P treatments (177%; Table 2.2.). The increased pneumatophore flux from the control treatment during the wet season is more than 4 times greater than the increase observed from the soil flux control treatment (23%). Furthermore, the +P treatment differed from both the control ($p = 0.003$) and +N treatments ($p = 0.004$) and displayed the greatest pneumatophore flux response across all treatments in the wet season (Figure 2.3).

During the dry season, the pneumatophore flux from the +N treatment was significantly greater than the control ($p = 0.003$) and promoted the greatest pneumatophore flux rate across all treatments in the dry season (Figure 2.3). This differs from the wet season results and further explains a shift in nutrient limitation between seasons that is opposite for soil microbes and live plant tissue. Furthermore, the

pneumatophore flux in the +N treatment did not differ between seasons and increased by only approximately 5% during the dry season (Table 2.2). The P+ treatment produced a pneumatophore flux response more than 20-fold that of the +N treatment between seasons, suggesting a higher sensitivity of live roots to +P fertilizer in this system.

	Control	Nitrogen	Phosphorus
Dry	0.122 ± 0.021	0.223 ± 0.027	0.146 ± 0.017
Wet	0.248 ± 0.018	0.213 ± 0.044	0.404 ± 0.052

Table 2.2: Mean pneumatophore flux rates ± 1 SE for each treatment x season combination.

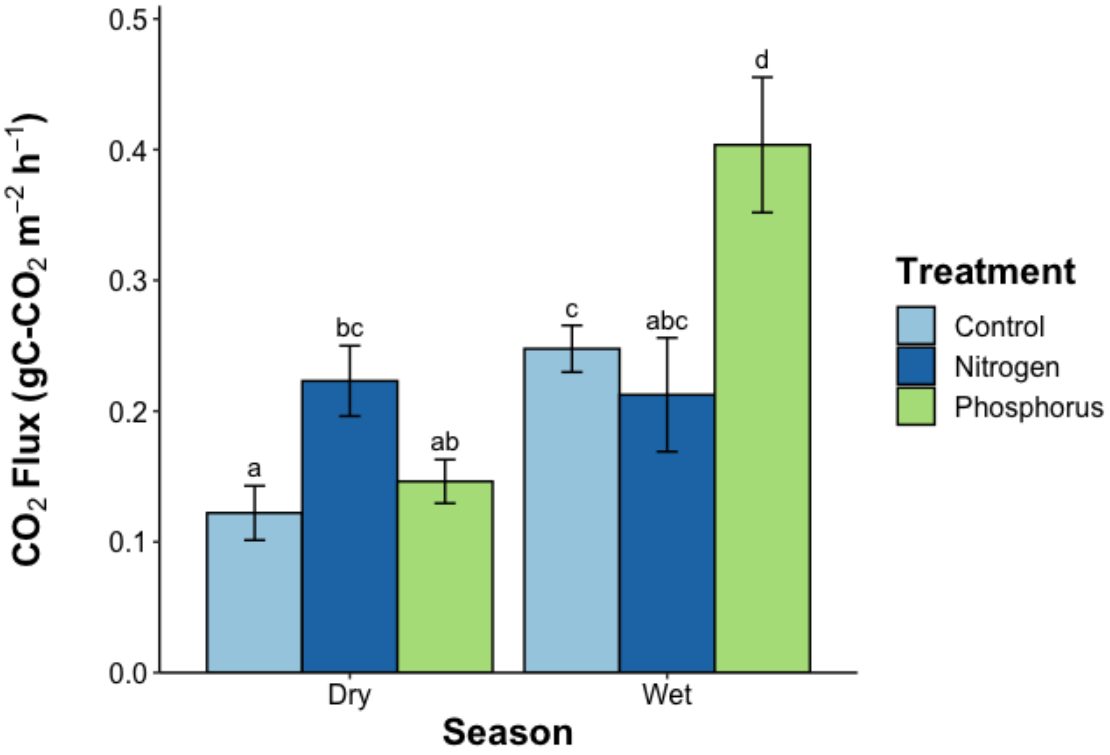

Figure 2.3: Pneumatophore flux response to fertilizer treatment and season. Error bars represent ± 1 SE and the standard error was computed separately for each cell. Bars with the same letter above are not significantly different at alpha = 0.05.

