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BEFORE THE FLORIDA PUBLIC SERVICE COMMISSION

IN RE: Application for certificate to provide wastewater service in Charlotte County, by Environmental Utilities, LLC
_____________________________________/

DOCKET NO. 20240032-SU

NOTICE OF FILING OF PREFILED REBUTTAL TESTIMONY OF BRIAN E. LAPOINTE ON BEHALF OF ENVIRONMENTAL UTILITIES, LLC

Environmental Utilities, LLC, by and through its undersigned counsel, hereby notices the filing of the attached Prefiled Rebuttal Testimony of Brian E. Lapointe.

Respectfully submitted this 6th day of December, 2024.

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CERTIFICATE OF SERVICE

I HEREBY CERTIFY that a true and correct copy of the foregoing Notice of Filing of Rebuttal Testimony has been furnished by electronic mail to the following parties this 6th day of December, 2024:

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BEFORE THE FLORIDA PUBLIC SERVICE COMMISSION

In re: Application for certificate to provide wastewater service in Charlotte County by Environmental Utilities, LLC

Docket No. 20240032-SU

REBUTTAL TESTIMONY

OF

BRIAN E. LAPOINTE, Ph.D.

on behalf of

Environmental Utilities, LLC

Q. What is the purpose of your Rebuttal Testimony?

A. The purpose of my Rebuttal Testimony is to provide comments on the critique by Robert J. Robbins, Ph.D. of my white paper (Lapointe 2024) "Science Supports a Septic-to-Sewer Conversion on the Barrier Islands on Charlotte County, Florida" and the Florida Atlantic University (FAU) - Harbor Branch (2016) report "Charlotte County Water Quality Assessment: Phase I Data Analysis and Recommendations for Long-Term Monitoring."

Q. Does Dr. Robbins have any experience studying septic systems and their environmental impacts on groundwaters and coastal waters?

A. Based on Dr. Robbins curriculum vitae, he has no research experience or peer-reviewed papers about septic systems or their environmental impacts on groundwaters or coastal waters. Although Dr. Robbins received his Ph.D. in 2005 from the University of Miami in fisheries science, he has not published as lead author a single peer-reviewed scientific paper.

Q. Was Dr. Robbins' claim true that my white paper was "devoid of any empirical data" from the Charlotte County Barrier Islands and was "misleading and erroneous?"

A. No. To understand why, one must understand that empirical evidence is evidence gathered directly or indirectly through observation or experimentation that may be used to confirm or reject a scientific theory or to help justify or establish as reasonable, a person's belief in a proposition. Although I did not collect on-site water quality data regarding the impacts of septic systems on the Charlotte County barrier islands, I did make personal observations and photos (see cover photo in Lapointe 2024) during a survey of these islands and coastal waters on December 6, 2023. This visual observations confirmed to me that the low elevations, high water tables, porous sandy soils, and high densities of septic systems in proximity to sensitive surface waters characterized poor conditions for septic system functioning on these barrier islands. All these factors are known to exacerbate septic system pollution of groundwaters and adjacent surface waters. The macroalgal overgrowth of seagrasses and abundant *Cassiopea* jellyfish along the shoreline in Gasparilla Sound were classic symptoms of nutrient pollution and eutrophication from septic system pollution. The Lapointe (2024) white paper cited numerous peer-reviewed scientific papers (42 peer reviewed papers) supporting my observations and conclusions regarding septic system pollution, including similar barrier islands in Florida. Furthermore, site-specific data and information for the Charlotte Harbor barrier islands regarding septic tank densities, age, soils, depth of water table, and septic nitrogen loading were obtained from the Charlotte County Sewer Master Plan (prepared by Jones & Edmunds) and other sources to further support my conclusions. The peer reviewed papers included my own recent studies in nearby Lee County that demonstrated how all these factors result in widespread sewage pollution of groundwaters and surface waters in the Caloosahatchee River and estuary with nutrients, fecal indicator bacteria (identified with the molecular tracer of human waste HF183), and human chemical tracers (sucralose, pharmaceuticals). On the other hand, Dr. Robbins provided no peer-reviewed publications that show septic systems on the Charlotte County barrier islands are not a source of pollution to groundwater and surface waters. Septic systems are well known to be a primary source of nitrogen pollution to groundwaters and surface waters in many urbanized areas in Florida and were identified as such by the Blue-Green Algae Task Force. The Brewton et al. (2022) and Tyre et al. (2023) studies, performed within the Charlotte Harbor National Estuary Program area, are provided as Exhibits BEL-2 and BEL-3 .

Q. Was the randomized monitor well sampling design used in the 2013 Tetra-Tech study appropriate for characterizing nutrient and fecal pollution derived from septic system effluent as suggested by Dr. Robbins?

A. No, a random sampling design will underestimate and obfuscate the impacts of septic systems on groundwater quality. Effluent from septic systems enters the groundwater below the drainfield and is then transported via groundwater flow downgradient to receiving surface waters. Over time, this results in a contaminant plume defined by groundwater flow, and not a randomized pattern of contamination on a given residential lot. To guide proper placement of monitoring wells in septic system research to characterize septic plumes, the direction of groundwater flow must be initially defined to accurately monitor the degree of nutrient and bacterial pollution. Without this critical approach, the random sampling design, such as in the TetraTech (2013) study, results in sampling bias. This biased monitoring design was recognized in section 1.6 "Significance of Test Results" (page 39) of the TetraTech (2013) report where it was stated "random placement provides an overview of the general study area but is not directly indicative of an issue with a failing OSTDS. However, it is noted that with this random sampling, it is difficult to achieve a true indication of the impact on the groundwater. The reason is that as effluent is released from a septic tank and migrates downward through the soil within the drainfield, once it makes it into the water table, it immediately begins to move in the direction of groundwater flow."

Q. Did the use three specific groundwater monitor wells in the FAU-Harbor Branch (2016) study prohibit drawing inferences about septic systems in the study area as claimed by Dr. Robbins?

A. No. Because of budget constraints, the FAU-Harbor Branch (2016) study only provided for limited reconnaissance sampling. As noted on page 19 of the FAU-Harbor Branch (2016) report, monitor wells (MW) 66, 67 and 68 were used in the reconnaissance sampling because "nutrient concentrations, especially nitrogen, were exceptionally high during the East and West Spring Lake Wastewater Pilot Program." The selection of these wells was based on discussions with Charlotte County Utilities Department (CCUD) staff who installed the wells. The "nuisance complaints" by the Florida Department of Health between 2010 and 2013 was based on sewage ponding on the ground surface, and was abated several years prior to the FAU-HBOI study. Because the TetraTech (2013) report noted that fertilizers and atmospheric deposition could also be contributing sources of nitrogen pollution in the East and West Spring Lake study area, discrimination between human waste, fertilizer, and atmospheric sources of groundwater nitrogen was a key objective in using these wells in the FAU-Harbor Branch (2016) study. Accordingly, the targeted sampling in these wells, which were located a distance away from the septic systems, included not just various forms of nitrogen, but also stable nitrogen isotopes of aqueous ammonium ($\partial^{15}N\text{-}NH_4^+$) and nitrate ($\partial^{15}N\text{-}NO_3^-$) to identify whether these nitrogen forms were sourced from human waste (septic systems, enriched $\partial^{15}N$ values between +3 to +30 o/oo) or fertilizers/atmospheric deposition (depleted $\partial^{15}N$ values < +3 o/oo). Sucralose concentrations were also measured to provide a conservative chemical tracer of human waste as this artificial sweetener is not removed by septic systems or during groundwater transport. The results showed very high sucralose concentrations (~ 10 µg/L) and enriched aqueous $\partial^{15}N\text{-}NH_4^+$ (+15 to +20 o/oo) and $\partial^{15}N\text{-}NO_3^-$ (+10 to +15 o/oo) values in the wells that are characteristic of human waste, not fertilizers or atmospheric deposition. These results of the FAU-Harbor Branch (2016) study are align with more extensive $\partial^{15}N$ sampling of macroalgae and particulate organic matter (POM) in the Indian River Lagoon (Lapointe et al. 2023) and Caloosahatchee River and estuary (Brewton et al. 2022; Tyre et al. 2023) that provided compelling evidence that the worsening eutrophication, harmful algal blooms (red tides, blue-green algae blooms), and seagrass die-offs are being driven to a large extent by human waste from septic systems in these urbanized estuaries. Furthermore, the TetraTech (2013) study did not sample the monitor wells for ammonia concentrations, which is the primary form of nitrogen in septic tank effluent and the preferred (reduced) form of nitrogen for growth of harmful algal blooms. The FAU-Harbor Branch (2016) study included ammonia data from the three monitoring wells (66, 67 and 68) in 2015 and 2016 and showed enriched values up to ~ 30 mg/L (Fig. 11). Higher ammonium concentrations compared to nitrate/nitrite were also found in the surface waters at four different sites during the 2016 reconnaissance sampling (Table 3), helping to explain why Charlotte Harbor is experiencing increasing phytoplankton biomass (chlorophyll *a*), macroalgal blooms, and seagrass loss. Understanding the nitrogen forms and transformations in septic plumes requires monitoring for ammonia as well as nitrate plus nitrite and is a necessary and fundamental aspect of septic system research. This key form of nitrogen was not monitored in the Tetra Tech (2013) study or addressed by Dr. Robbins. Lapointe et al. (2023) and a University of Florida (IFAS) report on the efficacy of seasonal fertilizer restrictions are attached as Exhibits BEL-4 and BEL-5.

Q. Was the sampling of stable nitrogen isotopes of aqueous ammonium and nitrate as well as sucralose from monitor wells 66, 67, and 68 reliable evidence of pollution from septic systems?

A. Yes. As noted above, data resulting from these analyses were consistent with many peer-reviewed papers, some cited in Lapointe (2024), which link septic system pollution to eutrophication and harmful algal blooms. Measurement of stable oxygen and nitrate isotopes ("dual isotope method") can be used for source identification of nitrate but not ammonium, the latter being the primary form of nitrogen in septic tank effluent. Unfortunately, the dual isotope method does not address the source of ammonium. Despite this shortcoming, the dual isotope method did provide another line of evidence beyond what was found using stable nitrogen isotopes in particulate organic matter (POM) and macroalgae, dissolved nutrients, and human tracers of contamination such as sucralose and the human molecular tracer HF183 in our recent Lee County studies (Tyre et al. 2023). Measurement of stable nitrogen isotopes in macroalgal tissue was also used in the FAU-Harbor Branch (2016) study. This is a proven method for nitrogen source identification in coastal waters and many peer-reviewed studies and reviews have established this. The nitrogen isotope values measured in the red macroalga *Gracilaria tikvahiae* in the FAU-Harbor Branch (2016) study ranged from +4 to +6 o/oo, which matches well with similar values for macroalgae in sewage polluted waters, such as the Indian River Lagoon (Lapointe et al. 2023). The sucralose data in the FAU-Harbor Branch study provided further evidence of contamination by human waste. Information from CCUD indicated that the groundwater monitor wells used for the isotope sampling were not being impacted by re-use water that is treated at the Eastport Water Reclamation Facility and has much lower total nitrogen concentrations (13.2 mg/L) compared to the incoming untreated wastewater (71.3 mg/L).

Q. Did the FAU-Harbor Branch (2016) study misrepresent the Tetra Tech (2013) fecal coliform dataset and distort the risk of fecal pollution from septic systems?

A. No. Apparently Dr. Robbins confused the TetraTech (2013) report with the larger follow up FAU-Harbor Branch (2016) study. The TetraTech (2013) study used fecal coliform data from 50 monitor wells sampled between 2012 and 2013. The FAU-Harbor Branch (2016) study included additional samples collected in the wells between 2014 and 2016 and provided very basic descriptive statistics of the data. Groundwater that is not polluted by human or animal waste should have zero fecal coliform, so positive values of 10 cfu/100 ml and above are of concern. The fecal coliform values from monitor wells (n = 39) in the FAU-Harbor Branch study (2016) were variable with many samples > 20 cfu/100 ml and eight samples in 2014 and 2015 ranging between the USEPA standard (200 cfu/100 ml) and approaching or exceeding the Florida surface water standards (400 cfu/100 ml). TetraTech (2013) reported higher fecal coliform levels in groundwater monitor wells (n=50) in the wet season (June through October; 1720 to 2940 cfu/100 ml) with lower values (10 cfu/100 ml) in the dry season. The random sampling design of the TetraTech (2013) study resulted in a statistical bias towards an overall lower range of fecal coliform values in groundwater and was not appropriate for monitoring septic system performance. TetraTech (2013) specifically noted this in stating "when a positive sample is obtained in a random location within the water table, such as where the initial 50 wells were set, it raises more concern that a point source such as an OSTDS likely was the cause of the "spike." As fecal coliform is an indicator of bacteria present in human waste, to have samples testing in the range 1720 and 2940 cfu/100 ml within groundwater away from OSTDS's, questions must be raised as to how the bacteria (which is not naturally occurring in the groundwater), was introduced. Having multiple samples testing with high levels raises more concern." Rainfall infiltration of soils in areas with high densities of septic systems and high-water tables can result in high fecal coliform values in groundwaters and storm drains so that stormwater runoff can carry high levels of fecal bacteria into surface waters. This was documented in the stormwater sampling analysis from the East and West Spring Lake Wastewater Pilot Program area in the FAU-Harbor Branch (2016) study. Fecal coliform values of the stormwater greatly exceeded the Florida and USEPA surface water standard, with mean values of 8,491 cfu/100ml (September 2015 to May 2016) and 11,033 cfu/100ml (September 2015), with maximum values of 48,000 cfu/100ml. This empirical evidence supports the conclusion of TetraTech (2013) that septic systems are linked to decreased water quality in the East & West Spring Lake area where test results showed a positive correlation between nutrients and bacterial loadings. This is consistent with the FAU-Harbor Branch (2016) conclusions that septic systems were a likely source contributing to fecal contamination in Charlotte Harbor. These conclusions align with a previous peer-reviewed study cited by Lapointe (2024) that concluded for microbial fecal pollution in northern Charlotte Harbor “sites within areas of high OSTDS density also tended to be more contaminated. This may be due to heavy loading of the systems and/or poor treatment of the effluent in the drainfield before reaching surface waters” (Lipp et al. 2001). A more intensive peer-reviewed study in nearby waters of Lee County found the human molecular marker HF183 in 50% of the surface water samples, which was positively correlated with enterococci, supporting the conclusion that septic systems were contributing to widespread contamination of surface waters with human waste. High levels of ammonium occurred in 55% of samples, fecal bacteria in 66% of the samples, and sucralose in 54% of the samples (Tyre et al. 2023).

Q. Was Dr. Robbins correct that there will be little environmental benefit from the estimated nitrogen load reduction from the proposed septic-to-sewer project compared to existing septic systems?

A. No, Dr. Robbins was incorrect. Conventional septic systems are not designed to remove nutrients like nitrogen and phosphorus. Their main function is on removing bacteria and solids and they only achieve limited removal of nutrients, even for for septic systems that are properly sited and maintained. The nitrogen load reduction estimate in Lapointe (2023) for the proposed barrier island project was based on information thought to be correct at the time. Based on new information, the “1,468 accounts” have been revised to 1,248 equivalent residential connections (ERCs) based on the most recent estimates by engineers and accountants. This new number would lower the expected nitrogen load reduction to 29,266 lbs per year. Because of the high-water tables, porous sandy soils with low contents of biologically available organic carbon content, and proximity to surface waters on the barrier islands, it is unlikely that nitrogen removal via denitrification would reduce much of this nitrogen load. Denitrification within a properly sited, designed, and operated conventional septic system is unlikely. Dr. Robbins was also incorrect in stating that existing Charlotte County wastewater treatment facilities "are not designed to remove nitrogen and phosphorus;" in fact, they do remove substantial amounts of these nutrients as noted in the FAU-Harbor Branch (2016) study. The mean total Kjeldahl nitrogen concentration of raw wastewater was 71.32 mg/L and total phosphorus (TP) was 6.87 mg/L, compared to treated effluent from the Charlotte County Eastport Water Reclamation Facility that had much lower concentrations of TN (13.3 mg/L) and TP (3.2 mg/L). However, current nutrient removal performance is not as high as the levels achieved with advanced wastewater treatment (AWT). Based on the CCUD 2023 Annual Report, design for expansion and upgrade to AWT (5:5:3:1) for the Rotonda WRF is already underway. CCUD intends to achieve AWT throughout its wastewater plants (including reuse water) to achieve the goals of House Bill 1379 (2023) by 2034 as directed by the Charlotte County Board of County Commissioners. So, by the time that the proposed barrier island septic-to-sewer project is completed, the diverted septic effluent will eventually receive AWT. Analysis and estimates like this are not for the immediate moment but rather for the long run at buildout, which will be years from now. This reduction in nitrogen loading will especially benefit the health of adjacent coastal waters surrounding the barrier islands that experience red tides and declining seagrass health. Similarly, TetraTech (2013) concluded for the Spring Lake area "numerous factors have been analyzed which have led to the conclusion that OSTDS's within East & West Spring Lake area are a contributor to elevated nutrient levels within adjoining water bodies, and hence, decreased water quality."

Q. Is it true that Lapointe (2024) described seagrass beds in Gasparilla Sound as "healthy" as Dr. Robbins claimed?

A. No. Lapointe (2024) described the seagrass beds in Gasparilla Sound as "some of the densest seagrass beds in the area," which was reported as such in the Charlotte Harbor National Estuary Program website summary of Seagrass in Gasparilla Sound/Cape Haze (CHNEP 2023). However, "dense" seagrass beds do not equate with "healthy" seagrass beds as Dr. Robbins implied, because dense seagrass beds can experience self-shading and light attenuation that results in low dissolved oxygen levels, which is exacerbated by eutrophication, algal blooms, and reduced light availability in the overlying water column.

Q. Can land based nutrient subsidies initially lead to dense seagrass beds and then followed by negative responses as disputed by Dr. Robbins?

A. Yes. It is well known in the seagrass literature that experimental nutrient enrichment can initially result in increased biomass and density of seagrasses because of nutrient limitation. Like all plants, seagrasses need nutrients to grow. However, continued nutrient enrichment can saturate growth demands of seagrasses and eventually result in negative effects from eutrophication such as algal blooms, reduced light, hypoxia, anoxia, and sulfide toxicity, resulting is seagrass decline and/or die-off. A published peer reviewed paper on this topic by Cabaco et al. (2013) concluded that "in general, shoot biomass of seagrasses increases with density, and nutrient enrichment enhances this effect." They also concluded that "the later, negative ones are mediated by whole ecosystem responses." These "whole ecosystem responses" include human nutrient pollution from fertilizers and human waste, which are well known to be a primary driving factor for seagrass decline in urbanized estuaries in Florida. See Cabaco et al. (2013) as Exhibit BEL-6

Q. Is it necessary to have a hypothesis to conduct scientific studies on septic systems as Dr. Robbins argues?

A. No. While hypothesis testing is appropriate for some scientific studies, it is not always required or the best approach. For example, some scientific studies are designed to explore a subject more thoroughly without a formal hypothesis. Some disciplines are entirely based on observations, and this does not make them obsolete or unscientific. Much of what we do in environmental science comes from observational research, such as water quality monitoring. The goal of these studies might be to make recommendations for future research, which was the case for the FAU-Harbor Branch (2016) study.

Q. Is the proposed septic-to-sewer project for the barrier islands misaligned with the sentiments of Charlotte County because of the lack of empirical evidence as claimed by Dr. Robbins?