Comparing soil and pneumatophore flux within seasons

Another analysis was performed to assess the differences between soil and pneumatophore responses to fertilizer treatment within each season. The dry season flux differed between treatments ($F_{2,75} = 4.843$, $p = 0.011$) and between flux types within each treatment ($F_{2,75} = 4.181$, $p = 0.019$). The pneumatophore flux response was significantly greater in the +N treatment compared to the control ($p = 0.003$; Figure 2.4a) by approximately 83% (Table 2.2). All other comparisons in the dry season were not significant, which suggests that soil and pneumatophore respiration responses to fertilizer treatment tend to behave more similarly in the dry season.

Further differences between flux type and treatment can be observed from the wet season results. The wet season flux differed between flux type ($F_{1,57} = 18.03$, $p < 0.001$) and between flux types within each treatment ($F_{2,57} = 6.4407$, $p = 0.003$). This differs from the dry season response that displayed no effect of flux type. The pneumatophore flux response from the control was approximately 31% greater (Table 2.1; Table 2.2) than the soil flux control during the wet season ($p = 0.043$; Figure 2.4b), suggesting that the microbial community and live aerial roots display divergent respiration rates during the wet season, regardless of fertilizer. Also, the pneumatophore +P treatment was approximately 90% greater (Table 2.2) than the pneumatophore +N treatment ($p = 0.015$) and approximately 122% greater (Table 2.1; Table 2.2) than the soil +P treatment during the wet season ($p = 0.001$; Figure 2.4b). This is consistent with results presented above that pneumatophore roots display the greatest flux response to +P fertilizer during the wet season.

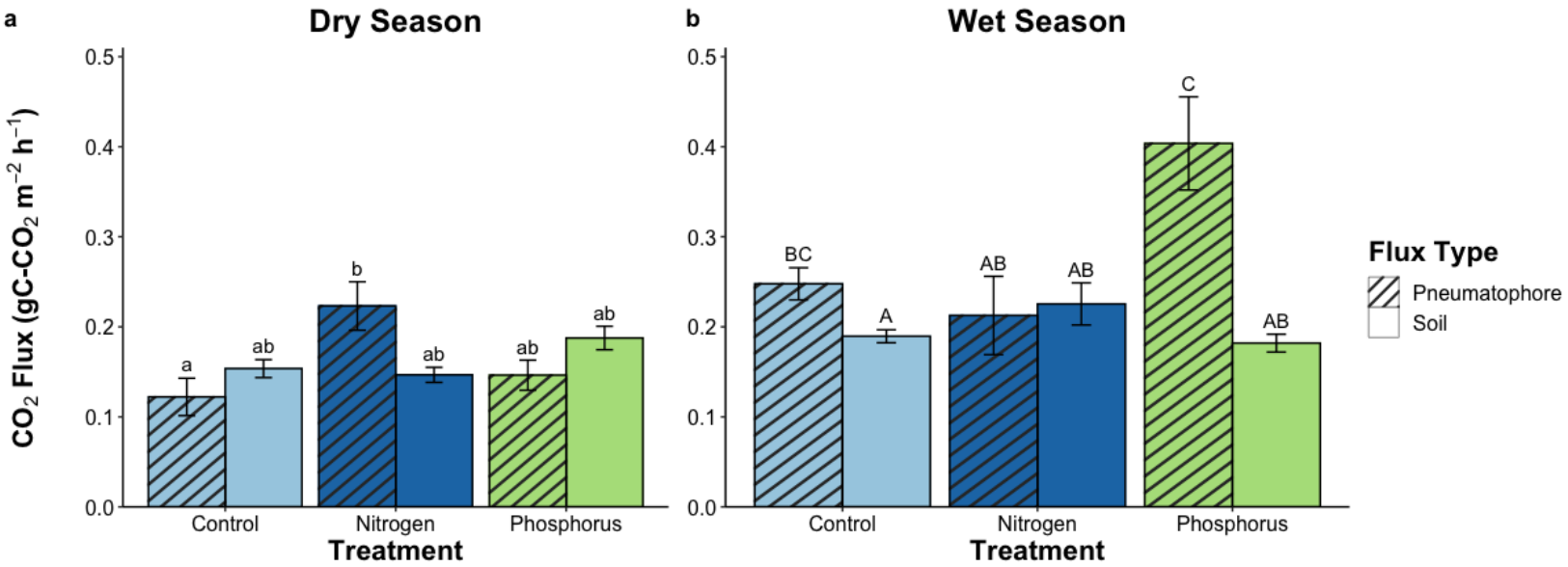

Figure 2.4: Flux responses compared between seasons as a function of treatment and flux type. Error bars represent ± 1 SE and the standard error was computed separately for each cell. Bars within a season with the same letter above are not significantly different at alpha = 0.05.