A. No. The Charlotte County Board of County Commissioners passed Resolution # 2023-155 that strongly supported the septic-to-sewer conversion on the barrier islands. Despite the lack of a site-specific study on the barrier islands, it is reasonable to assume from the peer-reviewed scientific literature that the high densities of septic systems, shallow water tables, porous sandy soils and proximity to sensitive surface waters allow for pollution of groundwaters and nearby surface waters such as the impaired waters in Lemon Bay. Septic systems are a widespread and growing source of human waste pollution in Florida and have been recognized as such by Florida's Blue-Green Algae Task Force. The need to mitigate septic system pollution was officially recognized by the unanimous vote for passage of HB1379 in both the Florida House and Senate in 2023. The septic-to-sewer project for the barrier islands was identified as a priority in the Charlotte County Sewer Master Plan (2017). The opportunity for considerable State and Federal funding for septic-to-sewer projects currently exists and many communities in Florida have already secured millions of dollars in funding that make the cost to homeowners reasonable. It would be unfortunate if Charlotte County missed the opportunity for cost-sharing this major infrastructure upgrade for the barrier islands, as these funds may not be available in future years.

Q. Are you sponsoring any rebuttal exhibits?

A. Yes, I am sponsoring several exhibits. Cabaco et al., 2013, Brewton et al. 2022, Lapointe et al. 2023, IFAS Fertilizer Report, Tyre et al. 2022.

Q. Does that conclude your rebuttal testimony?

A. Yes, it does.

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Research Paper

Septic system–groundwater–surface water couplings in waterfront communities contribute to harmful algal blooms in Southwest Florida

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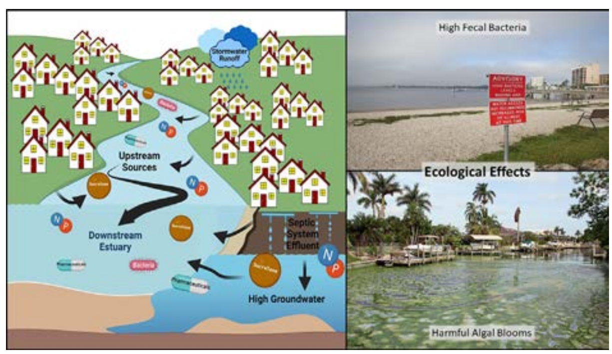
HIGHLIGHTS

- Groundwater is contaminated with nutrients and bacteria from septic systems.
- Septic systems do not function in high water tables.
- Surface waters are degraded due to human waste inputs.
- Sucralose was ubiquitous in groundwater and surface waters.
- Downstream harmful algal blooms may be mitigated through improved infrastructure.

GRAPHICAL ABSTRACT



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ABSTRACT

As human population growth has expanded in Southwest Florida, water quality has become degraded with an increased occurrence of harmful algal blooms (HABs). Red tide (*Karenia brevis*) originating offshore, intensifies in nearshore waters along Florida's Gulf Coast, and blue-green algae (*Microcystis* spp.) originating in Lake Okeechobee is discharged into the Caloosahatchee River. These HABs could be enhanced by anthropogenic nitrogen (N) and phosphorus (P) from adjacent watersheds. North Fort Myers is a heavily developed, low-lying city on the Caloosahatchee River Estuary serviced by septic systems with documented nutrient and bacterial pollution. To identify sources of pollution within North Fort Myers and determine connections with downstream HABs, this multiyear (2017–2020) study examined septic system- groundwater- surface water couplings through the analysis of water table depth, nutrients (N, P), fecal indicator bacteria (FIB), molecular markers (HF183, GFD, Gull2), chemical tracers (sucralose, pharmaceuticals, herbicides, pesticides), stable isotopes of groundwater (δ^{15}N-NH_4, δ^{15}N-NO_3) and particulate organic matter (POM; δ^{15}N, δ^{13}C), and POM elemental composition (C:N:P). POM samples were also collected during *K. brevis* and *Microcystis* spp. HAB events. Most (>80%) water table depth measurements were too shallow to support septic system functioning (<1.07 m). High concentrations of NH_4^+ and NO_x, up to 1094 μM and 482 μM respectively, were found in groundwater and surface water. δ^{15}N values of groundwater (+4.7‰) were similar to septic effluent (+4.9‰), POM (+4.7‰), and downstream HABs (+4.8 to 6.9‰), indicating a human waste N source. In surface water, FIB were elevated and HF183 was detected, while in groundwater and surface water sucralose, carbamazepine, primidone, and acetaminophen were detected. These data suggest that groundwater and surface water in North Fort Myers are coupled and contaminated by septic system effluent, which is negatively affecting water quality and contributing to the maintenance and intensification of downstream HABs.

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1. Introduction

Globally, coastal water quality degradation is an ongoing, evolving concern for urbanized watersheds with many ecological, economic, and public health implications (National Research Council, 2000). Excess nutrient inputs accelerate eutrophication, promote harmful algal blooms (HABs), and deplete dissolved oxygen (DO) in the water column, which can result in fish kills, habitat loss, and diminished water quality negatively impacting human health and the economy (Howarth et al., 2000). Many factors contribute to urban pollution, so there is high variability in water quality that is related to weather conditions, catchment rainfall, watershed characteristics, and drainage infrastructure (Walsh et al., 2005; Lapointe et al., 2012; Tran et al., 2015; Lapointe et al., 2017). These issues will be exacerbated by climate change, which is predicted to increase riverine nitrogen (N) loading by ~19% before the end of the century (Sinha et al., 2017).

Human waste contamination from onsite sewage treatment and disposal systems, commonly called "septic systems," can be a significant source of nutrient and microbial pollution to groundwater and surface water (Griffin et al., 1999; Lipp et al., 2001; Cahoon et al., 2006; Withers et al., 2011; Withers et al., 2014). As such, surface water contamination from septic system effluent has been observed in the coastal zone of all developed continents (Tuholske et al., 2021). In the United States, the state of Florida has ~2.67 million homes (~33%) serviced by septic systems for domestic waste management (Yang et al., 2016). Florida's coastal regions are particularly vulnerable to pollution from septic systems because of shallow water tables and porous soils that allow for rapid transport of contaminants to groundwater (Meeroff et al., 2008). Despite these poor conditions, Florida's coastal communities often contain high densities of septic systems (Flanagan et al., 2020) that can account for >50% of domestic waste disposal (Herren et al., 2021). As such, evidence of nutrient and bacterial pollution from septic system effluent contamination has been observed throughout the state (Lapointe et al., 1990; Lapointe and Krupa, 1995; Paul et al., 1995; Arnade, 1999; Corbett et al., 2000; Lipp et al., 2001; Bacchus and Barile, 2005; Lapointe and Bedford, 2007; Meeroff et al., 2008; Lapointe et al., 2015; Lapointe et al., 2017; Barile, 2018; Herren et al., 2021). Thus, source identification of nutrient and microbial pollution in Florida's coastal areas is necessary to determine the associated human health and environmental risks, as well as for the development of mitigation strategies.

Stable carbon (δ^{13}C) and N isotope (δ^{15}N) values are commonly used to identify nutrient pollution sources. For example, δ^{15}N values can help identify N sources, such as atmospheric deposition (<0‰), fertilizer (~−2 to +2‰), septic system effluent (+4.9‰) or processed human waste (>+3‰; Aravena et al., 1993; Costanzo et al., 2001; Kendall et al., 2007; Hinkle et al., 2008; Risk et al., 2009; Xue et al., 2009). Specifically, aqueous (dissolved) δ^{15}N from a water sample in the form of ammonium (δ^{15}N-NH_4^+) and nitrate (δ^{15}N-NO_3^-) can be used to distinguish between dissolved inorganic N (DIN) sources. Additionally, the δ^{15}N values of primary producers are often used to discriminate between natural and anthropogenic N sources (Costanzo et al., 2001; Cole et al., 2004; Lapointe et al., 2015). Finally, δ^{13}C values of plants can help identify carbon (C) sources because terrestrial C is more depleted (having lower values) than marine C, which is more enriched (Peterson and Fry, 1987).

Fecal indicator bacteria (FIB) are often used to assess watershed contamination, but this approach presents some challenges, including difficulty in discriminating between sources (e.g., human waste or environmental) and short survival times (Scott et al., 2002; Tran et al., 2015). Further, FIB are found in the feces of many animals and therefore provide no reliable indication regarding the source of fecal pollution where many potential non-point sources of fecal contaminants exist (Tran et al., 2015). Employing a suite of microbial source tracking tools can address these uncertainties and help to identify pollutant sources. For example, specific molecular markers can determine the source of bacterial contamination. HF183 is a molecular marker within the 16S rRNA genes of the *Bacteroides* species that reside in the human colon as part of the normal microbiota. Therefore, detection of HF183 in environmental waters serves as an indicator of human fecal pollution (Ahmed et al., 2008; Tran et al., 2015). BacR is a molecular marker within the 16S rRNA genes of *Bacteroides* species that reside in the digestive tract of most ruminants, such as deer, alpaca, cattle, llama, and goats. Thus, detection of BacR in environmental samples indicates ruminant fecal pollution (Reischer et al., 2006). Similarly, the avian marker GFD is an unclassified *Helicobacter* sp. and indicates the presence of many bird species, including gulls, goose, chicken, pigeon, egret, crow, etc. (Ahmed et al., 2016). Gull2 is associated with the bacteria species *Catellicoccus marimammalium* that is found in the feces of seagulls and other seabirds (Ryu et al., 2012).

Various chemical tracers can also help identify sources of nutrient and bacterial pollution. For example, the artificial sweetener sucralose, as well as certain pharmaceuticals, including the over the counter pain reliever acetaminophen and the prescription anticonvulsants carbamazepine and primidone are useful indicators of human waste contamination (Oppenheimer et al., 2011; Silvanima et al., 2018). Further, herbicide and pesticide chemical tracers can identify other sources of contamination to a waterbody, such as surficial runoff (Papadakis et al., 2018; Silvanima et al., 2018), which can contain nutrient and bacterial sources including pet waste, leaf litter, and grass clippings (Yang and Lusk, 2018; Krimsky et al., 2021).

A combination of the tools described above may be used to identify the sources of nutrient and bacterial pollution in urban areas with water quality concerns. Lee County, FL, is bisected by the Caloosahatchee River and Estuary, which historically was low in nutrients (Odum et al., 1955). However, the area has become highly developed since the 1950s (Fig. S1) and surface water in the Caloosahatchee River Estuary is now nutrient laden (Lapointe and Bedford, 2007; Vargo et al., 2008). Thus, some segments of the Caloosahatchee River Estuary are classified as impaired under Section 303(d) of the United States Clean Water Act of 1972 for nutrients, fecal coliforms, DO, and chlorophyll, while HABs are a recurring issue (see Fig. S2). For example, "red tide" blooms of the dinoflagellate *Karenia brevis* have become increasingly abundant, especially in nearshore environments (Brand and Compton, 2007), and have long been linked to nutrient enrichment from riverine inputs and estuarine flux (Slobodkin, 1953; Odum et al., 1955; Doig and Martin, 1974; Vargo et al., 2008; Yentsch et al., 2008; Medina et al., 2020; Medina et al., 2022), particularly during high flow years (Lapointe and Bedford, 2007; Heil et al., 2014). Additionally, beginning in 2003 red drift macroalgal HABs developed off the Lee County coast (Fig. S2) and were associated with increasing nutrient contributions from human waste, as well as rainfall and agricultural fertilizers (Lapointe and Bedford, 2007). Finally, extreme rainfall associated with hurricanes facilitated blooms of the freshwater blue-green alga *Microcystis aeruginosa* in the Caloosahatchee River Estuary and residential canals (Fig. S2) in 2005 (Lapointe et al., 2006), 2017, and 2018 (Glibert, 2020). Aside from the ecological and human health effects, these HABs can negatively impact local economies through the mortality of commercial seafood and by inhibiting ecotourism activities (Anderson et al., 2000). Despite these water quality issues, the population in Lee County continues to grow rapidly with a 24.5% increase from 2010 to 2019 (Fig. S1).

Within Lee County, the city of North Fort Myers has experienced degraded water quality over the last 30 years, including persistent nutrient and fecal bacterial pollution (W. Dexter Bender and Associates Inc., 1995). Therefore, to address the critical public health and water quality issues of nutrient and bacterial contamination in North Fort Myers, a multi-year microbial source tracking study was conducted to determine the sources of these impairments and assess connections with downstream HABs. Based on previous research, we hypothesized that effluent from septic systems was an important source of nutrient and bacterial pollution in North Fort Myers and thus location would be a more important factor for water quality than temporal factors, such as project year or season.

2. Materials and methods

2.1. Study area

Lee County encompasses 3139 km^2 and is bisected by the Caloosahatchee River and Estuary, which terminates into the Gulf of Mexico. The hydrology of the region has been highly modified over the last century with the conversion of the natural river into the C-43 canal and the connection to Lake Okeechobee via three lock-and-dam structures (S-77, S-78, and S-79) that allow the United States Army Corps of Engineers to control river flow with lake water discharges (Barnes, 2005). The unconfined water table aquifer in Lee County has high hydraulic conductivity with water flows from the topographic high to the southwest (Scott and Missimer, 2001). Rainfall is seasonally variable in subtropical Southwest Florida with wetter conditions from May to October (wet season) and drier conditions from November to April (dry season) and an average annual rainfall of ~155 cm (Liu et al., 2009), though variability is observed. Lee County Ordinance #08-08 limits fertilizer nutrient content and application rates and bans the application of fertilizers containing N and/or P during the wet season from June 1 to September 30.

There are ~39,768 "known" and ~57,054 "likely" septic systems in Lee County (~96,822 total estimated), however for many parcels there are no data available on domestic waste disposal (Florida Department of Health, 2020). Further, due to elevated seasonal high water tables (Arnade, 1999; Meeroff et al., 2008), many septic systems in Florida may not meet the state regulatory requirements. Septic systems require a minimum cover of 6″ over the drainfield, a drainfield depth of ~1′ (may be less in some soil types), and 2' of separation from the bottom of the drainfield to the high water table (FAC Rule 62E-6). Therefore, at least 3.5′ (1.07 m) of separation is needed from the ground surface to the water table to meet the minimum requirements. In some parcels with high water levels, the drainfield has been raised above the natural soil surface to help meet these requirements by adding additional separation between the initial effluent discharge and the groundwater ("mounding").

The urban areas of North Fort Myers were developed along natural creeks that flow into the tidal Caloosahatchee River Estuary that were modified by the addition of "finger" and drainage canals. Thus, North Fort Myers has three major drainage basins: Hancock Creek, Powell Creek, and a central drainage feature (Fig. 1). The primary land-use in North Fort Myers is residential with a high abundance of waterfront homes. The study area is serviced by an estimated 2164 septic systems (Fig. 1) and there is no application of reuse water.

In September 2017, ten shallow groundwater wells were installed using a hand auger to a depth of ~2.1 m. The wells were constructed from 2-in. (50.8 mm) diameter PVC with 1.5 m well screen and were sand packed between the well bore and casing to 0.31 m above the well screen with a 0.31 m thick bentonite cap on top of the sand pack. The groundwater monitoring wells were installed in Lee County right of way or at private residences where the owner consented. Nine wells were installed in high density, low elevation residential areas serviced by septic systems amended with finger canals in the drainage basins of Hancock Creek (GW1-GW3; 136 septic systems/km^2), a central drainage feature (GW4-GW6; 236 septic systems/km^2), and Powell Creek (GW7-GW9; 279 septic systems/km^2; Table 1). Unfortunately, the intensity of development in North Fort Myers prevented the inclusion of a completely "natural" location, so one "reference" groundwater well (GW10) was installed in a less densely developed upland area within the Hancock Creek watershed with no canals serviced by both sewer and septic systems (Fig. 1). Four of the ten surface water sites were along Powell Creek (SW1-SW4), one was in the central drainage feature (SW5), and five were along Hancock Creek (SW6-SW10; Fig. 1). The sites were freshwater with greater estuarine influence near the Caloosahatchee River Estuary.

2.2. Rainfall

Rainfall data over the study period (January 2017 to May 2020) was obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (https://www.ncdc.noaa.gov/data-access). Fort Myers station US1FLLE0037 was selected as the primary data source due to its proximity to the study area and high temporal coverage (92.6%). These data are supplemented with data from other nearby stations in Fort Myers (US1FLLE0055, 3.1%; USW00012835, 2.0%; US1FLLE0056, 0.1%; US1FLLE0039, 0.1%) and Cape Coral (US1FLLE0053, 2.2%) for days in which data at the primary station are missing. Sampling was conducted during wet (October 2017, November 2017, August 2019, and September 2019) and dry (February 2018, March 2018, February 2020, and March 2020) seasons that were determined based on rainfall and seasonal water table fluctuations.

2.3. Sample collection and analyses

Samples were collected four times during the wet season (October 17–18, 2017, November 14–15, 2017, August 27–28, 2019, and September 25–26, 2019) and four times during the dry season (February 13–14, 2018, March 13–14, 2018, February 10–11, 2020, and March 10–11, 2020). Groundwater and surface water sampling were conducted simultaneously by two teams: Florida Atlantic University-Harbor Branch Oceanographic Institute researchers and Lee County Environmental Laboratory (LCEL) staff. Additionally, approximately weekly throughout the study period and during sampling events, depth to water in the groundwater wells was measured with a clean 100 m Geotech Water Meter Reader. Environmental parameters of groundwater and surface water, including pH, salinity (ppt), conductivity (μS), temperature (°C), and DO (%, mg/L) were measured during sampling events using calibrated multiparameter probes.

For groundwater sampling, a peristaltic pump and clean Tygon tubing were used by LCEL staff to purge triple the well volume before collecting samples per Florida Department of Environmental Protection (FDEP) standard operating procedures (FS2200). After purging, triplicate water samples were collected into HDPE bottles to determine concentrations of ammonium (NH_4^+), nitrate + nitrite (NO_x), soluble reactive P (SRP), total N (TN), and total P (TP). Singular water samples were collected into high-density polyethylene (HDPE) bottles for determination of enterococci and *Escherichia coli* counts, 5-day biochemical oxygen demand (BOD) concentrations, and color. These samples were submerged in ice and delivered to LCEL, Fort Myers, FL for analyses. For detailed methods and method detection limits (MDLs), see the Supplemental Methods. Groundwater was also collected for determination of aqueous stable N isotope values of ammonium ($\delta^{15}N$-NH_4) and nitrate ($\delta^{15}N$-NO_3). These samples were collected into 1 L HDPE bottles and stored on ice until shipment to various labs for processing and stable isotope analyses (see Supplemental Methods).