Environmental variables

Due to a strong seasonality effect found for both flux types, further analysis was required to test the effect of soil temperature and moisture on flux responses within each treatment. Values were analyzed across both seasons. Relationships were found between the soil flux rate and both temperature and moisture, which varied among the three fertilizer treatments (Control $R^2 = 0.082$: $F_{2,104} = 2.344$, $p = 0.021$ and $F_{2,104} = -3.346$, $p = 0.001$, respectively; Nitrogen $R^2 = 0.453$: $F_{2,43} = 5.969$, $p < 0.001$ and $F_{2,43} = -2.379$, $p = 0.022$, respectively; Phosphorus $R^2 = 0.090$: $F_{2,46} = 2.439$, $p = 0.019$ and $F_{2,46} = -2.473$, $p = 0.017$, respectively).

In general, as soil temperature increases, soil flux rates tend to increase (Figure 2.5). This trend was most clearly demonstrated in the soil +N treatment. The effect of soil temperature on soil flux rate was strongest in the +N treatment (Figure 2.5b) and coincides with increased CO_2 fluxes from the soil +N treatment during the wet season (Figure 2.2). In general, as soil moisture content increases, soil flux rates tend to decrease (Figure 2.5d-f). This trend was not observed in the soil +N treatment which displayed an increase in soil flux values as soil moisture increases. Furthermore, soil temperature and moisture are positively correlated in the soil control, +N, and +P treatments ($r = 0.80$, $p < 0.001$; $r = 0.64$, p 0.001; and $r = 0.78$, $p < 0.001$, respectively).

No relationship between the pneumatophore flux rate and soil moisture was found (Control: $R^2 = 0.764$, $F_{2,23} = 1.901$, $p = 0.070$; Nitrogen: $R^2 = -0.122$, $F_{2,16} = -0.001$, $p = 0.999$; Phosphorus: $R^2 = 0.385$, $F_{2,18} = -1.517$, $p = 0.147$). Temperature was related to pneumatophore flux in the control and +P treatments ($R^2 = 0.764$, $F_{2,23} = 3.091$, $p = 0.005$ and $R^2 = 0.385$, $F_{2,18} = 3.490$, $p = 0.003$, respectively). No relationships were found in the

pneumatophore +N treatment (Temperature: $R^2 = -0.1223$, $F_{2,16} = -0.123$, $p = 0.90$; Moisture: $R^2 = -0.1223$, $F_{2,16} = -0.001$, $p = 0.999$). In general, as soil temperature increases, pneumatophore flux rates tend to increase in controlled environments and when exposed to elevated phosphorus (Figure 2.6). Furthermore, soil temperature and moisture are positively correlated in the pneumatophore control, +N, and +P treatments ($r = 0.85$, $p < 0.001$; $r = 0.77$, $p < 0.001$; and $r = 0.73$, $p < 0.001$, respectively). Higher soil temperatures increase pneumatophore flux in the control and +P treatments but show no effect on pneumatophore flux rates in the +N treatment (Figure 2.6). By adding nitrogen fertilizer, the pneumatophore flux response deviates from the baseline response (control) and display no relationship between soil temperature and flux. The strong seasonality effect found for both soil and pneumatophore flux responses may be partially attributed to changes in soil temperature and moisture content and this relationship can change when different fertilizers are added to the system.

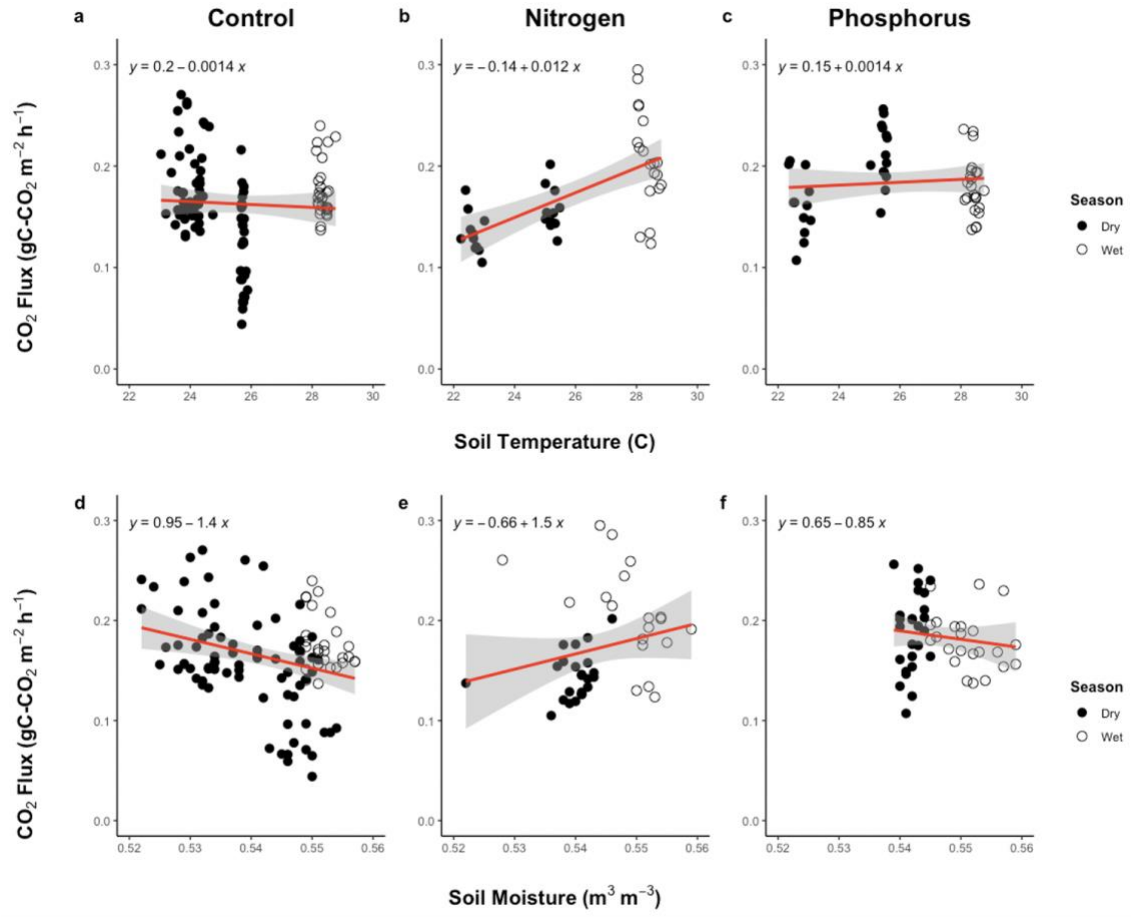

Figure 2.5: Relationships between soil temperature (a-c), soil moisture (d-f), and soil CO_2 flux within fertilizer treatments. Only significant relationships are displayed. The shaded gray area represents the 95% confidence interval.