Surface water samples were collected during outgoing tides and handled similarly to groundwater for determination of NH_4, NO_x, SRP, TN, TP, enterococci, *E. coli*, BOD, and color. Additionally, at surface water sites and during HAB events, particulate organic matter (POM) was collected as a proxy for phytoplankton (see Supplemental Methods). Additionally, macroalgae was collected at sites when present, rinsed briefly with DI water, dried, and ground into a homogenous powder. The POM filters and tissue were analyzed for $\delta^{13}C$ and $\delta^{15}N$, as well as elemental composition (%C, %N) on a Thermo Delta V Environmental Analysis – Isotope Ratio Mass Spectrometer coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo Conflo III Interface (see the following for methods: http://sisbl.uga.edu/ratio.html#top). In year 1, %P was analyzed at UGA following the methodology of Aspila et al. (1976) on a Technicon Autoanalyzer II with an IBM-compatible, Labtronics, Inc. DP500 software data collection system (D'Elia et al., 1997). In year 2, because UGA ceased to perform these services, %P samples were analyzed at the University of Missouri Soil and Plant Testing Laboratory by Inductively Coupled Plasma Atomic Emission Spectroscopy (Viso and Zachariadis, 2018). C:N:P data were compared to a modified Redfield ratio of 360:30:1 (Redfield, 1958) to characterize temporal and spatial variation in nutrient status. Additionally, surface water samples for determination of molecular markers were collected at each site into 500 mL HDPE bottles, stored on ice, and shipped overnight to the FDEP lab. At the FDEP lab, they were analyzed using qPCR

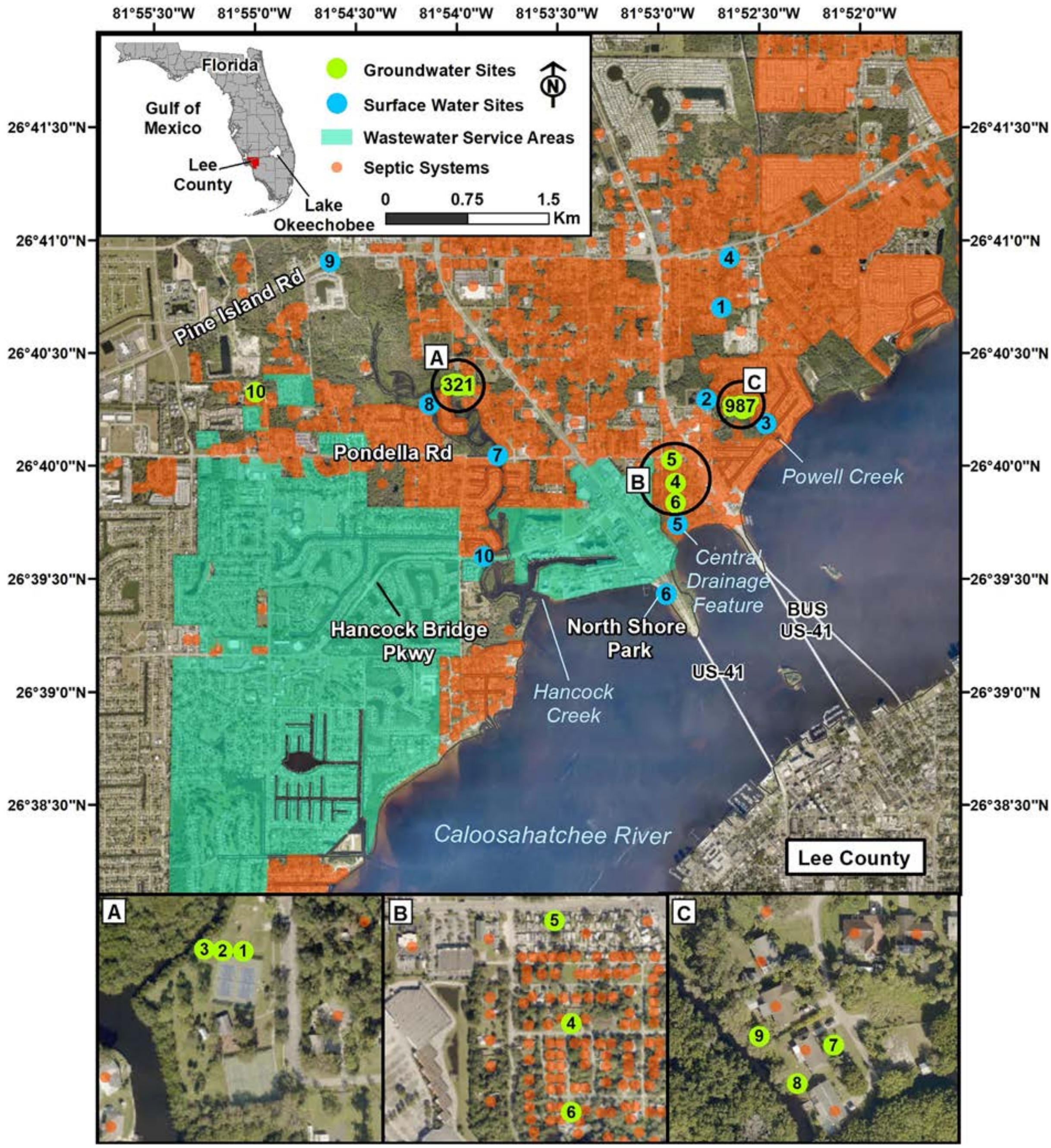

Fig. 1. Satellite imagery of the study area in North Fort Myers, FL, showing locations of groundwater wells (GW 1–10, green circles) in A) Hancock Creek drainage basin, B) the central drainage feature, and C) Powell Creek drainage basin, and surface water collection sites (SW 1–10, blue circles), as well as areas connected to centralized sewer for domestic waste disposal (teal shading) and parcels with septic systems (orange circles).

Table 1
Characteristics of watersheds within the North Fort Myers, FL study area, including total area (in km^2 and acre), septic system count, and number of septic systems per km^2 and acre. Estimated population using septic systems in each drainage basin is also shown using an average occupancy rate for the study area of 1.89 persons per unit based on Lee County census data.

Drainage basin	Area (km^2)	Area (acre)	Septic system count	Septic systems per km^2	Septic systems per acre	Estimated population using septic systems
Hancock Creek	5.22	1290	712	136	0.55	1346
Central drainage basin	2.54	628	599	236	0.95	1132
Powell Creek	2.63	650	735	279	1.13	1389
Overall	*10.39*	*2567*	*2046*	*197*	*0.80*	*3867*

to determine concentrations of the human marker HF183, the ruminant marker BacR, and the bird markers GFD and Gull2.

Finally, groundwater and surface water samples for analysis of chemical tracers were collected into amber glass bottles, stored on ice, and shipped overnight to the FDEP Laboratory, Tallahassee, FL. At the FDEP lab, samples were analyzed by high performance liquid chromatography, coupled with both thermospray-mass spectrometry and an ultraviolet detector (EPA method 8321B; see Supplemental Methods). Due to what was offered by the analytical lab, various chemicals were tested for during different sampling events. During all events, concentrations of the human waste tracers sucralose, carbamazepine, primidone, and acetaminophen were determined. The herbicides, 2,4-dichlorophenoxyacetic acid (2,4-D), bentazon, and triclopyr, as well as the psychoactive stimulant meta-Chlorophenylpiperazine (mCPP) were tested for in both seasons of year 1 (2017–2018). In the dry season of year 1 (2018), water samples were also analyzed to determine concentrations of an insecticide (imidacloprid), agricultural fungicide (pyraclostrobin), and other herbicides, including diuron, fenuron, fluridone, imazapyr, and linuron. Additional human waste tracers, ibuprofen, hydrocodone, and naproxen were also included in the suite of analytes during both seasons in year 2 (2019–2020).

2.4. Statistical analyses

For nutrient concentrations and bacterial counts, results flagged as below detection limits or less than the criterion of detection were substituted with a value equal to half the MDL for calculation of means, while original values reported by the lab were used in rank ordered, non-parametric analyses (Helsel, 2005). The following replacements were made for NH_4^+: one groundwater and 57 surface water samples and for NO_x: 148 groundwater and 61 surface water samples. For chemical tracers, any results below the MDL were considered as non-detects, per Silvanima et al. (2018) and replaced with zeros for data analyses. Estimated values and results flagged as between the MDL and the practical quantitation limit were included in data analyses (Helsel, 2005). To determine what factors were influential on water quality in the study area, parameters were compared between watersheds, project year (year 1, year 2), and season (dry, wet) with analysis of variance (ANOVA) if assumptions were met. The non-parametric Kruskal-Wallis test (groups of three or more, adjusted for ties) or Mann-Whitney *U* test (groups of two) were used if ANOVA assumptions were not met or if too many values (>15%) were below the MDL (USEPA, 2000; Helsel, 2005). Log transformation was attempted for all parameters having <15% non-detects before non-parametric statistics were employed. Significant main tests for ANOVA were followed by Tukey Honest Significant Difference test (HSD) and significant Kruskal-Wallis tests were followed by Dunn's test with a Bonferroni correction. Spearman's rank-order correlation was used to assess monotonic associations between groundwater and surface water variables. Only significant correlations with $r > 0.30$ are discussed in the Results and correlations between mathematically related variables (i.e., DIN and DIN:SRP) are not discussed. Analyses were conducted in SPSS 27, maps were made using ArcMap 10.8.1, and figures were created in GraphPad Prism 8. For all parameters and tests, differences were considered significant at $p < 0.05$ and data are presented as means with standard error (±S.E.) unless otherwise noted.

3. Results

3.1. Rainfall

All sampling events were conducted on days with little to no precipitation (Fig. 2). Mean annual rainfall in the study area ranged from a low of 1.24 m in 2018 to a high of 2.08 m in 2017. The most significant rainfall occurred from September 10–11, 2017, when Hurricane Irma passed through, contributing ~258 mm of precipitation over a two-day period. The cumulative rainfall ten days prior to the October 2017, November 2017, February 2018, and March 2018 sampling events was 18.54 mm, 5.33 mm, 22.86 mm, and 8.38 mm, respectively. The cumulative rainfall ten days prior to the August 2019, September 2019, February 2020, and March 2020 sampling events was 48.01 mm, 14.22 mm, 45.97 mm, and <0.01 mm, respectively.

3.2. Depth to water table

The depth to water table observed during the sampling events ranged from 0.19 to 2.08 m (Fig. 3) with an overall mean depth of 0.86 ± 0.04 m. During sampling events, depth to water table was variable by season (ANOVA, $F = _{1,\,64}$ 7.00, $p = 0.01$) and watershed (ANOVA, $F = _{3,\,64}$ 15.5, $p < 0.01$), but not project year (ANOVA, $F = _{1,\,64}$ 0.06, $p = 0.80$) with no significant interactions (all $p > 0.05$). By season, depth to water table was significantly shallower in the wet season (0.75 ± 0.06 m) than the dry season (0.96 ± 0.05 m). By watershed, depth to water table was significantly deeper at the reference well (GW10 = 1.29 ± 0.03 m) and in Hancock Creek watershed (1.06 ± 0.09 m) than in the central drainage watershed (0.70 ± 0.05 m) or Powell Creek (0.67 ± 0.04 m; Fig. 3). In the central drainage watershed and Powell Creek, 100% of the depth to water measurements recorded during sampling events were too shallow to

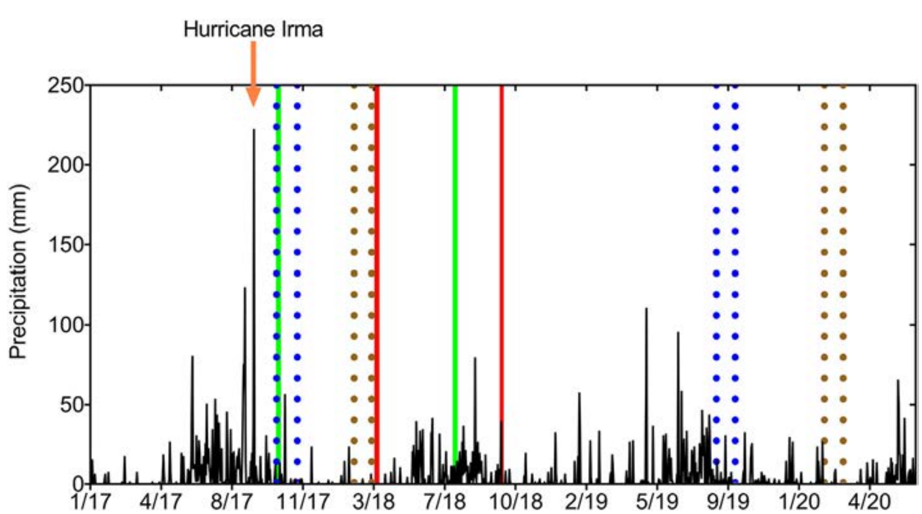

Fig. 2. Daily rainfall (mm) measured in North Fort Myers, FL from January 2017 through May 2020, showing sampling events conducted during the wet season (blue dotted lines), dry season (brown dotted lines), blue-green algae blooms (*Microcystis* spp.; green solid lines), and red tide blooms (*Karenia brevis*; red solid lines), as well as Hurricane Irma (orange arrow).

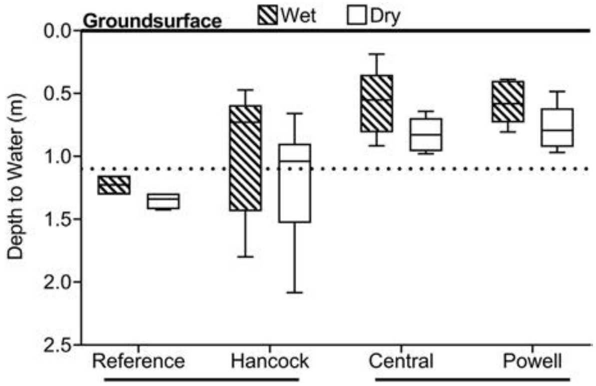

Fig. 3. Depth to water table measured in groundwater wells during sampling events by watershed (reference well, Hancock Creek, central drainage feature, and Powell Creek) and season (wet/dry); the dotted line indicates the approximate minimum separation required from the ground surface (at zero) to the water table required by FAC Rule 62E-6 (~1.07 m), values above this line indicate septic systems in this area may not be compliant with current requirements for new septic systems and do not have the separation needed to function properly. Lines below x-axis labels represent significant groupings.

support adequate treatment of effluent from a septic system without the addition of mounding (all <1.07 m). In Hancock Creek, 58% of depth to water measurements were too shallow and varied seasonally with 50% of dry season measurements <1.07 m and 67% of wet season measurements <1.07 m. In the reference well, 100% of depth to water measurements recorded during sampling events were >1.07 m (Fig. 3).

3.3. Nutrient concentrations

NH_4^+ concentrations were significantly higher in groundwater (153.6 ± 15 μM) than in surface water (2.82 ± 0.22 μM; Mann-Whitney *U* test, U = 1812, n = 478, p < 0.001). In groundwater, NH_4^+ concentrations ranged from below detection to 482 μM and were variable by watershed (Kruskal-Wallis test, H = 173, n = 238, df = 3, p < 0.001) and project year (Mann-Whitney *U* test, U = 5160, n = 238, p < 0.001), but not season (Mann-Whitney *U* test, U = 7786, n = 238, p = 0.184). Powell Creek groundwater had a significantly higher NH_4^+ concentration (395 ± 38 μM) than the other watersheds, while the central drainage watershed (97.0 ± 3.3 μM) was significantly higher than Hancock Creek watershed (20.8 ± 1.5 μM) and the reference well (17.4 ± 0.7 μM; Fig. 4). Groundwater NH_4^+ concentrations were significantly higher in year 1 (208 ± 26 μM) than in year 2 (98.3 ± 14 μM).

In surface water, NH_4^+ concentrations ranged from below detection to 21.1 μM and were variable by watershed (Kruskal-Wallis test, H = 36.5, n = 240, df = 2, p < 0.001) and season (Mann-Whitney *U* test, U = 9883, n = 240, p < 0.001), but not project year (Mann-Whitney U test, U = 6204, n = 240, p = 0.062). The central drainage feature (8.27 ± 1.6 μM) and Powell Creek (2.73 ± 0.14 μM) had significantly higher NH_4^+ concentrations than Hancock Creek (1.80 ± 0.13 μM; Fig. 4). Surface water NH_4^+ concentrations in the wet season were significantly higher (3.78 ± 0.04 μM) than the dry season (1.85 ± 0.13 μM).

NO_x concentrations were significantly higher in groundwater (23.7 ± 5.1 μM) than in surface water (3.97 ± 0.26 μM; Mann-Whitney *U* test, U = 40,185, n = 478, p < 0.001). In groundwater NO_x concentrations ranged from 0.36 to 482 μM and were variable by watershed (Kruskal-Wallis test, H = 51.9, n = 238, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 6339, n = 238, p = 0.094) or project year (Mann-Whitney *U* test, U = 7623, n = 238, p = 0.221). Groundwater in the Hancock Creek watershed had significantly higher concentrations of NO_x (76.9 ± 15 μM) than the other watersheds, which were similar (all <0.90 μM; Fig. 4).

In surface water, NOx concentrations ranged from 0.36 to 19.6 μM and were variable by watershed (Kruskal-Wallis test, H = 27.5, n = 240, df = 2, p < 0.001) and season (Mann-Whitney *U* test, U = 11,104, n = 240, p < 0.001), but not project year (Mann-Whitney *U* test, U = 6203, n = 240, p = 0.061). Powell Creek had significantly higher surface water NO_x concentrations (4.60 ± 0.26 μM) than Hancock Creek (3.31 + 0.42 μM), while the central drainage feature had the highest average NO_x concentrations with high variability (4.74 ± 1.1 μM; Fig. 4). Wet season surface water NO_x concentrations were significantly higher (5.81 ± 0.42 μM) than in the dry season (2.13 ± 0.20 μM).

DIN concentrations were higher in groundwater (177 ± 15 μM) than surface water (6.79 ± 0.41 μM; Mann-Whitney *U* test, U = 983, n = 478, p < 0.001). Groundwater DIN concentrations were variable by watershed (Kruskal-Wallis test, H = 111, n = 238, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 6830, n = 238, p = 0.637) or project year (Mann-Whitney *U* test, U = 6321, n = 238, p = 0.153). The groundwater DIN concentrations of all watersheds were significantly different from each other (Fig. 4). The highest groundwater DIN was observed in the Powell Creek watershed (396 ± 38 μM), followed by Hancock Creek watershed (97.7 ± 15 μM), the central drainage watershed (97.6 ± 3.3 μM), and the reference well (17. 9 ± 0.64 μM; Fig. 4).

Surface water DIN concentrations were variable by watershed (Kruskal-Wallis test, H = 30.9, n = 240, df = 2, p < 0.001), season (Mann-Whitney

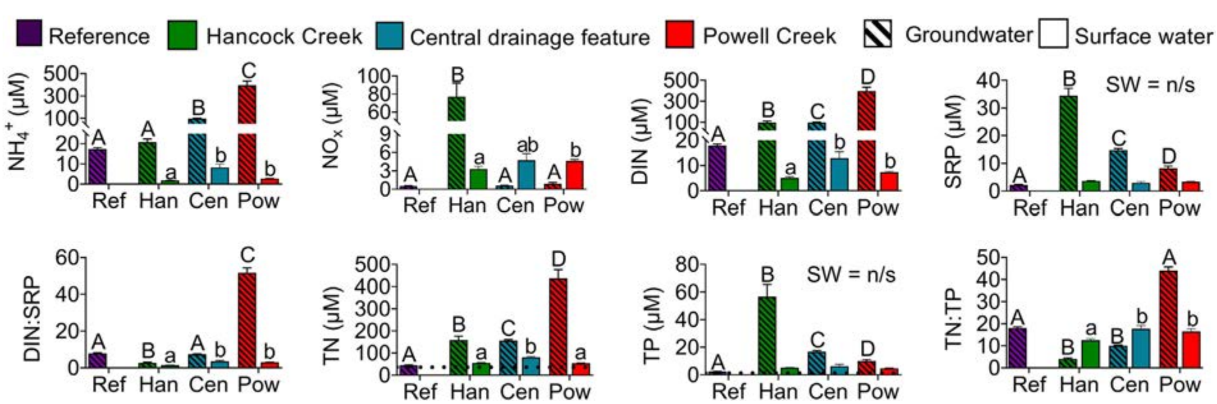

Fig. 4. Nutrient concentrations and molar ratios (mean ± SE) of groundwater (striped) and surface water (no pattern) in North Fort Myers, FL by watershed (reference, Hancock Creek, central drainage feature, and Powell Creek) including ammonium (NH_4^+), nitrate + nitrite (NO_x), dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), the molar ratio of DIN:SRP, total nitrogen (TN) with a black dotted line indicating the Florida Department of Environmental Protection (FDEP) surface water target concentrations for the Lower Caloosahatchee Tidal Segments 1 and 2 (<35.7 μM), total phosphorus (TP) with a black dotted line indicating the FDEP surface water standard for the Lower Caloosahatchee (<1.29 μM), and the molar ratio of TN:TP. Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison.

U test, U = 10985, n = 240, p < 0.001), and project year (Mann-Whitney *U* test, U = 6053, n = 240, p = 0.033). Surface water DIN concentrations were significantly higher in the central drainage feature (13.0 ± 2.6 μM) and Powell Creek (7.33 ± 0.35 μM), than in Hancock Creek (5.11 ± 0.49 μM; Fig. 4). Surface water DIN concentrations were higher in the wet season (9.59 ± 0.68 μM) than in the dry season (3.99 ± 0.26 μM), while year 1 (7.63 ± 0.60 μM) was significantly higher than year 2 (5.94 ± 0.54 μM).

SRP concentrations were significantly higher in groundwater (17.5 ± 1.2 μM) than in surface water (3.47 ± 0.11 μM; Mann-Whitney *U* test, U = 9591, n = 478, p < 0.001). In groundwater, SRP concentrations ranged from 0.97 to 96.7 μM and were variable by watershed (Kruskal-Wallis test, H = 130, n = 238, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 7267, n = 238, p = 0.725) or project year (Mann-Whitney *U* test, U = 7746, n = 238, p = 0.210). SRP concentrations of groundwater by watershed were all significantly different (Fig. 4). The highest groundwater SRP concentration was in Hancock Creek watershed (34.4 ± 2.7 μM), followed by the central drainage watershed (14.8 ± 0.53 μM), Powell Creek watershed (8.20 ± 0.83 μM), and the reference well (2.27 ± 0.06 μM).

In surface water, SRP concentrations ranged from 0.45 to 7.65 μM and were variable by watershed (Kruskal-Wallis test, H = 6.80, n = 240, df = 2, p = 0.033), season (Mann-Whitney *U* test, U = 5033, n = 240, p < 0.001), and project year (Mann-Whitney *U* test, U = 5439, n = 240, p < 0.001). Multiple comparisons did not find significant differences in SRP concentrations by watershed (Dunn's test, all p > 0.05; Fig. 4), however the highest surface water SRP concentrations were in Hancock Creek (3.66 ± 0.14 μM), followed by Powell Creek (3.35 ± 0.19 μM), and the central drainage feature (3.00 ± 0.37 μM). Dry season surface water SRP concentrations were significantly higher (3.96 ± 0.16 μM) than in the wet season (2.99 ± 0.14 μM), while SRP concentrations were significantly higher in year 1 (3.84 ± 0.16 μM) than year 2 (3.10 ± 0.15 μM).

DIN:SRP were significantly higher in groundwater (19.0 ± 1.6) than in surface water (2.31 ± 0.13; Mann-Whitney *U* test, U = 10,000, n = 478, p < 0.001). Groundwater DIN:SRP ranged from 0.30 to 106 and were variable by watershed (Kruskal-Wallis test, H = 180, n = 238, df = 3, p < 0.001), but not project year (Mann-Whitney *U* test, U = 6,161, n = 238, p = 0.084) or season (Mann-Whitney *U* test, U = 6715, n = 238, p = 0.492). The highest groundwater DIN:SRP was in the Powell Creek watershed (51.6 ± 2.7), followed by the reference well (7.94 ± 0.30) and central drainage watershed (7.34 ± 0.37), which were similar, and then Hancock Creek watershed (2.80 ± 0.32; Fig. 4).

Surface water DIN:SRP were generally low (<5) for the North Fort Myers area (Fig. 4). DIN:SRP of surface water ranged from 0.17 to 8.57 and were variable by watershed (Kruskal-Wallis test, H = 45.5, n = 240, df = 2, p < 0.001) and season (Mann-Whitney *U* test, U = 12,343, n = 240, p < 0.001), but not project year (Mann-Whitney *U* test, U = 7293, n = 240, p = 0.863). Surface water DIN:SRP were significantly lower in Hancock Creek (1.48 ± 0.15), than in the central drainage basin (3.57 ± 0.49) or Powell Creek (3.04 ± 0.20; Fig. 4). Wet season surface water DIN:SRP water was higher (3.58 ± 0.20) than dry season (1.05 ± 0.07).

TN concentrations were significantly higher in groundwater (228 ± 16 μM) than in surface water (57.3 ± 1.2 μM; Mann-Whitney *U* test, U = 7822, n = 478, p < 0.001). In groundwater, TN concentrations ranged from 34.3 to 1143 μM and were variable by watershed (Kruskal-Wallis test, H = 98.8, n = 238, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 7304, n = 238, p = 0.673) or project year (Mann-Whitney *U* test, U = 6530, n = 238, p = 0.300). Groundwater TN concentrations of all watersheds were significantly different from each other (Fig. 4) with the highest in the Powell Creek watershed (437 ± 39 μM), followed by Hancock Creek watershed (159 ± 17 μM), the central drainage watershed (156 ± 4.5 μM), and the reference well (44.4 ± 1.6 μM).

Surface water TN often exceeded the FDEP target concentrations for the Lower Caloosahatchee Tidal Segments 1 and 2 of <0.5 mg/L (~35.7 μM) throughout the study area with a range of 17.9 to 114 μM. TN concentrations of surface water were variable by watershed (Kruskal-Wallis test, H = 34.0, n = 240, df = 2, p < 0.001), season (Mann-Whitney *U* test, U = 10,197, n = 240, p < 0.001), and project year (Mann-Whitney *U* test, U = 9155, n = 240, p < 0.001). By drainage basin, surface water TN concentrations were significantly higher in the central drainage feature (80.7 ± 3.7 μM) than in Hancock Creek (55.4 ± 1.5 μM) or Powell Creek (53.7 ± 1.8 μM; Fig. 4). Surface water TN concentrations were significantly higher in the wet season (64.3 ± 1.7 μM) than the dry season (50.3 ± 1.5 μM) and were significantly higher in year 2 (61.3 + 1.7 μM) than year 1 (53.3 ± 1.7 μM).

TP concentrations were significantly higher in groundwater (25.5 ± 3.0 μM) than in surface water (5.14 ± 0.19 μM; Mann-Whitney *U* test, U = 10,329, n = 478, p < 0.001). In groundwater, TP concentrations ranged from 1.65 to 549 μM and were variable by watershed (Kruskal-Wallis test, H = 145, n = 238, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 6693, n = 238, p = 0.466) or project year (Mann-Whitney *U* test, U = 1.09, n = 238, p = 0.297; Fig. 4). By watershed, groundwater TP concentrations were all significantly different (Fig. 4) with the highest groundwater TP concentration in the Hancock Creek watershed (56.7 ± 8.8 μM), followed by the central drainage watershed (16.8 ± 0.86 μM), Powell Creek watershed (10.1 ± 0.89 μM), and the reference well (2.49 ± 0.09 μM; Fig. 4).

Surface water TP concentrations exceeded the FDEP estuary-specific Numeric Interpretations of the Narrative Nutrient Criterion (F.S. Chapter 62-302) water quality standard for the Lower Caloosahatchee of <0.04 mg/L (~1.29 μM) throughout the study area with a range of 1.39 to 35.5 μM. TP concentrations were variable by season (Mann-Whitney *U* test, U = 4580, n = 240, p < 0.001) and project year (Mann-Whitney *U* test, U = 4766, n = 240, p < 0.001), but not by watershed (Kruskal-Wallis test, H = 5.28, n = 240, df = 2, p = 0.071). Surface water TP concentrations were highest in the central drainage feature (6.25 ± 1.3 μM), followed by Hancock Creek (5.21 ± 0.18 μM), and Powell Creek (4.79 ± 0.25 μM; Fig. 4). Surface water TP concentrations were significantly higher in the dry season (5.97 ± 0.33 μM) than the wet season (4.31 ± 0.16 μM) and were significantly higher in year 1 (5.59 ± 0.18 μM) than year 2 (4.70 ± 0.33 μM).

TN:TP of groundwater (19.0 ± 1.2) were not significantly different from surface water TN:TP (14.6 ± 0.64; Mann-Whitney *U* test, U = 28,834, n = 478, p = 0.856). In groundwater, TN:TP ranged from 0.37 to 76.0 μM and were variable by watershed (Kruskal-Wallis test, H = 197, n = 238, df = 3, p < 0.001) and project year (Mann-Whitney *U* test, U = 5952, n = 238, p < 0.034), but not season (Mann-Whitney *U* test, U = 7264, n = 238, p = 0.730). The highest TN:TP was observed in the Powell Creek watershed (44.0 ± 1.8) and the reference well (18.0 ± 0.6), which were significantly higher than the central drainage (10.0 ± 0.3) and Hancock Creek (4.11 ± 0.38) watersheds (Fig. 4). Year 1 groundwater TN:TP were significantly higher (21.3 ± 1.8) than year 2 (16.7 ± 1.6; Fig. 4).

TN:TP of surface water ranged from 2.21 to 50.1 and were variable by watershed (Kruskal-Wallis test, H = 9.21, n = 240, df = 2, p = 0.010), season (Mann-Whitney *U* test, U = 10,442, n = 240, p < 0.001), and project year (Mann-Whitney *U* test, U = 9676, n = 240, p < 0.001). Surface water TN:TP in Hancock Creek (12.6 ± 0.64) were significantly lower than either Powell Creek (16.4 ± 1.3) or the central drainage feature (17.7 ± 1.5; Fig. 4). Surface water TN:TP were significantly higher in the wet season (18.5 ± 1.0) than the dry season (10.8 ± 0.6), while year 2 (17.9 ± 1.1) was significantly higher than year 1 (11.3 ± 0.60).

3.4. Stable isotopes and C:N:P

δ^{15}N-NH_4^+ values of groundwater in North Fort Myers were generally enriched (Fig. 5a) with a mean of +4.06 ± 0.4‰ and range of −5.72 to +12.1‰. Groundwater δ^{15}N-NH_4^+ values were variable by project year (ANOVA, $F = _{1,\,62}$ 17.6, p < 0.001) and watershed (ANOVA, $F = _{3,\,62}$ 5.74, p = 0.002), but not season (ANOVA, $F = _{1,\,64}$ 3.00, p = 0.088). There was also a significant interaction between project year and watershed (ANOVA, $F = _{3,\,64}$ 2.97, p = 0.039), but no other interactions were

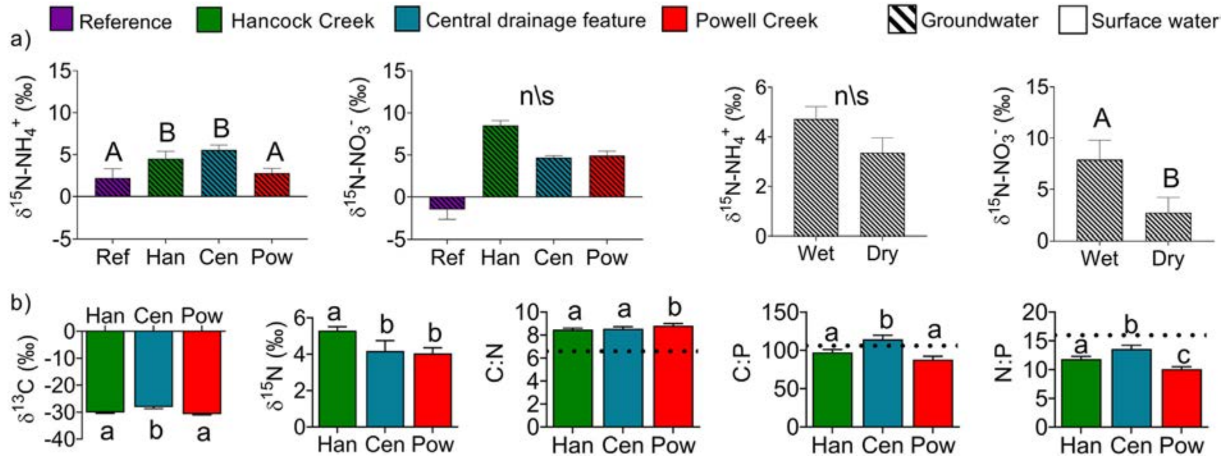

Fig. 5. Stable isotope values and elemental composition (mean ± SE) from North Fort Myers, FL including a) ammonium (δ^{15}N-NH$_4^+$) and nitrate (δ^{15}N-NO$_3^-$) isotopes of groundwater by watershed (reference well, Hancock Creek, central drainage watershed, and Powell Creek) and season (wet/dry), b) as well as carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes and molar C:N:P of particulate organic matter (POM), a proxy for phytoplankton, collected from surface water sites by watershed; dotted lines indicate shifts in nutrient limitation where C:N ratios >6.6 indicate increasing N-limitation, C:P ratios >106 indicate increasing P-limitation, and N:P ratios >16 indicate increasing P-limitation (Atkinson and Smith, 1983; Lapointe, 1987; Lapointe et al., 2015). Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison.

significant (ANOVA, all $p > 0.050$). Multiple comparisons between project years did not reveal significant differences between year 1 (+5.57 ± 0.5‰) and year 2 (+2.62 ± 0.5‰; Tukey HSD pairwise comparison, $p > 0.05$). Multiple comparisons by watershed revealed that groundwater δ^{15}N-NH$_4^+$ values were significantly higher in the central drainage watershed (+5.54 ± 0.6‰) and Hancock Creek watershed (+4.51 ± 0.9‰), than in Powell Creek watershed (+2.77 ± 0.6‰) or the reference well (+2.20 ± 1.1‰; Fig. 5a). In year 2, Powell Creek watershed had significantly higher δ^{15}N-NH$_4^+$ values (+2.51 ± 1.1‰) than the reference well (+0.98 ± 1.1‰). Interestingly, in year 2 the central drainage watershed had the highest average groundwater δ^{15}N-NH$_4^+$ values (+3.92 ± 0.6‰), but due to the high variability observed (range from −0.75 to 5.96‰), the difference was not significant. Similarly, in year 2 Hancock Creek had a large groundwater δ^{15}N-NH$_4^+$ range (from −5.72 to +6.74‰), which lowered the watershed mean in that year (+1.99 ± 1.0‰). The reference well had the tightest range in year 2 (from −1.08 to +3.06‰).

Aqueous δ^{15}N-NO$_3^-$ values of groundwater in North Fort Myers were variable with a mean of +5.37 ± 1.2‰ and range of −10.5 to +44.1‰. Groundwater δ^{15}N-NO$_3^-$ values were variable by season (ANOVA, $F = {}_{1,\,58}\ 6.16$, $p = 0.016$), but not by project year (ANOVA, $F = {}_{1,\,58}\ 0.017$, $p = 0.896$) or watershed (ANOVA, $F = {}_{3,\,58}\ 1.95$, $p = 0.131$). The interaction of project year and season was significant (ANOVA, $F = {}_{3,\,58}\ 14.7$, $p < 0.001$). Wet season groundwater δ^{15}N-NO$_3^-$ values (+7.89 ± 1.9‰) were significantly higher than dry season values (+2.71 ± 1.5‰; Fig. 5a). While watershed was not a significant factor, δ^{15}N-NO$_3^-$ values of groundwater were highest in Hancock Creek watershed (+8.49 ± 2.7‰), followed by Powell Creek watershed (+4.90 ± 2.6‰), the central drainage watershed (+4.65 ± 1.2‰), and the reference well (−1.52 ± 2.8‰; Fig. 5a). The interaction of project year and season revealed that groundwater δ^{15}N-NO$_3^-$ values from the year 2 wet season (+14.3 ± 2.9‰) were significantly higher than the year 1 wet season (+0.82 ± 0.6‰) and the year 2 dry season (+0.08 ± 1.8‰), while the year 1 dry season was not significantly different from any sampling events (+6.00 ± 2.2‰).

Surface water POM δ^{13}C values were generally depleted throughout the study area (Fig. 5b) with values ranging from −38.3 to −24.8‰ and an overall mean of −30.2 ± 0.19‰. POM δ^{13}C values were variable by project year (Mann-Whitney *U* test, $U = 10{,}355$, $n = 240$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 15.9$, $n = 240$, $p < 0.001$), but not season (Mann-Whitney *U* test, $U = 7.189$, $n = 240$, $p = 0.983$). Surface water POM δ^{13}C values were significantly higher in year 2 (−29.0 ± 0.19‰) than in year 1 (−31.3 ± 0.28‰). By watershed, the central drainage feature had significantly more enriched POM δ^{13}C values (−28.1 ± 0.56‰), than Hancock Creek (−30.1 ± 0.25‰) or Powell Creek (−30.7 ± 0.29‰).

Surface water POM δ^{15}N values were generally enriched (>+3‰) throughout the study area with values ranging from −8.45 to +11.9‰ and an overall mean of +4.69 ± 0.18‰. POM δ^{15}N values were variable by season (Mann-Whitney *U* test, $U = 5117$ $n = 239$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 10.3$, $n = 239$, $p = 0.006$), but not project year (Mann-Whitney *U* test, $U = 7306$, $n = 240$, $p = 0.756$). The dry season had significantly higher surface water POM δ^{15}N values (+5.28 ± 0.22‰) than the wet season (+4.10 ± 0.27‰). Surface water POM δ^{15}N values were significantly higher in Hancock Creek (+5.30 ± 0.22‰), than the central drainage feature (+4.18 ± 0.57‰) and Powell Creek (+4.05 ± 0.31‰; Fig. 5b).

Surface water POM C:N ratios ranged from 0.96 to 13.7 with an overall mean of 8.63 ± 0.09. POM C:N ratios were variable by project year (Mann-Whitney *U* test, $U = 5222$, $n = 240$, $p < 0.001$), season (Mann-Whitney *U* test, $U = 9250$, $n = 240$, $p < 0.001$), and watershed (Kruskal-Wallis test, $H = 8.21$, $n = 240$, $p = 0.016$). POM C:N ratios were significantly higher in year 1 (8.83 ± 0.11) than in year 2 (8.43 ± 0.15). Wet season POM C:N ratios (9.04 ± 0.14) were significantly higher than in the dry season (8.22 ± 0.11). Powell Creek POM C:N ratios (8.83 ± 0.17) were significantly higher than the central drainage feature (8.54 ± 0.19) and Hancock Creek (8.49 ± 0.12; Fig. 5b).

Surface water POM C:P ratios ranged from 21.9 to 221 with an overall mean of 95.5 ± 2.5. The POM C:P ratios were variable by project year (Mann-Whitney *U* test, $U = 10{,}371$, $n = 240$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 13.5$, $n = 240$, $p = 0.001$), but not season (Mann-Whitney *U* test, $U = 7690$, $n = 240$, $p = 0.362$). Surface water POM C:P ratios were significantly higher in year 2 (110 ± 0.38) than year 1 (81.1 ± 2.9). The central drainage feature had significantly higher POM C:P ratios (115 ± 5.0) than Hancock Creek (97.7 ± 3.6) and Powell Creek (88.0 ± 4.2; Fig. 5b).