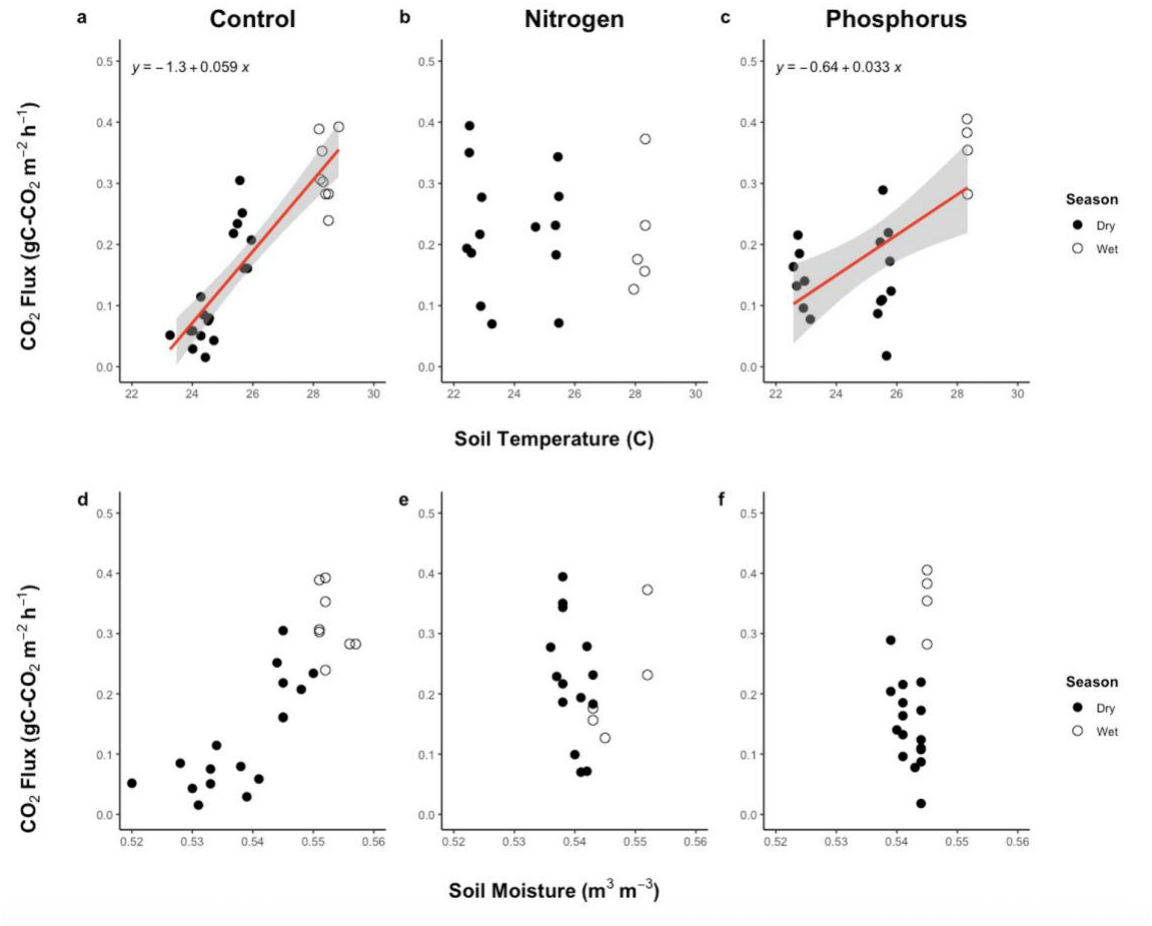

Figure 2.6: Relationships between soil temperature (a-c), soil moisture (d-f), and pneumatophore CO_2 flux within fertilizer treatments. Only significant relationships are displayed. The shaded gray area represents the 95% confidence interval.

Discussion

Discovering links between nutrient additions and belowground respiration processes improve carbon exchange models across a variety of forest types. Mangroves represent productive tropical forests that provide important ecosystems services and several ecological functions, one of which is carbon burial and storage (Alongi 2002; Chmura et al. 2003). Mangrove trees, with their associated above- and belowground root system and anoxic soils, act as ecosystem engineers by trapping organic material at the sediment surface and gaining elevation to offset sea level rise (Krauss et al. 2014; Lovelock et al. 2015) while also creating large carbon stocks and contributing to the global carbon cycle (Alongi 2012; Donato et al. 2011; Jennerjahn and Ittekkot 2002). This study aimed to discover those links between nutrient enrichment and belowground respiration of soil microbes versus live roots within a mangrove forest. The results obtained here will advance our knowledge about nutrient limitation between different ecological functions within tropical forests and highlight the importance of mangrove root adaptions.

Microbial respiration response to fertilizer

Mangrove soil respiration across the three fertilizer treatments was variable and the response to fertilizer depended on the season. Soil respiration rates were generally higher and more variable than those reported from both unfertilized and fertilized mangroves forests (Hien et al. 2018; Lovelock 2008; Lovelock et al. 2014) and can likely be explained by site-specific characteristics. Soil composition, structure, and characteristics, in addition to tidal regimes and biotic factors (e.g. pneumatophores), along with proximity to human development, can all influence soil respiration rates by

affecting root growth and microbial metabolism (Lovelock et al. 2014). The current study measured respiration rates from a single basin mangrove forest dominated by *Avicennia Germinans* in Southwest Florida. This forest is rich in organic soil and receives eutrophic outflows from the neighboring watershed serving as the main freshwater drainage system from Lake Okeechobee and the Northern Everglades, which likely contributes to a higher baseline soil nutrient content and consequently increased soil respiration rates compared to other mangrove habitats.

Soil respiration rates were generally greater in the wet season likely due to a combination of increased soil temperatures and increased anthropogenic nutrient loading into the system. Nitrogen fertilizer produced the greatest soil respiration rate during the wet season and the response to nitrogen fertilizer differed the most between seasons (Figure 2.2). This may be due to increases in soil temperature and moisture which positively affect soil flux rates in the nitrogen treatment (Figure 2.5b,e) and supports observations of nitrogen limitation in soil microbial communities (Deegan et al. 2012; Bulseco et al. 2019). However, this system experiences nutrient loading, predominantly phosphorus, from the Caloosahatchee watershed and its neighboring estuaries during the wet season, due to surface runoff from agricultural, industrial, and residential processes, higher temperatures, and the intense management of freshwater outflows from Lake Okeechobee. It's likely that this system becomes saturated with phosphorus during the wet season and soil microbes appear to be unaffected by our phosphorus fertilizer. As a result, the soil microbial community is presumed to be nitrogen limited during the wet season in this system when eutrophic outflows in this system peak during the hot summer months and the soil respiration response to +N fertilization is at its greatest (Figure 2.5b).

The limiting nutrient for soil respiration shifts between seasons. Phosphorus fertilizer produced the greatest soil respiration rate during the dry season but the response to phosphorus fertilizer did not differ between seasons (Figure 2.2). This suggests a change in the natural soil nutrient content of this system between seasons and demonstrates a shift in nutrient limitation for belowground decomposers. Soil temperature and moisture displayed a weaker relationship to soil flux in the phosphorus treatment (Figure 2.5c,f) and cannot be solely attributed to the observed increase in soil flux rates. Soil respiration response to phosphorus fertilizer is consistent with previous studies that have determined phosphorus to be the limiting nutrient in basin mangrove forests (Feller 1995; Feller et al. 2003a; Lovelock et al. 2015). Phosphorus fertilization has generated increases in belowground microbial activity in some coastal ecosystems (Lovelock et al. 2014) but the increase in soil respiration tended to follow an increase in aboveground growth and canopy development (Lovelock 2008). Soil respiration rates can vary greatly within and between mangrove systems and the response to nutrient enrichment can vary by forest zone due to shifting hydrological regimes and mineral inputs (Feller et al. 2003a; Lovelock et al. 2014). Mangrove forests with rich organic soil comprised mostly of roots, like observed at the Refuge, have displayed phosphorus limitation where heavily disturbed mangrove forests comprised mostly of sandy soils have displayed nitrogen limitation (Feller et al. 2007). These results may be more representative of mangrove forests since system specific nutrient loading and eutrophic freshwater inputs tend to decrease in dry season.

Live root respiration response to fertilizer

Mangrove aerial root respiration across the three fertilizer treatments was variable and the response to fertilizer depended on the season. No studies have measured the pneumatophore respiration response to nitrogen and phosphorus fertilization, but the results generated in this study are slightly higher and more variable than those reported from the pneumatophore respiration rates of four mangrove species in an unfertilized forest (Kitaya et al. 2002). Pneumatophore flux rates were generally greater in the wet season and nearly doubled in the control treatment whereas the soil control rates increased by only approximately 23% during the wet season. Pneumatophore flux rates displayed a stronger relationship to soil temperature in the control plot compared to soil flux (Figure 2.6a) and could explain the drastic increase in the pneumatophore control flux during the wet season. Live root respiration may be more affected by changes in soil temperature than microbial respiration when fertilizer is not considered.

Soil microbial metabolism and live root respiration responded differently to nitrogen and phosphorus fertilizer due to variations in nutrient limitation between different ecological processes (Feller et al. 2007). Phosphorus fertilizer produced the greatest pneumatophore flux rate during the wet season (Figure 2.3), which can likely be explained by both differences in phosphorus retention capabilities between plant roots versus soil microbes and increased soil temperatures. The strong relationship between soil temperature and pneumatophore flux rate observed in the phosphorus treatment (Figure 2.6c) could partially explain why mangrove aerial roots are able to respond to our phosphorus fertilizer during the wet season. Another reason is that the aerial roots and attached belowground cable roots likely have a higher capacity for phosphorus uptake

and can avoid saturation, unlike the soil microbial community, while still responding to our phosphorus fertilizer. As the system becomes inundated with phosphorus loading during the wet season, mangrove roots can take up, utilize, and eventually store phosphorus within the plant where the soil microbes cannot. Consequently, phosphorus retention by mangrove roots may affect respiration responses to fertilizer treatment during the dry season. Nitrogen fertilizer produced the greatest pneumatophore flux rate during the dry season (Figure 2.3). Once eutrophic outflows into the system cease, the mangrove roots utilize the phosphorus stored in tissues from the wet season and become limited by nitrogen once those phosphorus reserves are depleted. These major divergences between soil and root respiration response to fertilizer treatment prove that not all ecological processes within a mangrove forest are limited by the same nutrient and responses to fertilizer treatment are more variable in already eutrophic ecosystems.

Differences between microbial and root respiration rates within seasons

Further discrepancies between soil microbe and aerial root behavior are observed when both respiration types are analyzed together within each season. Overall, pneumatophore roots displayed greater flux rates than the exposed soil surface. Pneumatophores act as CO_2 conduits from deep sediments to the atmosphere and areas with abundant pneumatophores have demonstrated larger total CO_2 releases compared to areas with few aboveground root structures (Kristensen et al. 2011). It's likely that only a portion of respired CO_2 can be measured at the soil surface and aerial pneumatophore roots are responsible for a large portion of the total CO_2 lost to the atmosphere from mangrove sediments (Kristensen et al. 2011; Lovelock et al. 2014).