Surface water POM N:P ratios ranged from 2.05 to 26.6 with an overall mean of 11.3 ± 0.31. POM N:P ratios were variable by project year (Mann-Whitney *U* test, $U = 10{,}758$, $n = 240$, $p < 0.001$) and watershed (Kruskal-Wallis test, $H = 15.1$, $n = 240$, $p = 0.001$), but not season (Mann-Whitney *U* test, $U = 6983$, $n = 240$, $p = 0.687$). POM N:P ratios were significantly higher in year 2 (13.3 ± 0.44) than year 1 (9.36 ± 0.35). All watersheds had significantly different POM N:P ratios (Fig. 5c). The central drainage feature had the highest POM N:P ratio (13.6 ± 0.65), followed by Hancock Creek (11.8 ± 0.47), and Powell Creek (10.19 ± 0.44; Fig. 5b).

Green macroalgae in the order Ulotrichales were collected at SW6 (North Shore Park) on October 17, 2017 and March 14, 2018 (unidentifiable to genus). No other sites had macroalgae present and/or accessible during sampling events. In October 2017, the $\delta^{13}C$ and $\delta^{15}N$ values for macroalgae were − 18.3 ± 0.24‰ and + 7.74 ± 0.09‰, respectively. In March 2018, the $\delta^{13}C$ and $\delta^{15}N$ values were − 29.7 ± 0.03‰ and + 5.01 ± 0.06‰, respectively. Overall, the $\delta^{13}C$ and $\delta^{15}N$ values for green macroalgae collected from SW6 were − 24.0 ± 2.54‰ and + 6.38 ± 0.61‰, respectively.

Microcystis spp. POM samples collected from Davis Boat Ramp on October 19, 2017, had depleted $\delta^{13}C$ values similar to those from the study area (−29.9 ± 0.19‰) and enriched $\delta^{15}N$ values within the range of human waste (+6.93 ± 0.81‰; Fig. 6a, b). Further, *Microcystis* spp. POM samples collected during a bloom on July 19, 2018, at North Shore Park (SW6) also had depleted $\delta^{13}C$ values (−28.8 ± 0.02‰) and enriched $\delta^{15}N$ values (+8.79 ± 0.04‰; Fig. 6a, b). Another localized *Microcystis* spp. bloom ~12 km downstream from the study area in the Caloosahatchee River Estuary was sampled on July 19, 2018, from a residential finger canal near Normandy Court in Cape Coral, FL. This very high biomass HAB event (see Fig. S2b) had $\delta^{13}C$ values that were slightly higher than the study area (−26.5 ± 0.08‰) and enriched $\delta^{15}N$ values (+7.42 ± 0.04‰).

Karenia brevis bloom POM samples collected in coastal areas of Lee County during 2018 had more enriched $\delta^{13}C$ values (−15.9 ± 1.2‰) than the samples collected in the study area (−30.2 ± 0.19‰), while the $\delta^{15}N$ values were similar (+4.94 ± 0.24‰ vs. 4.69 ± 0.18%, respectively; Fig. 6a,c). The *K. brevis* POM samples collected from several beaches on March 14, 2018, had more enriched $\delta^{13}C$ values (−11.3 ± 1.7‰) and lower $\delta^{15}N$ values (+3.85 ± 0.23‰) than those collected from Lighthouse Beach Park, Sanibel, FL, on September 29, 2018, which had more depleted $\delta^{13}C$ values (−18.7 ± 0.57‰) and more enriched $\delta^{15}N$ values (+5.60 ± 0.11‰). On March 14, 2018, *K. brevis* POM samples from Lighthouse Beach had more enriched $\delta^{13}C$ values (−9.83‰) and more depleted $\delta^{15}N$ values (+3.33‰) than those collected on September 29, 2018, which had more depleted $\delta^{13}C$ values (−18.7 ± 0.57‰) and more enriched $\delta^{15}N$ values (+5.60 ± 0.11‰; Fig. 6a,c).

3.5. Bacterial abundance

Enterococci counts were significantly lower in groundwater (39.2 ± 30 MPN/100 mL) than in surface water (594 ± 88 MPN/100 mL; Mann-Whitney *U* test, U = 6163, n = 160, p < 0.001). Groundwater enterococci

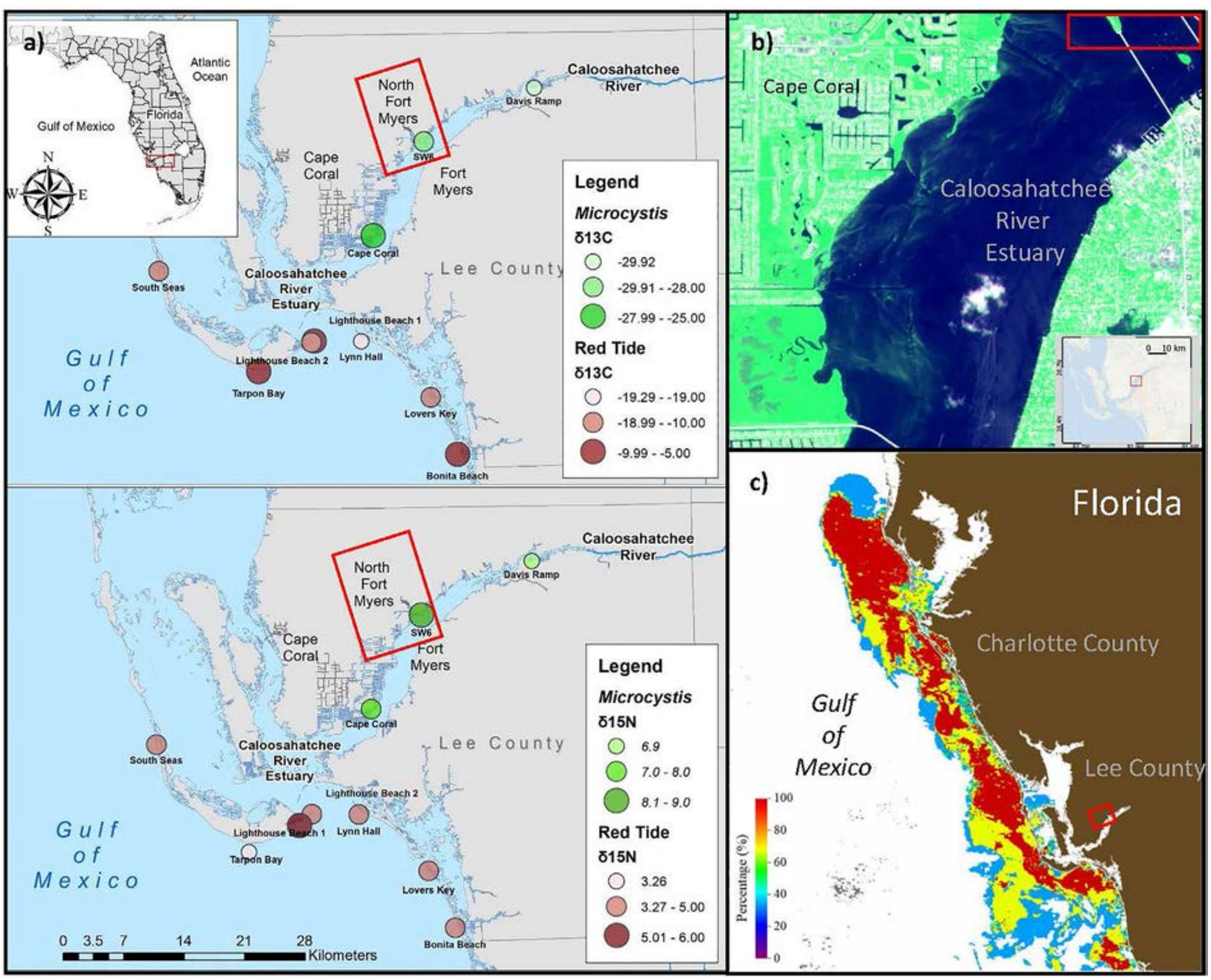

Fig. 6. Harmful algal bloom event sampling locations (dots) in Lee County, FL for blue-green algae, primarily *Microcystis* spp., (green) and red tide (*Karenia brevis*; red), a) showing average (n = 3; Davis Ramp, SW6, Cape Coral, Lighthouse Beach 2) or single sample values (Bonita Beach, Lovers Key, Lynn Hall, Lighthouse Beach 1, Tarpon Bay, and South Seas) by location for $\delta^{13}C$ and $\delta^{15}N$. b) A false-color Red-Green-Blue image from the PlanetScope DOVE on July 11, 2018, showing surface cyanobacterial scums in the Caloosahatchee River Estuary. In this image, clouds appear white, land appears green, water appears blue, and cyanobacterial scums appear greenish over the water background. and c) Frequency of red tide observations between September 24–30, 2018, estimated from satellite and field observations. Here, "red tide" is defined as *K. brevis* concentrations >10^5 cells/L. Panels b and c courtesy of Chuanmin Hu. The approximate North Fort Myers study area is denoted in each panel by a red box.

counts ranged from <0.5 to >2420 MPN/100 mL and were not significantly different by watershed (Kruskal-Wallis test, $H = 2.28$, $n = 80$, $df = 3$, $p = 0.516$), season (Mann-Whitney *U* test, $U = 0.03$, $n = 80$, $p = 0.862$), or project year (Mann-Whitney *U* test, $U = 0.27$, $n = 80$, $p = 0.603$). Groundwater enterococci counts were highest in the Powell Creek watershed (114 ± 100 MPN/100 mL), followed by the Hancock Creek (11.0 ± 8.1 MPN/100 mL), central drainage (5.3 ± 2.3 MPN/100 mL), and reference (2.3 ± 0.69 MPN/100 mL) watersheds (Fig. 7a).

Surface water enterococci counts ranged from <5 to >2420 MPN/100 mL and were variable by watershed (Kruskal-Wallis test, $H = 7.58$, $n = 80$, $df = 2$, $p = 0.023$) and season (Mann-Whitney *U* test, $U = 7.32$, $n = 80$, $p = 0.007$), but not by project year (Mann-Whitney *U* test, $U = 598$, $n = 80$, $p = 0.051$). Pairwise comparisons revealed no significant differences in surface water enterococci counts between watersheds (Dunn's test, all $p > 0.05$). The highest surface water enterococci counts were observed in the central drainage feature (883 ± 278 MPN/100 mL), followed by Powell Creek (767 ± 162 MPN/100 mL), and Hancock Creek (397 ± 97 MPN/100 mL; Fig. 7a). Seasonal effects were observed in surface water enterococci counts with a higher count in the dry (907 ± 150 MPN/100 mL), than the wet (280 ± 59 MPN/100 mL) season (Fig. 7b).

Escherichia coli counts were significantly lower in groundwater (8.60 ± 7.2 MPN/100 mL) than in surface water (650 ± 91 MPN/100 mL; Mann-Whitney *U* test, $U = 6345$, $n = 160$, $p < 0.001$). Groundwater *E. coli* counts ranged from 0.5 to 579 MPN/100 mL and were not variable by watershed (Kruskal-Wallis test, $H = 2.06$, $n = 80$, $df = 3$, $p = 0.560$), season (Mann-Whitney *U* test, $U = 1.00$, $n = 80$, $p = 0.317$), or project year (Mann-Whitney *U* test, $U = 0.07$, $n = 80$, $p = 0.786$). Though not significantly different, the highest groundwater *E. coli* counts were observed in the central drainage watershed (25 ± 24 MPN/100 mL), followed by the Powell Creek (2.4 ± 1.9 MPN/100 mL) and Hancock Creek (0.75 ± 0.18 MPN/100 mL) watersheds, as well the reference well (0.5 ± 0 MPN/100 mL; Fig. 7a).

Surface water *E. coli* counts ranged from 38 to >4840 MPN/100 mL and were variable by watershed (Kruskal-Wallis test, $H = 16.3$, $n = 80$, $df = 2$, $p < 0.001$) and season (Mann-Whitney *U* test, $U = 9.82$, $n = 80$, $p = 0.002$), but not by project year (Mann-Whitney *U* test, $U = 704$, $n = 80$, $p = 0.355$). Powell Creek had significantly higher surface water *E. coli* counts (893 ± 147 MPN/100 mL) than Hancock Creek (323 ± 44 MPN/100 mL; Fig. 7a). The central drainage feature had the highest surface water *E. coli* counts (1310 ± 570 MPN/100 mL), but the variability in that watershed was also largest and the number of sites lowest ($n = 1$); therefore, differences between the central drainage watershed and the other watersheds were not statistically significant. Significantly higher surface water *E. coli* counts were observed in the dry season (969 ± 164 MPN/100 mL) than the wet season (331 ± 37 MPN/100 mL; Fig. 7b).

BOD concentrations were significantly higher in groundwater (1.19 ± 0.16 mg/L) than in surface water (1.02 ± 0.08 mg/L; Mann-Whitney *U* test, $U = 3806$, $n = 160$, $p = 0.036$). Groundwater BOD concentrations ranged from 0.15 to 5.2 mg/L and were variable by watershed (Kruskal-Wallis test, $H = 40.0$, $n = 80$, $df = 3$, $p < 0.001$), but not by season (Mann-Whitney *U* test, $U = 1.61$, $n = 80$, $p = 0.204$) or project year (Mann-Whitney *U* test, $U = 0.57$, $n = 80$, $p = 0.451$). Powell Creek watershed had the highest groundwater BOD concentration (2.77 ± 0.31 mg/L) and was significantly higher than the other watersheds, which all had similar BOD concentrations (reference well = 0.59 ± 0.19 mg/L, central drainage watershed = 0.57 ± 0.12 mg/L, and Hancock Creek watershed = 0.43 ± 0.14 mg/L; Fig. 7a).

Surface water BOD concentrations ranged from 0.15 to 4.4 mg/L and were variable by season (Mann-Whitney *U* test, $U = 15.6$, $n = 80$, $p < 0.001$), but not by watershed (Kruskal-Wallis test, $H = 2.32$, $n = 80$, $df = 2$, $p = 0.314$) or project year (Mann-Whitney *U* test, $U = 609$, $n = 80$, $p = 0.065$). The highest surface water BOD by watershed was at the central drainage feature (1.49 ± 0.43 mg/L), followed by Hancock Creek (0.98 ± 0.11 mg/L), and Powell Creek (0.96 ± 0.08 mg/L; Fig. 7a). Surface water BOD concentrations were significantly higher in the dry season (1.30 ± 0.12 mg/L) than the wet season (0.74 ± 0.07 mg/L; Fig. 7b).

Average FIB concentrations were relatively low in groundwater, though individual samples were occasionally high. For example, in Hancock Creek groundwater, an elevated enterococci concentration of 195 MPN/100 mL was observed on 08/27/2019, while a high BOD concentration of 3.20 mg/L was observed on 02/10/2020. In the central drainage basin *E. coli* concentrations up to 579 MPN/100 mL were observed with corresponding

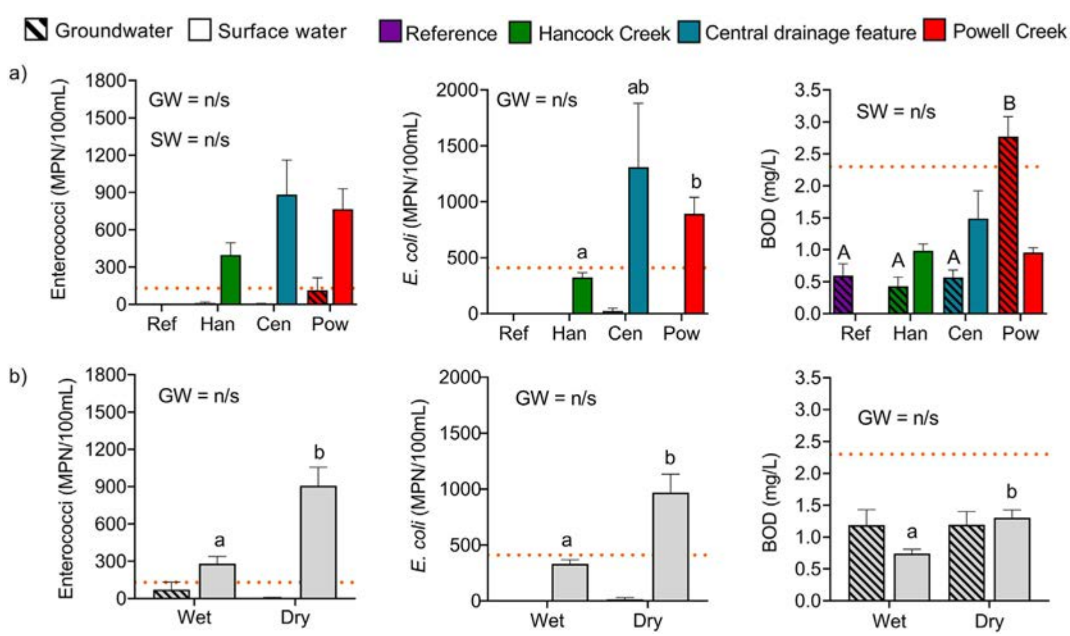

Fig. 7. Fecal indicator bacteria abundance, including enterococci and *Escherichia coli* (*E. coli*), as well as biochemical oxygen demand (BOD) concentrations of groundwater (striped) and surface water (no pattern) by a) watershed (reference well, Hancock Creek, central drainage feature, and Powell Creek), and b) season (wet/dry); dotted lines indicate Florida Department of Environmental Protection surface water criteria for enterococci (marine water ≥130 MPN/100 mL), BOD (2.4 mg/L), and *E. coli* (fresh water ≥410 MPN/100 mL). Some surface water standards do not apply to groundwater or the study area because it is freshwater and are shown as a point of reference only. Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison. All show mean count ± SE.

enterococci concentrations of 49 MPN/100 mL from the same date (02/13/2018), while elevated BOD concentrations (>1 mg/L) were observed in six of 24 groundwater samples. Finally, in Powell Creek basin a very high enterococci concentration of >2420 MPN/100 mL was observed on 10/18/2017, while elevated BOD concentrations (>1 mg/L) were consistently observed in 20 of 24 groundwater samples, with 16 of those being >2 mg/L. Thus, in Powell Creek groundwater, average BOD concentrations were particularly high (2.77 ± 0.31 mg/L).

3.6. Molecular markers

Concentrations of the human marker HF183 spanned from below detection to 1820 GEU/100 mL in surface water. HF183 concentrations were variable by watershed (Kruskal-Wallis test, H = 13.3, n = 80, df = 2, p = 0.001), but not by season (Mann-Whitney *U* test, U = 1.34, n = 80, p = 0.247) or project year (Mann-Whitney *U* test, U = 0.476, n = 80, p = 0.490). HF183 concentrations were significantly higher in the central drainage feature (406 ± 175 GEU/100 mL) than in Hancock Creek (31.3 ± 114 GEU/100 mL), but not Powell Creek (177 ± 76 GEU/100 mL; Fig. 8).