The wet season indicated a strong influence of flux type on flux rates whereas the dry season did not, which could be explained by varying sensitivities to increased temperature and moisture between soil microbes and aerial roots. The pneumatophore nitrogen treatment displayed the greatest flux rate during the dry season, but all other comparisons were not significant, which suggests that soil and pneumatophore respiration responses to fertilizer are more similar in the dry season. During the wet season, the flux rate from the controlled pneumatophore treatment was 31% greater than the controlled soil treatment. This further strengthens the observation that soil microbes and live aerial roots display divergent respiration rates during the wet season, regardless of fertilizer, with more CO_2 being respired from live root respiration. Lastly, soil respiration responses did not differ between treatments for either season when analyzed in combination with the pneumatophore results and suggests that the mangrove tree may be more sensitive to nutrient enrichment compared to the belowground soil microbial community.

Influence of soil temperature and moisture on respiration

Some of the variation in respiration rates between seasons could be explained by variation in soil temperature and moisture. Soil respiration has been found to vary seasonally with shifting soil temperature and water content across a wide range of wetland environments (Hien et al. 2018; Lovelock 2008; Lu et al. 2017). Due to a large seasonality affect observed in this study, we must consider that soil temperature and moisture may drive respiration responses and act alongside fertilizer treatments. Soil temperature was most influential in the soil N treatment and pneumatophore P treatment, which coincides with the greatest respiration rate observed for each flux type during the wet season when temperatures have increased. A significant correlation between soil

temperature and moisture was found across all treatments and both flux types. Furthermore, temperature and moisture were more strongly correlated in the control treatment for both soil and pneumatophore flux ($r = 0.80$ and $r = 0.85$, respectively), which suggests that fertilizer treatments may interact with the relationship between soil temperature and moisture. There is no doubt that fertilization affects respiration from soil microbes and pneumatophore roots differently, but that effect may be accelerated by increased soil temperatures during the wet season.

Conclusions

Nutrient enrichment can increase aboveground growth and belowground decomposition in mangrove forests. The results from this study indicate that nitrogen and phosphorus enrichment can also increase the amount of CO_2 lost to the atmosphere from microbial and live root respiration, but the effect of nutrient enrichment depends on the ecological process and the baseline soil nutrient content of the system. Within a eutrophic subtropical barrier island in Southwest Florida, mangrove soil respiration rates increased when exposed to elevated levels of nitrogen during the wet season and elevated levels of phosphorus during the dry season. Pneumatophore aerial root respiration rates increased when exposed to elevated levels of phosphorus during the wet season and elevated levels of nitrogen during the dry season. Improved estimates of mangrove soil versus root respiration rates require observations across spatial, temporal, and seasonal scales that are based in site-specific characteristics. The influence of soil type, soil nutrient content, and hydrological regimes on soil respiration requires more research as these factors will likely have a strong influence on the mangrove carbon budget and the contribution of carbon to adjacent ecosystems.

The results obtained from this study will contribute to our understanding of how further increases in nutrient availability can affect belowground processes in mangrove forests. The respiration responses from mangrove soils and aerial pneumatophore roots to fertilizer treatments demonstrate that essential nutrients are not evenly distributed throughout mangrove forests and not all ecological processes are limited by the same nutrient. The data presented in this study can be combined with aboveground growth measurements and belowground decomposition rates to estimate changes in the mangrove carbon cycle due to increased nutrient loading of our coastal waters. Future studies including year-round respiration measurements, comparing rates between different forest zones, and comparisons of plant versus soil activity will further create a comprehensive understanding of belowground responses to elevated nutrients in mangrove systems. Understanding the implications of increased nutrients in coastal environments will improve ecosystem models of carbon exchange in estuaries and other coastal habitats and provides useful information for ecological management of these systems.

CHAPTER 3 – IMPLICATIONS OF EUTROPHIC WATERS ON THE HEALTH OF COASTAL ECOSYSTEMS

Changes in the quantity and quality of essential nutrients within estuarine habitats can affect a variety of biological and ecological processes and cause alterations in the movement of carbon throughout these systems. The net effect of eutrophication on coastal ecosystems is highly debated and the answer remains unclear. This uncertainty can be explained by the simultaneous effect that nutrients have on soil carbon inputs and outputs. Some studies suggest that nutrient additions will enhance the carbon storage ability of coastal habitats by increasing aboveground productivity and growth, along with increasing belowground plant biomass by sediment accumulation (Morris and Bradley 1999; Pastore et al. 2017). Other studies indicate a neutral effect because eutrophication simultaneously increases both the production and decomposition of organic matter (Feller et al. 1999; Keuskamp et al. 2015a; Simpson et al. 2020). Furthermore, a growing number of studies report that nutrient enrichment can reduce the carbon sequestration ability of coastal ecosystems by boosting the microbial decomposition of organic matter (Liu et al. 2017; Liu et al. 2020; Lovelock et al. 2014; Morris and Bradley 1999) and enhancing the mortality of plants and roots (Lovelock et al. 2009). Disturbances in the natural cycling of nutrients throughout coastal ecosystems have the potential to alter various ecological processes, such as above aboveground production and belowground decomposition, and ultimately affect ecosystem functions.

Here, I use J.N. “Ding” Darling National Wildlife Refuge (JDNWR) as a case study to estimate exposed soil and aerial root respiration rates from a basin mangrove forest to demonstrate belowground responses to nitrogen and phosphorus enrichment and illustrate potential alterations in the carbon cycle of these systems. I found that soil respiration rates tend to be higher at JDNWR compared to other mangrove forests due to a higher baseline soil nutrient content in this estuarine system. Overall, nitrogen fertilization produced the greatest mean soil respiration rate and the response to phosphorus fertilizer did not change between seasons. Increased soil temperatures also accelerate the influence of nitrogen fertilizer on microbial respiration rates during the wet season. On the other hand, phosphorus fertilization produced the greatest mean pneumatophore respiration rate and the response to nitrogen fertilizer did not change between seasons, which is opposite to the soil respiration results. Likewise, increased soil temperatures also accelerate the influence of phosphorus fertilizer on live root respiration rates during the wet season where no relationship between soil temperature and nitrogen fertilizer exists for the pneumatophore roots. The results from this study indicate that mangrove microbial communities found in JDNWR are more sensitive to changes in soil nitrogen content, but the actual mangrove tree is more sensitive to changes in phosphorus content and the influence of fertilizer on these two ecological processes is better observed in the wet season. Fewer differences in fertilizer treatment were observed between soil and pneumatophore respiration rates in the dry season and suggests these two processes respond to fertilizer more similarly at lower soil temperatures but divergent respiration responses to fertilizer can be observed when soil temperatures are elevated, and each process becomes more sensitive to either nitrogen or phosphorus enrichment.

The lack of consensus from nutrient enrichment studies is further complicated when considering the variable effects of eutrophication on plants versus microbes. Soil microbes must respond quickly to changes in the environment and are more dependent on outside nutrient inputs whereas the roots are connected to the aboveground plant system and display greater storage capabilities. The transformation of coastal ecosystems into carbon sources is often attributed to changes in the microbiome community structure and/or the cycling of essential nutrients (Holguin et al. 2001). Nitrogen loading may threaten the ability of some coastal habitats to act as carbon sinks by increasing microbial activity and reducing the burial rate of organic sediments. Likewise, phosphorus loading stimulates aboveground growth and canopy development in basin mangrove forests (Feller 1995; Feller et al. 2003a; Lovelock et al. 2015) which often promotes an increased belowground respiration response from tree roots (Lovelock 2008; Lovelock et al. 2014) like observed in this study.

Since coastal nutrient loading consists of mainly excessive nitrogen and phosphorus, eutrophication has the potential change the way carbon is transported through mangrove forests by altering microbial activity and thus influencing the belowground decomposition rate and by altering aboveground growth along with the subsequent increase in live root respiration. Partitioning soil CO_2 emissions into microbial respiration and root respiration is necessary to calculate the total soil carbon budget and determine whether soils are acting as a CO_2 sinks or sources (Sapronov and Kuzyakov 2007). Nutrient additions affect the balance between productivity and decomposition in mangrove forests and a better understanding of that relationship will be vital for the management of eutrophic outflows into our coastal habitats. Future studies

should include multi-year respiration measurements, assess the natural soil nutrient content prior to fertilization treatment, and compare respiration rates between different forest zones experiencing different tidal influence and mineral inputs. Creating a comprehensive understanding of belowground responses to elevated nutrients in mangrove systems and the implications of increased nutrients in coastal environments will improve ecosystem models of carbon exchange in estuaries and other coastal habitats and provides useful information for ecological management of these systems.

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