Avian markers were also detected in surface water. For example, concentrations of GFD fluctuated from below detection to 32,600 TSC/100 mL. GFD concentrations were variable by watershed (Kruskal-Wallis test, H = 8.58, n = 80, df = 2, p = 0.014) and season (Mann-Whitney *U* test, U = 7.32, n = 80, p = 0.007), but not by project year (Mann-Whitney *U* test, U = 0.755, n = 80, p = 0.385). Multiple comparisons by watershed did not find any significant differences in GFD concentrations (Dunn's test, all p > 0.05; Fig. 8). Significantly higher GFD concentrations were observed in the wet season (2472 ± 961 TSC/100 mL) than in the dry season (488 ± 223 TSC/100 mL). Gull2 was only detected in Hancock Creek and concentrations ranged from below detection to 107,000 TSC/100 mL (Fig. 8). Gull2 concentrations were not variable by season (Mann-Whitney *U* test, U < 0.001, n = 80, p = 0.992) or project year (Mann-Whitney *U* test, U = 2.81, n = 80, p = 0.094). The ruminant marker BacR was not detected during the study.

3.7. Chemical tracers

Though apparently higher, groundwater sucralose concentrations (5769 ± 1291 ng/L) were not significantly different from surface water concentrations (635.3 ± 37.6 ng/L; Mann-Whitney *U* test, U = 2897, n = 160, p = 0.300). In groundwater, sucralose was detected at least once in all wells with individual concentrations ranging from below detection to 58,000 ng/L. Concentrations of sucralose in groundwater were variable by watershed (Kruskal-Wallis test, H = 42.3, n = 80, df = 3, p < 0.001), but not by season (Mann-Whitney *U* test, U = 2.51, n = 80, p = 0.113) or project year (Mann-Whitney *U* test, U = 0.65, n = 80, p = 0.419). By watershed, groundwater sucralose concentrations in the reference well (195 ± 26 ng/L) and Powell Creek (580 ± 349 ng/L) were significantly lower than in the central drainage basin (5554 ± 1665 ng/L) or Hancock Creek (13,030 ± 3507 ng/L; Fig. 9).

Sucralose was ubiquitous in surface water of the study area with detections occurring at every site and individual concentrations ranging from 86 to 1600 ng/L. Sucralose varied by watershed (Kruskal-Wallis test, H = 6.81, n = 80, df = 2, p = 0.033), but not season (Mann-Whitney *U* test, U = 1.54, n = 80, p = 0.214) or project year (Mann-Whitney *U* test, U = 0.265, n = 80, p = 0.607). Multiple comparisons by watershed showed no significant differences in surface water sucralose concentrations (Dunn's test, all p > 0.05). Though no significant differences were found between watersheds, the highest surface water sucralose concentrations were observed in Powell Creek (749 ± 66 ng/L), followed by the central drainage feature (710 ± 74 ng/L) and Hancock Creek (529 ± 46 ng/L; Fig. 9).

Carbamazepine concentrations were significantly higher in groundwater (12.4 ± 4.9 ng/L) than in surface water (5.77 ± 0.7 ng/L; Mann-Whitney *U* test, U = 4070, n = 160, p = 0.003). Carbamazepine was not detected in the reference well, but was detected in groundwater of all other watersheds in low concentrations (Fig. 9) with individual values ranging from below detection to 310 ng/L. Groundwater carbamazepine concentrations were variable by watershed (Kruskal-Wallis test, H = 50.3, n = 80, df = 3, p < 0.001), but not season (Mann-Whitney *U* test, U = 0.450, n = 80, p = 0.501) or project year (Mann-Whitney *U* test, U = 0.020, n = 80, p = 0.892). The highest groundwater carbamazepine concentrations were found in the Hancock Creek watershed (36.8 ± 15 ng/L) and the central drainage watershed (4.13 ± 0.6 ng/L), which were significantly higher than Powell Creek (0.28 ± 0.28 ng/L) and the reference well (no detection; Fig. 9).

In surface water, carbamazepine was detected at least once at every site in low concentrations with an overall range from below detection to 28 ng/L (Fig. 9). Surface water carbamazepine concentrations varied by watershed (Kruskal-Wallis test, H = 52.9, n = 80, df = 2, p < 0.001), but not season (Mann-Whitney *U* test, U = 0.854, n = 80, p = 0.355) or project year (Mann-Whitney *U* test, U = 0.946, n = 80, p = 0.331). Powell Creek had significantly higher carbamazepine concentrations in surface water (11.9 ± 1.1 ng/L) than Hancock Creek (1.71 ± 0.18 ng/L) or the central drainage feature (1.44 ± 0.26 ng/L; Fig. 9).

Groundwater primidone concentrations (6.16 ± 1.3 ng/L) were not significantly different from those in surface water (3.04 ± 0.4 ng/L; Mann-Whitney *U* test, U = 3382, n = 160, p = 0.481). Primidone was not detected in the reference well but was detected in groundwater of all other watersheds with individual concentrations ranging from below detection to 47 ng/L. Concentrations of primidone in groundwater were not variable by project year (Mann-Whitney *U* test, U = 2.35, n = 80, p = 0.125), watershed (Kruskal-Wallis test, H = 4.28, n = 80, df = 3, p = 0.233), or season (Mann-Whitney *U* test, U = 0.003, n = 80, p = 0.953). All watersheds with detections had similar groundwater primidone concentrations (Powell Creek = 5.50 ± 2.2 ng/L, Hancock Creek = 5.27 ± 1.75 ng/L, and central drainage = 9.75 ± 3.3 ng/L; Fig. 9).

In surface water, primidone was detected at least once at every site in low levels with an overall range from below detection to 14 ng/L (Fig. 9). Surface water primidone concentrations varied by season (Mann-Whitney *U* test, U = 31.7, n = 80, p < 0.001), but not by watershed (Kruskal-Wallis test, H = 1.48, n = 80, df = 2, p = 0.478) or project year (Mann-Whitney *U* test, U = 0.328, n = 80, p = 0.567). Surface water primidone

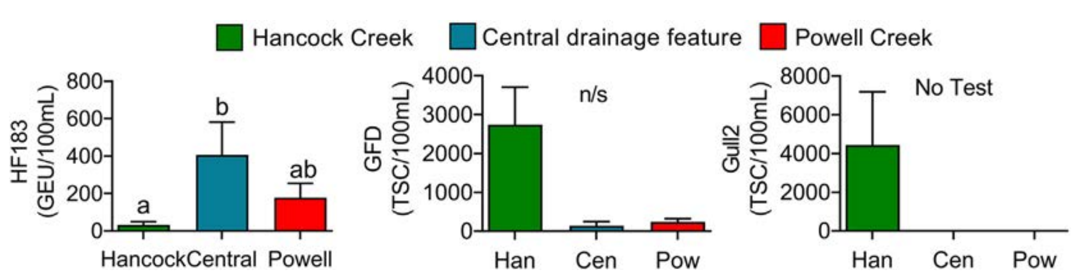

Fig. 8. Molecular markers concentrations in surface water (mean ± SE) of North Fort Myers, FL by watershed (Hancock Creek, a central drainage feature, and Powell Creek) including the human marker, HF183, and the avian markers GFD and Gull2. Significant differences are represented by lowercase letters, while "n/s" represents a non-significant statistical comparison.

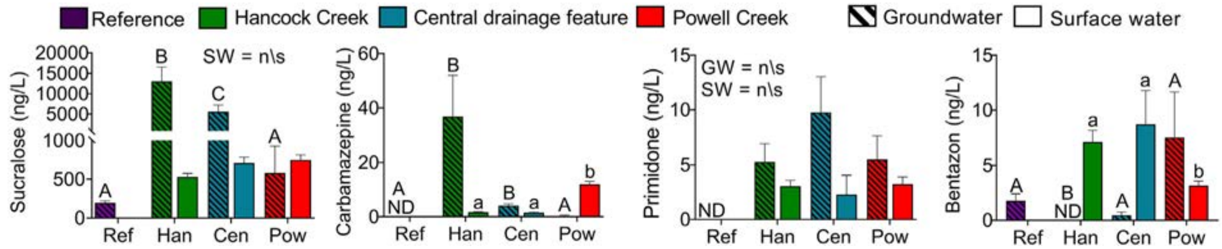

Fig. 9. Chemical tracer concentrations (mean ± SE) of groundwater (striped) and surface water (no pattern) observed in North Fort Myers, FL by watershed (reference well, Hancock Creek, central drainage basin, and Powell Creek), including the artificial sweetener sucralose, the anticonvulsant pharmaceuticals carbamazepine and primidone, and the herbicide bentazon. Significant differences are represented by uppercase letters for groundwater (GW) and lowercase letters for surface water (SW), while "n/s" represents a non-significant statistical comparison.

concentrations were significantly higher in the dry (5.28 ± 0.58 ng/L) than the wet (0.80 ± 0.31 ng/L) season. Though no significant differences were found between watersheds, the highest surface water primidone concentrations were observed in Powell Creek (3.23 ± 0.66 ng/L), followed by Hancock Creek (3.04 ± 0.54 ng/L) and the central drainage feature (2.29 ± 1.8 ng/L; Fig. 9).

Acetaminophen was detected in groundwater and surface water in the central drainage and Powell Creek basins with no significant difference between groundwater (3.63 ± 3.0 ng/L) and surface water concentrations (1.92 ± 0.76 ng/L; Mann-Whitney *U* test, U = 3394, n = 80, p = 0.131). Acetaminophen was only detected three times in groundwater during the study with one detection in Powell Creek (GW7) on 11/15/217 (23 ng/L) and two detections from the same well (GW5) in the central drainage watershed on 08/27/2019 (27 ng/L) and 09/16/2019 (240 ng/L). Surface water acetaminophen concentrations ranged from below detection to 38 ng/L. Acetaminophen concentrations in surface water varied by watershed (Kruskal-Wallis test, H = 12.0, n = 80, df = 2, p = 0.002), but not season (Mann-Whitney *U* test, U = 0.533, n = 80, p = 0.465) or project year (Mann-Whitney *U* test, U = 0.506, n = 80, p = 0.477). Surface water acetaminophen concentrations were significantly higher in the central drainage feature (6.50 ± 3.6 ng/L) and Powell Creek (3.18 ± 0.16 ng/L), than in Hancock Creek (no detection).

Groundwater bentazon concentrations were significantly higher (2.59 ± 1.3 ng/L) than those in surface water (0.01 ± 0.001 ng/L; Mann-Whitney *U* test, U = 1185, n = 80, p < 0.001). Bentazon was detected in groundwater in three of the four watersheds in very low concentrations with a range from below detection to 44 ng/L. In groundwater, concentrations of bentazon were variable by watershed (Kruskal-Wallis test, H = 8.92. n = 40, df = 3, p = 0.030), but not season (Mann-Whitney *U* test, U = 216, n = 40, p = 0.678). Due to the high variability observed in Powell Creek (7.56 ± 4.1 ng/L) and the low concentration observed in the central drainage basin (0.47 ± 0.27 ng/L), only the reference well (1.78 ± 0.64 ng/L) and Hancock Creek (no detections) had significantly different bentazon concentrations from each other (Fig. 9).

Surface water bentazon concentrations ranged from below detection to 17 ng/L. In surface water, bentazon concentrations varied by watershed (Kruskal-Wallis test, H = 9.33, n = 40, df = 2, p = 0.009) and season (Mann-Whitney *U* test, U = 4.86, n = 40, p = 0.027). Surface water bentazon concentrations were significantly higher in the central drainage feature (8.7 ± 3.1 ng/L) and Hancock Creek (7.15 ± 1.1 ng/L) than in Powell Creek (3.16 ± 0.41 ng/L; Fig. 9). By season, bentazon concentrations were significantly higher in the wet season (7.23 ± 1.1 ng/L) than the dry season (4.19 ± 0.8 ng/L).

Diuron concentrations in surface water ranged from below detection to 4.1 ng/L and did not vary by watershed (Kruskal-Wallis test, H = 5.67, n = 10, df = 2, p = 0.059). Though no significant differences were found between watersheds, the highest surface water diuron concentrations were observed in the central drainage feature (one detection = 3.9 ng/L), followed by Hancock Creek (2.48 ± 0.70 ng/L), and Powell Creek (no detections).

Fluoridone was detected at all sites throughout the study area with concentrations ranging from 3 to 95 ng/L. Surface water fluoridone concentrations did not vary by watershed (Kruskal-Wallis test, H = 3.18, n = 101, df = 2, p = 0.203). Though no significant differences were found between watersheds, the highest surface water fluoridone concentrations were observed in Hancock Creek (36.4 ± 17 ng/L), followed by Powell Creek (4.13 ± 0.62 ng/L) and the central drainage feature (one detection = 3.30 ng/L).

Imazapyr was detected at all surface water sites throughout the study area with concentrations ranging from 16 to 38 ng/L. Surface water imazapyr concentrations did not vary by watershed (Kruskal-Wallis test, H = 2.48, n = 10, df = 2, p = 0.290) Though no significant differences were found between watersheds, the highest surface water imazapyr concentrations were observed in Powell Creek (32.3 ± 3.2 ng/L), followed by Hancock Creek (26.2 ± 3.6 ng/L), and the central drainage feature (one detection = 22 ng/L).

Imidacloprid was detected at all surface water sites throughout the study area, except for SW9, with concentrations ranging from below detection to 8.6 ng/L. Surface water imidacloprid concentrations did not vary by watershed (Kruskal-Wallis test, H = 1.50, n = 10, df = 2, p = 0.474). Though no significant differences were found between watersheds, the highest surface water imidacloprid concentrations were observed in the central drainage feature (one detection = 7.90 ng/L), followed by Powell Creek (4.87 ± 0.80 ng/L) and Hancock Creek (4.38 ± 1.4 ng/L).

Some chemical tracers were not widely detected in the North Fort Myers study area. The chemical tracers mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any groundwater wells during the study, while fluoridone was only detected at the reference well. The chemical tracers hydrocodone, ibuprofen, naproxen, mCPP, triclopyr, fenuron, linuron, and pyraclostrobin were not detected at any surface water sites during the study.

3.8. Correlations between variables

Fecal indicator bacteria had no strong (≥0.30) correlations with each other or human waste tracers (Fig. S11). In fact, enterococci and *E. coli* had weak (<0.30) negative correlations with some of the human waste tracers (Fig. S11). Further, groundwater BOD concentrations were negatively correlated with carbamazepine (Spearman r = −0.51, n = 74, p < 0.001), sucralose (Spearman r = −0.39, n = 74, p = 0.001), SRP (Spearman r = −0.35, n = 74, p = 0.002), TP (Spearman r = −0.37, n = 74, p = 0.001), and δ^{15}N-NH_4^+ (Spearman r = −0.38, n = 72, p = 0.001), while positive correlations were observed between groundwater BOD concentrations and NH_4^+ (Spearman r = 0.51, n = 74, p < 0.001), DIN (Spearman r = 54, n = 74, p < 0.001), DIN:SRP (Spearman r = 70, n = 74, p < 0.001), TN (Spearman r = 48, n = 74, p < 0.001), and TN:TP (Spearman r = 67, n = 74, p < 0.001). Additionally, groundwater NH_4^+ concentrations were negatively correlated with carbamazepine (Spearman r = −0.45, n = 74, p < 0.001), sucralose (Spearman r = −0.37, n = 74, p = 0.001), NO_x (Spearman r = −0.32, n = 74, p = 0.005), and SRP (Spearman r

= −0.31, n = 74, p = 0.007). Groundwater NOx concentrations were positively correlated with carbamazepine (Spearman r = 0.42, n = 74, $p < 0.001$), SRP (Spearman r = 0.35, n = 74, p = 0.002), and $\delta^{15}N\text{-}NH_4^+$ (Spearman r = 0.32, n = 74, p = 0.002). Groundwater SRP and TP concentrations were also positively correlated with carbamazepine (Spearman r = 0.62, n = 74, $p < 0.001$; Spearman r = 0.68, n = 74, $p < 0.001$, respectively) and sucralose (Spearman r = 0.72, n = 74, $p < 0.001$; Spearman r = 0.67, n = 74, $p < 0.001$, respectively). Groundwater $\delta^{15}N\text{-}NH_4^+$ was positively correlated with sucralose (Spearman r = 0.30, n = 74, p = 0.010) and color (Spearman r = 0.33, n = 72, p = 0.010). The color of groundwater was also positively correlated with sucralose (Spearman r = 0.30, n = 74, p = 0.020). Groundwater pH was negatively correlated with sucralose (Spearman r = −0.65, n = 74, $p < 0.001$), carbamazepine (Spearman r = −0.46, n = 74, $p < 0.001$), and SRP (Spearman r = −0.45, n = 74, $p < 0.001$). Finally, groundwater salinity was positively correlated with primidone (Spearman r = 0.51, n = 74, $p < 0.001$), DIN (Spearman r = 0.36, n = 74, p = 0.002), and TN (Spearman r = 0.30, n = 74, p = 0.011).

Enterococci concentrations were positively correlated with *E. coli* (Spearman r = 0.78, n = 74, $p < 0.001$) and in surface water fecal indicator bacteria had strong positive correlations with human waste tracers (Fig. S12). Specifically, surface water enterococci concentrations were positively correlated with primidone (Spearman r = 0.32, n = 74, p = 0.005), sucralose (Spearman r = 0.306, n = 74, p = 0.008), and HF183 (Spearman r = 0.30, n = 74, p = 0.008). Positive correlations were also observed between surface water *E. coli* concentrations and carbamazepine (Spearman r = 0.37, n = 74, p = 0.001), primidone (Spearman r = 0.376, n = 74, p = 0.001), sucralose (Spearman r = 0.30, n = 74, p = 0.008), and HF183 (Spearman r = 0.32, n = 74, p = 0.009). Similarly, BOD was positively correlated with primidone (Spearman r = 0.40, n = 74, $p < 0.000$), as well as salinity (Spearman r = 0.38, n = 74, p = 0.001). HF183 concentrations in surface water were positively correlated with acetaminophen (Spearman r = 0.43, n = 74, $p < 0.001$), NH_4^+ (Spearman r = 0.43, n = 74, $p < 0.001$), DIN (Spearman r = 0.31, n = 74, p = 0.007), and TN (Spearman r = 0.35, n = 74, p = 0.002), but negatively correlated with pH (Spearman r = −0.36, n = 74, p = 0.002). Surface water NH_4^+ concentrations were positively correlated with acetaminophen (Spearman r = 0.30, n = 74, p = 0.009) and NO_x (Spearman r = 0.55, n = 74, $p < 0.001$), while surface water NO_x and DIN concentrations were positively correlated with carbamazepine concentrations (Spearman r = 0.42, n = 74, $p < 0.001$; Spearman r = 0.38, n = 74, p = 0.001, respectively). Surface water SRP concentrations were positively correlated with primidone (Spearman r = 0.35, n = 74, p = 0.002), but negatively correlated with TN (Spearman r = −0.55, n = 74, $p < 0.001$). Surface water pH was negatively correlated with reactive N, including NH_4^+ (Spearman r = −0.52, n = 74, $p < 0.001$), NO_x (Spearman r = −0.51, n = 74, $p < 0.001$), DIN (Spearman r = −0.57, n = 74, $p < 0.001$), and DIN:SRP (Spearman r = −0.47, n = 74, $p < 0.001$). Finally, surface water color was negatively correlated with primidone (Spearman r = −0.33, n = 74, p = 0.004), but positively correlated with DIN:SRP (Spearman r = 0.31, n = 74, p = 0.008) and TN (Spearman r = 0.30, n = 74, p = 0.010).

4. Discussion

This study demonstrated the nutrient and microbial couplings between septic systems, groundwater, surface water, and HABs in the highly modified Caloosahatchee River Estuary and downstream coastal waters. This multi-year study revealed that these human waste contaminant sources remain relatively consistent over time with location generally being the most influential factor for water quality. In each watershed there were multiple lines of evidence that indicated septic system effluent was adversely affecting water quality. Notably, shallow water tables demonstrated that these systems did not have the physical separation required for adequate treatment of effluent (>1 m separation from the ground surface to the seasonally high table). Evidence of human waste contamination observed in all three affected watersheds included high groundwater and surface water DIN concentrations, enriched $\delta^{15}N$ values of groundwater and POM that closely matched septic effluent (+4.9‰; Hinkle et al., 2008), and elevated surface water FIB with the presence of HF183, as well as detections of sucralose, carbamazepine, and primidone in groundwater and surface water. These findings demonstrate that septic systems are not protective of water quality in these low elevation watersheds, which has also been found in other urbanized waterfront residential areas, such as Michigan's Lower Peninsula (Verhougstraete et al., 2015), North Carolina (Humphrey et al., 2011; Cahoon et al., 2016), and Jepara, Indonesia (Adyasari et al., 2018). The presence of the chemical tracers 2,4-D, bentazon, diuron, fluoridone, imazapyr, and imidacloprid provided evidence that stormwater runoff was also adversely affecting surface water quality. Finally, detections of GFD and Gull2 indicated that avian fecal matter may also negatively affect water quality at some sites in North Fort Myers. Thus, these findings may be useful for understanding water quality and HAB drivers in other waterfront communities.

4.1. Nitrogen enrichment and harmful algal blooms

The high nutrient concentrations observed in North Fort Myers groundwater and surface water reflects enrichment from septic system effluent. Groundwater NH_4^+ concentrations were particularly high in the central drainage and Powell Creek basins, while NO_x concentrations were at background levels (Fig. 4). These drainage basins had the highest water tables (Fig. 3), which would likely suppress coupled nitrification-denitrification (Lapointe et al., 1990; Mallin, 2013). Conversely, in Hancock Creek where water tables were deeper, groundwater NO_x concentrations were highest and NH_4^+ concentrations were lower, indicating that some nitrification was occurring. $\delta^{15}N\text{-}NO_3^-$ values were also highest in Hancock Creek groundwater (+8.49‰), further linking these high NO_x concentrations to a human waste source. Despite the variability between reactive N species, DIN, SRP, TN, and TP concentrations were all significantly higher in groundwater near high density septic systems than in the less densely developed, upland reference well. Surface water in the drainage basins with the shallower water tables also had higher reactive N concentrations and N:P. The various significant correlations of HF183, carbamazepine, and acetaminophen with surface water NH_4^+, NO_x, and TN concentrations help to connect this N loading to effluent from septic systems (Fig. S12). Conversely, surface water P concentrations were not variable between watersheds and may not be as affected by septic system effluent loading because some of these additional P inputs are likely adsorbed by sediments (Lapointe et al., 1990; Weiskel and Howes, 1992). The study region has P-rich phosphorus deposits in the Bone Valley Formation, which provide for natural elevation of P concentrations (Odum, 1953). Despite this, P was elevated above FDEP standards throughout the study area (Fig. 4), which may be reflective of anthropogenic inputs from human waste. These findings support previous work that identified local basin nutrient sources as more "severe" than Lake Okeechobee discharges and proposed that improving water in local basins would have the greatest effect on estuarine water quality (Doering and Chamberlain, 1999; Lapointe and Bedford, 2007).

As such, nutrient enrichment from the local North Fort Myers basin may support HAB events, such as those that occurred during this study in 2017–2018 following Hurricane Irma (Glibert, 2020). While this study is one of few to connect downstream HABs with upstream nutrient loading from septic systems, this has been observed in other locations, such as the St. Lucie Estuary, FL (Lapointe et al., 2017), Waquoit Bay, Massachusetts (Valiela et al., 1992) and Lake Huron, Ontario, Canada (Rakhimbekova et al., 2021). In many areas of Florida, due to the high background P (Odum, 1953), N has been identified as the element most capable of promoting algal growth and toxicity– especially for cyanobacteria (Kramer et al., 2018), which has been observed in other locations as well (Gobler et al., 2016). In the estuarine and coastal environments adjacent to Lee County, *M. aeruginosa* and *K. brevis* blooms thrive in low N:P conditions (Ketchum and Keen, 1948; Odum, 1953; Lapointe et al., 2006; Yentsch et al., 2008; Lapointe et al., 2012; Lapointe et al., 2017), but *K. brevis* can also be sustained at higher N:P (Odum et al., 1955). At SRP concentrations typical of coastal Southwest Florida, growth of *K. brevis* in lab cultures was a linear function of ammonia in sewage effluent (Doig and Martin, 1974). In addition to the exacerbating effects of excess N loading to HABs, reduced salinity has also been shown to favor *K. brevis* and *Microcystis* spp. blooms (Slobodkin, 1953; Brand and Compton, 2007; des Aulnois et al., 2019; Medina et al., 2020). During wet periods, such as following tropical storms or hurricanes, the downstream Caloosahatchee River Estuary can be seeded with *M. aeruginosa* from Lake Okeechobee freshwater discharges. Similarly, blooms of *M. aeruginosa* occurred in Southeast Florida's St. Lucie River Estuary in 2005, 2013, and 2016 that were attributed to algal "seeding" from Lake Okeechobee discharges combined with high biomass local basin blooms supported by urban nutrient loading (Lapointe et al., 2012; Phlips et al., 2012; Lapointe et al., 2017; Kramer et al., 2018). In 2016, the phytoplankton biomass in Lake Okeechobee was N-limited during *M. aeruginosa* bloom conditions (Kramer et al., 2018); thus after heavy rainfall and discharges these blooms experienced exponential growth in urbanized estuaries with low salinities and high levels of DIN, especially NH_4^+ (Lapointe et al., 2017; Kramer et al., 2018). This is consistent with this study, where cyanobacterial bloom samples collected from nearby Cape Coral residential canals (Fig. S2) had very high concentrations of NH_4^+ (2107 μM) and SRP (148 μM) with enriched POM $\delta^{15}N$ values (+7.41‰; Fig. 6). Comparable $\delta^{15}N$ values (+6.93‰) were observed in *M. aeruginosa* samples collected from the Caloosahatchee River Estuary in 2005 (Lapointe et al., 2006) demonstrating the consistency of this issue and nutrient source over time.

There were similarities observed between the study area and coastal HABs. For example, in North Fort Myers $\delta^{15}N$ values were enriched in cyanobacteria (+8.79‰) and green macroalgae (+6.38) samples collected at North Shore Park, as well as in groundwater aqueous NH_4^+ samples (+3.90‰), groundwater aqueous NO_3^- samples (+6.36‰), and surface water POM samples (+4.69‰). NH_4^+ is often the dominant inorganic N species in the study area (Lapointe and Bedford, 2007; Dixon et al., 2014; Heil et al., 2014), which could have implications for bloom development. This connection is supported by enriched $\delta^{15}N$ values observed in *K. brevis* samples (+4.94‰) collected from coastal areas of Lee County in 2018 that are similar to those of septic effluent (+4.9‰; Hinkle et al., 2008). Further, alongside urban development in the study area, NH_4^+ and *K. brevis* bloom concentrations have increased (Brand and Compton, 2007). Interestingly, many of the *K. brevis* blooms between 1998 and 2001 initiated offshore of Lee County before moving north (Vargo et al., 2008). Additionally, in this study, *K. brevis* $\delta^{13}C$ values from the most terrestrially influenced site, Lighthouse Beach, were more depleted during the wet season (September 2018) than in the dry season (March 2018) and at the other more coastal sites, indicative of a more freshwater C source. The $\delta^{15}N$ values were also the most enriched during this sampling event, further demonstrating the link between upstream freshwater sources and N enrichment. These values are similar to $\delta^{15}N$ values of POM collected during cruises between 1998 and 2001 at stations with >50,000 *K. brevis* cells/L^{-1} (~+4.4‰), which led the authors to conclude that a common seasonal N supply was available for *K. brevis* blooms (Havens et al., 2004). Enriched $\delta^{15}N$ values were also observed in red drift macroalgae (+4.86‰) and *K. brevis* samples (+7.83‰) collected from Lee County coastal waters in 2004 and 2005 following hurricanes Charley, Frances, and Jeanne (Lapointe et al., 2006; Lapointe and Bedford, 2007). Therefore, it is reasonable to expect that HABs in estuarine and coastal environments of Southwest Florida may be intensified following periods of heavy or prolonged rain events that reduce salinity and increase local basin N loading, including from less conspicuous sources like submarine groundwater discharge (Hu et al., 2006).

4.2. Bacterial abundance

The low groundwater FIB concentrations observed near septic systems was not unprecedented. Other studies in Florida (Herren et al., 2021), along Chesapeake Bay (Reay, 2004), and in Australia (Ahmed et al., 2005) have observed similar patterns where groundwater FIB are lower than in adjacent surface water. For example, Herren et al. (2021) found very low to non-detectable *E. coli*, fecal coliform, and enterococci concentrations in groundwater near septic systems and elevated concentrations in adjacent surface water that they attributed to the well distance from septic systems and the diffuse nature of the sub-surface septic plume. Additionally, bacteria can move quickly in saturated conditions and rapidly permeable soils (Cogger, 1988; Bicki and Brown, 1990; Harris, 1995), such as what was observed in North Fort Myers. These circumstances can lead to low FIB groundwater residence time due to preferential conduit flow (Andreo et al., 2006). Previous monitoring in North Fort Myers observed patchiness in "the power and magnitude" of groundwater bacteria concentrations and noted difficulty in definitively discriminating contributing sources (W. Dexter Bender and Associates Inc., 1995) These issues in the use of FIB as human waste tracer are well established and support the importance of using a multifaceted microbial source tracking approach to track human fecal contamination (Scott et al., 2002; Field and Samadpour, 2007; Tran et al., 2015; Kelly et al., 2018). In this study because wells were installed in parks or private residences, no septic system plume tracking was conducted prior to well installation and well locations were haphazardly selected, thus groundwater samples may not reflect the full magnitude of impacts to groundwater from septic systems. Despite these issues, even the relatively low concentrations of FIB observed in North Fort Myers groundwater are concerning as they exceed the United States Safe Drinking Water Act, which has been observed in other locations near septic systems (Hunter et al., 2021). The North Fort Myers study area has ~141 domestic wells that could be affected by this contamination.

Surface water FIB concentrations in the study area were higher than groundwater, often exceeding FDEP water quality standards. In warm environments, such as the study area, bacteria can be sourced from the gut of an animal, including humans, but become "naturalized" allowing them to persist and multiply in the environment if conditions are favorable (Devane et al., 2020). In North Fort Myers, the high background P may help to maintain these high surface water bacterial abundances (Mallin and Cahoon, 2020). Additionally, submarine groundwater discharge from septic systems can promote bacterial survival in surface water through delivery of nutrients and fresh water (Reay, 2004). In this study, the surface water sites near dense residential areas with high water tables tended to have higher microbial densities, which can be linked to human waste inputs because of the presence of chemical human waste tracers and HF183 (Figs. 5, 6, and S12). For example, the watersheds with greater densities of septic systems, Powell Creek and the central drainage feature (279 and 236 septic systems/km^2, respectively; Table 1) also had the highest enterococci and *E. coli* concentrations. In Hancock Creek, the densely residential site, SW10 had the highest enterococci and *E. coli* counts, while in Powell Creek, an upstream to downstream gradient was observed for both enterococci and *E. coli* counts, which may indicate dilution from exchange with the Caloosahatchee River Estuary. FIB in the central drainage feature was as high as the most upstream site in Powell Creek, which could indicate a higher initial FIB concentration due to the high density of septic systems in this drainage basin or a closer human waste source to the sampling location in this watershed.

Septic contamination of surface water with FIB is further supported by the high concentration of DIN (especially NH_4^+), sucralose, and HF183 observed at the central drainage feature (Figs. 4–6). The positive correlation between groundwater BOD and NH_4^+ (Fig. S11) provides additional evidence of non-functioning septic systems, particularly in Powell Creek where both were highest. While there was a negative correlation between surface water BOD and NH_4^+, surface water BOD was highest in the central drainage feature (1.49 ± 0.43 mg/L), which similarly had the highest NH_4^+ concentrations (8.27 ± 1.6 μM) further supporting this relationship. High NH_4^+ concentrations exert a BOD on receiving waters (referred to as nitrogenous BOD or NBOD) because DO is consumed as bacteria and other microbes oxidize NH_4^+ into NO_x.

Like other locations in Florida, tidal pumping likely increases nutrient and bacterial loading from septic systems during outgoing tides (Lapointe et al., 1990; Lipp et al., 2001; Buszka and Reeves, 2021). This connectivity between septic systems, groundwater, and surface water is supported by significant positive correlations between FIB and human waste tracers observed in surface water during outgoing tides. For example, enterococci and *E. coli* were positively correlated with carbamazepine, primidone, and sucralose, while acetaminophen was weakly correlated with both (Fig. S12). Further supporting this connection, downstream bacteria populations in other locations have been matched to septic tank source populations using biochemical fingerprinting (Ahmed et al., 2005).

4.3. Molecular markers

The detection of HF183 in surface water of all three watersheds (Fig. 6) demonstrated the ubiquitous presence of human fecal bacteria in North Fort Myers. Because there is no application of reuse water in the study area groundwater contaminated with human enteric bacteria from septic systems is the most likely source of HF183. Similar fecal pollution has been observed in the St. Lucie Estuary, another Florida ecosystem affected by high-density, waterfront septic systems at low elevation (Lapointe et al., 2012; Lapointe et al., 2017; Kelly et al., 2020), as well as in other locations, including North Carolina (Cahoon et al., 2006), Georgia (Sowah et al., 2014), Michigan's Lower Peninsula (Verhougstraete et al., 2015), and Puerto Rico (Jent et al., 2013). In future studies, HF183 should be sampled in both groundwater and adjacent surface water to better connect septic system bacterial contamination to surface water concentrations.

Bird molecular markers were also detected in all the surface water surveyed in North Fort Myers, but were particularly prevalent in the Hancock Creek watershed (Fig. 6). Seabird feces contain high amounts of enterococci and can be a significant source of bacteria to beaches (Grant et al., 2001). While elevated bacteria levels are concerning and seabird feces have the potential to transfer zoonotic agents to humans, such as bacteria and viruses (Epstein et al., 2006), the risk of seabird fecal contamination to humans is relatively unknown (Field and Samadpour, 2007). For example, in South Florida, transmission of salmonella from white ibis (*Eudocimus albus*) to humans in park environments has been suspected, but not confirmed (Hernandez et al., 2016). Further, bird feces also likely contribute nutrients to the study area. Thus the exclusion of gulls and other seabirds from beaches can significantly improve water quality (Goodwin et al., 2017), which suggests that discouragement of human food provisioning within beach areas might have similar effects and positive benefits for both humans and birds (Murray et al., 2021). As such, at North Shore Park where the Gull2 molecular marker was observed, FIB and nutrient concentrations might be reduced through a public education campaign, such as informational signs or social media campaigns, designed to inform park visitors of the water quality implications to feeding wild bird populations (Murray et al., 2021). Further, trash receptacles could be modified with heavy sealing lids that would lessen the availability of discarded food materials attracting birds to the area. Analyses of long-term databases on Florida beaches showed that while covered trash receptacles alone were not associated with a change in FIB, the discouragement of bird groupings was associated with a decrease in FIB (Kelly et al., 2018). These types of programs would likely be helpful in reducing bird FIB and nutrient contributions to waterfront parks in other locations.

4.4. Chemical tracers

There are some advantages to using chemical tracers over FIB and molecular markers, including source specificity, stability, and higher probability of detection (Lim et al., 2017). In this study chemical human waste tracers were useful for connecting nutrient and bacterial pollution to a source. For example, sucralose, carbamazepine, and primidone were present in groundwater and surface water in all three basins serviced primarily by septic systems (Fig. 9) confirming the ubiquitous influence of human waste throughout North Fort Myers. Similar widespread detection of sucralose in surface water has been observed in other regions with septic system influence (Watanabe et al., 2016; Spoelstra et al., 2020). Sucralose concentrations were similar to values observed in groundwaters in other areas of Florida, including Indian River (Herren et al., 2021), Martin, Charlotte, and St. Lucie Counties (Lapointe et al., 2016; Lapointe and Herren, 2016; Lapointe et al., 2017; Lapointe et al., 2020a), as well as North Carolina (Hunter et al., 2021), Vietnam and the Philippines (Watanabe et al., 2016). Acetaminophen was also detected in the central drainage and Powell Creek basins in both groundwater and surface water further demonstrating the presence of poorly treated human waste in these basins, which could be a result of the shallow water tables in these basins combined with higher densities of septic systems (Table 1). Despite the subtle differences between watersheds, the ubiquitous presence of these human waste tracers and their positive correlations with other indicators in surface water (Fig. S12) confirms that effluent from septic systems is contaminating both the groundwater and surface water of North Fort Myers.

In North Fort Myers, the presence of herbicides and pesticides provided evidence that stormwater runoff was also contributing to water quality degradation. Impervious surface area has been correlated with estuarine fecal coliform abundance, indicating that smart urban planning and stormwater mitigation techniques, including constructed wetlands and green areas, can be useful for improving urban water quality (Mallin et al., 2000). Thus, reducing N and P in stormwater from urban residential catchments has been identified as a primary opportunity to reduce nutrient loading (Yang and Toor, 2018). In Florida, turfgrass has been estimated to take up ~80 to 90% of applied fertilizers within the turf thatch with minimal amounts reaching groundwater and surface water, even in the wet season (Hochmuth et al., 2009; Shaddox and Unruh, 2018), so this is likely not a major nutrient source in the study area. However, a detailed study in Florida documented shifting patterns of $\delta^{15}N\text{-}NO_3^-$ throughout individual storm events, ranging from atmospheric deposition, inorganic fertilizers, soil, manure, and human waste (Jani et al., 2020), so multiple sources may contribute nutrients to stormwater runoff depending on conditions. In South Florida, climate change is expected to greatly exacerbate stormwater runoff issues in the next thirty years, which will demand drastic improvements of existing stormwater drainage infrastructure through retrofitting, rehabilitations, and new construction in order to minimize local basin pollutant loading (Huq and Abdul-Aziz, 2021). Therefore, working to minimize the amount of untreated stormwater runoff flowing into surface water represents another important component of improving water quality in North Fort Myers.

4.5. High groundwater

High groundwater levels were ubiquitous in the study area and may provide an accurate indicator for locations where septic systems may not provide adequate domestic waste treatment. This is because proper functioning of a septic system can only occur when a sufficient volume of unsaturated soil is present to absorb and treat the effluent (Bicki and Brown, 1990). Thus, in these areas, septic systems may not be an appropriate option for domestic waste management, particularly when they are located close to surface water important for recreation and fisheries. High groundwater tables were first documented in North Fort Myers almost 30 years ago (W. Dexter Bender and Associates Inc., 1995) and this study confirms that poor conditions for septic systems persist. Resource managers and civic leaders could use groundwater levels and proximity to surface water to help prioritize locations for septic to sewer conversions or other mitigation strategies, such as the use of Distributed Wastewater Systems that provide onsite advanced wastewater treatment (Lapointe and Brewton, 2021). High water table mapping in Florida coastal regions has recently been improved using fine resolution Lidar Digital Elevation Model data coupled with spatial interpolators like multiple linear regression and support vector machine techniques (Zhang et al., 2021) that could make these efforts more efficient.

This study supports the findings of previous research that septic systems contribute to the human waste contamination of groundwater and adjacent surface water. For example, shallow seasonal high water tables that interfere with the functioning of septic systems (Bicki and Brown, 1990) have also been observed throughout Florida in Jupiter and Tequesta (Lapointe and Krupa, 1995), St. George Island (Corbett et al., 2002), Dania Beach

(Meeroff et al., 2008), and Port St. Lucie (Lapointe et al., 2020a). Similar issues with shallow water tables interfering with the performance of septic systems have been observed in many other United States coastal communities, such as in North Carolina (Humphrey et al., 2011), Rhode Island (Cox et al., 2020), and Texas (Forbis-Stokes et al., 2016). Thus, there are far ranging implications of this work for other waterfront communities seeking to better manage human waste inputs and improve water quality.

4.6. Seasonality

While generally not as important as proximity to septic systems, some seasonal effects were also observed during this study, particularly for surface water. To begin with, water tables were shallower in the wet season compared to the dry season (Fig. 3 and Fig. S4). While groundwater concentrations of FIB and nutrients did not vary seasonally, there were seasonal differences observed in surface water. For example, in surface water higher concentrations of enterococci, *E. coli*, and BOD were observed in the dry season compared to the wet season (Fig. 4b). In the wet season, surface water concentrations of NH_4^+, NO_x, DIN, and TN were higher, while SRP and TP concentrations were higher in the dry season. Due to the relative variability observed in reactive nutrient concentrations by season, DIN:SRP was also higher in the wet season. These differences between seasons support a higher rate of N-loading from septic systems when water tables are elevated in the wet season, while elevated P in the dry season may support the higher surface water bacterial abundances observed during these sampling events (Mallin and Cahoon, 2020). Groundwater and surface water concentrations of sucralose and carbamazepine, as well as surface water concentrations of acetaminophen and HF183 were not variable by season, suggesting that septic systems are providing a year-round source of human waste in North Fort Myers. Interestingly, while groundwater primidone concentrations were not seasonally variable, surface water concentrations were higher in the dry season. This could be an effect of seasonal residency increasing loading of this specific tracers in the dry season or slower degradation due to cooler temperatures in the dry season. While concentrations of bentazon in groundwater were not seasonally variable, those in surface water were, suggesting an increase in surficial runoff during the wet season.

Increased surficial runoff may also contribute to the elevated N observed during the wet season. For example, rainfall can also carry animal (bird, dog, cat, etc.) waste and fertilizers into the adjacent surface water (Krimsky et al., 2021). This is supported by higher concentrations of the avian GFD marker in the wet season, though Gull 2 was not seasonally variable. Future studies could also seek to quantify the effect of pet waste on water quality by including molecular markers for dog and cat fecal bacteria. $\delta^{15}N$-NH_4^+ values were not seasonally variable, suggesting a constant NH_4^+ source in the groundwater, such as septic system effluent. However, $\delta^{15}N$-NO_3^- were significantly higher in the wet season (+7.89 ± 1.9‰) than the dry season (+2.71 ± 1.5‰), suggesting a more enriched NO_x source, such as human waste, is dominant in groundwater in the wet season (Fig. 7a). POM $\delta^{13}C$ values were not seasonally variable reflecting a consistent C source, however POM $\delta^{15}N$ values were significantly higher in the dry season (+5.28 ± 0.22‰) than the wet season (+4.10 ± 0.27‰; Fig. 7b), which could reflect more diversity in N sources from increased surficial runoff, as opposed to primarily septic system effluent. Lapointe and Bedford (2007) found a similar seasonal pattern for macroalgal $\delta^{15}N$ in Lee County coastal waters during 2004, with higher values in the dry season (+5.84‰) compared to the wet season (+3.89‰), which they attributed to greater inputs of isotopically depleted atmospheric and fertilizer N in the wet season. POM C:N were also higher in dry season than the wet season, reflecting the demonstrated higher N availability in the wet season.

4.7. Ecological impacts

There are many ecological effects of nutrient loading to the downstream estuary. Excess N loading represents a threat to seagrasses in Florida (Lapointe et al., 2020b) and other coastal areas globally (Hauxwell and Valiela, 2004; Orth et al., 2006; Short et al., 2014; Thomsen et al., 2020). As such, seagrasses are among the most threatened ecosystems on earth (Waycott et al., 2009) and it is important to consider threats to seagrass that stakeholders perceive as persistent (Unsworth et al., 2019). During sampling events, residents in the Powell Creek study area shared anecdotal accounts of large-scale seagrass losses where the creek terminates into the Caloosahatchee River Estuary that they attributed to poor water quality. This was likely tape grass (*Vallisneria americana*), as most of its coverage is located upstream of the Fort Myers bridges (Doering et al., 2002). Loss of seagrass habitats can potentially have cascading effects on ecologically and recreationally important fish populations, such as gag grouper (*Mycteroperca microlepis*) and spotted seatrout (*Cynoscion nebulosus*) that are dependent on this structure during juvenile life history stages (Barnes, 2005). Further, the vulnerable Florida manatee (*Trichechus manatus*) depends on seagrasses and adequate water quality to preserve their essential habitat (Barnes, 2005), while the critically endangered smalltooth sawfish (*Pristis pectinate*) uses the remaining natural shoreline along the Caloosahatchee River Estuary dominated by the red mangrove (*Rhizophora mangle*) as essential habitat (Poulakis et al., 2011). In fact, telemetry studies have defined the area in the Caloosahatchee River Estuary between the North Fort Myers bridges into which the study area drains as a *P. pectinate* "nursery hotspot" (Scharer et al., 2017). Accordingly, a juvenile sawfish (species undetermined) was observed and reported to Florida Fish and Wildlife Conservation Commission during the March 2020 sampling event of this study at North Shore Park.

4.8. Implications for management

Preserving and improving water quality in urbanized areas is an ongoing challenge for waterfront communities globally that will intensify as climate change progresses. This multi-year study indicates that septic systems are not protective of water quality in North Fort Myers, which has also been observed in other locations, including England (Withers et al., 2011, 2014) and Cape Cod, MA (Howes et al., 2004). Thus, in these locations there is a unique opportunity to significantly improve water quality by reducing dependence upon aging septic systems adjacent to surface water. These issues in North Fort Myers are caused by aging septic systems installed in high densities (~0.63/acre; Bicki and Brown, 1991) in areas with shallow water tables (Bicki and Brown, 1990). Additionally, the presence of canals in these residential areas with septic systems may increase the rate of pollutant transfer from groundwater to surface water via tidal pumping (Lapointe et al., 1990; Lipp et al., 2001; Buszka and Reeves, 2021). The amount of impervious ground cover, such as concrete or asphalt, in urban areas is another contributing factor to water quality issues (Chelsea Nagy et al., 2012; Flanagan et al., 2020). The combination of these conditions is likely to degrade water quality, which may continue to worsen with climate change. Municipalities should consider all these factors when planning sustainable development and infrastructure improvements, such as septic to sewer programs and stormwater treatment areas. For example, it would be beneficial for coastal areas with high densities of septic systems and canals to be prioritized for septic to sewer conversions (Buszka and Reeves, 2021) or other advanced wastewater treatment options, such as Distributed Wastewater Treatment Systems (Lapointe and Brewton, 2021). Finally, the susceptibility of these systems to localized HABs may be reduced by balancing the ecological stoichiometry of the watershed to achieve a nutrient load and N:P ratio where these microalgae will not thrive, leading to fewer HAB events. The scale of the red tide issue demands watershed-scale solutions and N management based on a holistic view, considering both oceanographic and anthropogenic processes (Medina et al., 2022).

5. Conclusions

Urban water quality is complex because it is affected by myriad environmental, economic, and political issues. This means that resource managers must be able to identify sources contributing to water quality decline and then prioritize mitigation and abatement strategies. Due to the nature of human waste inputs (i.e., reactive nutrients, pathogens, bacteria, pharmaceuticals, etc.), improved wastewater infrastructure and management, including advanced wastewater treatment (nutrient removal), in low elevation, high density waterfront communities serviced by septic systems should be prioritized as climate change will continue to exacerbate water quality issues and HABs. Further, stormwater improvements and citizen education campaigns on subjects such as feeding wild birds, removal of pet waste, and fertilizer use may be other methods to help minimize the effect of urbanization on water quality.

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CRediT authorship contribution statement

Rachel A. Brewton: conceptualization, data curation, methodology, data visualization, project administration, writing- original draft, writing-review and editing. **Lisa B. Kreiger**: conceptualization, data curation, methodology, writing- review and editing. **Kevin N. Tyre**: data curation, methodology, formal analyses, writing- review and editing. **Diana Baladi**: data curation, writing- review and editing. **Lynn E. Wilking**: formal analyses, writing- original draft. **Laura W. Herren**: conceptualization, data visualization, project administration, writing- review and editing. **Brian E. Lapointe**: conceptualization, methodology, writing- review and editing, project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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Widespread human waste pollution in surface waters observed throughout the urbanized, coastal communities of Lee County, Florida, USA

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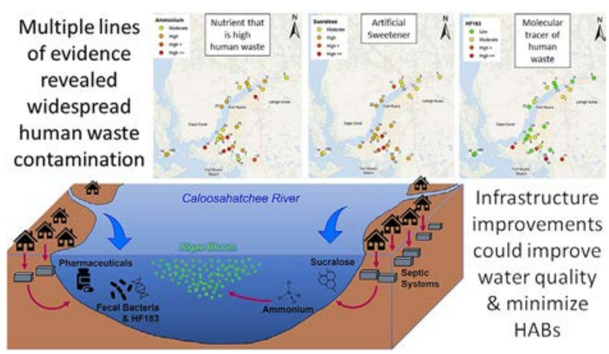
HIGHLIGHTS

- HF183 was detected in 50 % of samples and positively correlated with enterococci.
- Widespread sucralose and carbamazepine demonstrated the influence of human waste.
- High $\delta^{15}N$ in surface water, POM, and macrophytes corroborated a human waste N source.
- Infrastructure improvements may help improve water quality issues in Lee County.

GRAPHICAL ABSTRACT



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ABSTRACT

The coastal communities of Lee County, Florida, USA have grown rapidly since the 1970s. In this county, drainage ditches, canals, creeks, and the Caloosahatchee River Estuary often have high concentrations of nutrients and bacteria limiting their designated uses. Septic systems have previously been identified as a major pollution source in some areas of Lee County; therefore, this study sought to identify the extent of this issue throughout the county. To accomplish this, surface water samples were collected at 25 ditch, creek, or canal sites suspected of human waste contamination from septic systems in various drainage basins throughout Lee County during January 2020–January 2021. Water samples were analyzed for nutrients, dual stable nitrate isotopes ($\delta^{15}N\text{-}NO_3^-$, $\delta^{18}O\text{-}NO_3^-$), fecal indicator bacteria (enterococci, *Escherichia coli*), a molecular tracer of human waste (HF183), and chemical tracers of human waste (the artificial sweetener sucralose, pharmaceuticals). Particulate organic matter (POM) and macrophytes were also collected and analyzed for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, as well as elemental composition (C:N:P). To broaden the assessment of stable isotope values and C:N:P, archived macrophyte samples from 2019 were also included in analyses. Ammonium concentrations were high (> 4.3 μM) in 55 % of samples. Fecal bacteria were high in 66 % of samples. HF183 was detected in 50 % of samples and positively correlated with enterococci ($r = 0.32$). Sucralose concentrations were high (> 380 ng/L) in 54 % of samples, while carbamazepine was detected in 40 % of samples. Human waste N sources were indicated by $\delta^{15}N > 3.00$ ‰ at 44 % of sites by $\delta^{15}N\text{-}NO_3^-$, 68 % of sites by POM, and at 100 % of sites where macrophyte samples were collected. This large-scale study provides evidence of widespread human waste pollution throughout Lee County and can help guide infrastructure improvements to promote sustainable development. These findings should be applicable to urbanized regions globally that are experiencing declines in water quality and harmful algal blooms due to development with inadequate infrastructure.

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1. Introduction

Human activities have significantly degraded water quality in rivers, estuaries, and coastal oceans resulting in environmental and human health issues (Meybeck, 2004). One anthropogenic driver of degradation to aquatic environments is poorly treated human waste. For example, in some environments onsite sewage treatment and disposal systems (septic systems) can be a significant source of nutrient and bacterial pollution via subsurface transport of contaminated groundwater to nearby waterbodies (Lapointe et al., 1990; Lipp et al., 2001; Lapointe et al., 2017; Brewton et al., 2022). In the United States, 20 % of homes (26.1 million) currently rely on septic systems to treat domestic waste (USEPA, 2008).

In Florida, ~40 % of septic systems are found in coastal areas with high water tables (Toor et al., 2011). Septic systems in Lee County, FL exceed 100,000 units (FDOH, 2018) and the Caloosahatchee River Estuary, which bisects the county, is a major discharge area for contaminated groundwater and surface water, especially during the wet season (Wedderburn et al., 1982). High concentrations of nitrogen (N) and phosphorus (P) are characteristic of the urbanized estuary and contribute to decreased dissolved oxygen (DO) levels (Liu et al., 2009), as well as the development and maintenance of harmful algal blooms (HABs; Lapointe and Bedford, 2007; Brewton et al., 2022). Accordingly, there is value in identifying pollution sources to minimize loading into these coastal ecosystems.

Stable N isotope values ($\delta^{15}N$) provide an effective tool for N source identification (Luu et al., 2020). Dual stable isotope analysis of nitrate ($\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$) can discriminate between nitrate (NO_3^-) sources, such as fertilizers and human waste (Heaton et al., 2012). Further, assessment of stable N isotope values ($\delta^{13}C$ and $\delta^{15}N$) in macrophyte tissue can be used to distinguish natural vs. anthropogenic N sources (Lapointe et al., 2015). Specifically, enriched $\delta^{15}N$ values > +3.0 ‰ are indicative of a human/animal waste N source (Costanzo et al., 2001), whereas N from synthetic fertilizers range from −2 ‰ to +2 ‰ (Bateman and Kelly, 2007), atmospheric N ranges from −3 ‰ to +1 ‰ (Paerl and Fogel, 1994), and natural N-fixation source values are close to 0 ‰ (Heaton, 1986; Costanzo et al., 2001). In Great South Bay, Long Island, human waste from septic systems accounts for 67 % of the terrestrial N loads to the coast compared to 7 % for fertilizers (Kinney and Valiela, 2011). While on Cape Cod, MA, septic systems contribute 48 % of the N loads to Waquoit Bay, compared to 29 % for atmospheric deposition and 16 % for fertilizers (Valiela et al., 1997; Valiela et al., 2000).

Microbial source tracking methods are also useful for determining pollution sources, and commonly involve the use of the HF183 molecular marker. HF183 is found within the 16S rRNA gene of the *Bacteroides* sp. that resides exclusively in the human colon as part of the normal microbiota. Thus, detection of the HF183 molecular marker serves as a definitive indicator of human fecal pollution (Ahmed et al., 2019) with some limitations. Humic acid, which can be prevalent in freshwater, is detrimental to qPCR analysis (Green and Field, 2012) and the half-life of HF183 is relatively short from 0.4 to 8 days (Walters and Field, 2009). Therefore, it is useful to supplement HF183 testing with other methods.

Chemical tracers of human waste provide another method for determining pollution sources. The artificial sweetener sucralose is an organochlorine that is not recognized by the human body as sugar, therefore 85 to 92 % of the amount consumed is excreted primarily in the feces with smaller losses in urine (Lewis and Tzilivakis, 2021). Thus, widespread consumption of sucralose in conjunction with its resistance to degradation makes it a reliable indicator of human waste contamination (Scheurer et al., 2009). In Florida, multiple studies have used sucralose to identify areas where septic systems are leaching into groundwater and surface water (Lapointe et al., 2017; Herren et al., 2021; Brewton et al., 2022). Similarly, the detection of pharmaceutical compounds in the environment provides another useful tracer of human waste pollution (Tran et al., 2014). For example, the anti-seizure medicine, carbamazepine is regularly detected in domestic waste (Luo et al., 2014). Additionally, the pain medicine, acetaminophen has low removal in septic systems with detection rates up to 83 % in effluent (Yang et al., 2017). This makes acetaminophen another useful tracer of septic system contamination.

This study combines the use of stable isotopes, molecular markers, and chemical tracers of human waste to identify sources of nutrient and bacterial pollution in Lee County surface water. These data will allow for the identification of locations contaminated by human waste from septic system inputs. Additionally, this information will help to better understand the drivers of degraded surface water quality and worsening HABs throughout Lee County.

2. Materials and methods

2.1. Study sites and sample collection

Twenty-four surface water sites near septic systems and one “reference” site without nearby septic systems (Riverside Lane) were selected in Lee County in an area spanning ~800 km^2 (Fig. 1; see Supplemental Figs. S1–23). All sites had primarily residential / urban land use and were known or suspected to be impaired for nutrients and/or bacteria based on long-term water quality monitoring by Lee County.

Rainfall data for Lee County from December 1, 2019, to January 31, 2021, were downloaded from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (ncdc.noaa.gov/data-access). Fourteen weather stations within Lee County were selected to calculate daily rainfall. These stations included US1FLLE0006, US1FLLE0010, US1FLLE0022, US1FLLE0029, US1FLLE0037, US1FLLE0055, US1FLLE0057, US1FLLE0058, US1FLLE0059, US1FLLE0062, US1FLLE0065, US1FLLE0068, USW00012835, and USW00012894.

Sampling was conducted eight times with dry season events in January, February, late November/December 2020, and January 2021, while wet season sampling occurred in July, August, September, and October/early November 2020. Ligon Court was replaced by a Pine Island 2 in August 2020 due to low water levels that prevented adequate sample collection. At each site, salinity, pH, DO, and temperature were measured in situ by placing a calibrated YSI ProPlus or Pro130 multiparameter sonde 0.3 m below the surface of the water and waiting for readings to stabilize.

Singular water samples were collected into clean high-density polyethylene bottles for analysis of HF183, enterococci, *Escherichia coli*, ammonium (NH_4^+), nitrate + nitrite (NO_x), total Kjeldahl nitrogen (TKN), soluble reactive phosphorus (SRP), and total phosphorus (TP). Upon collection, enterococci and *E. coli* samples were preserved with sodium thiosulfate, NH_4^+, NO_x, TKN, and TP samples were acidified to a pH of <2 with sulfuric acid, and SRP samples were field filtered (0.45 μm). Water samples were also collected into amber glass bottles for analysis of sucralose, acetaminophen, carbamazepine, ibuprofen, and naproxen. All water samples were immediately submerged in ice in a dark cooler. Samples were transported to the lab within required holding times for analysis of target analytes. During the five sampling events between August 2020 – January 2021, additional water samples were field-filtered (0.45-μm) into vials containing 18 % aqueous hydrochloric acid solution to lower the pH to <2 for analysis of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$. Particulate organic matter (POM) samples were collected in triplicate during all eight sampling events for analysis of $\delta^{13}C$, $\delta^{15}N$, %C, %N, and %P. POM samples were coarse filtered (200 μm) filter to remove macrodetritus, collected into HDPE bottles, and transported on ice to the lab. When present, algae and/or aquatic plants (macrophytes hereafter) were collected in triplicate (if biomass was sufficient) at sampling sites and other additional locations throughout the Lee County study area for analysis of %C, %N, %P, $\delta^{13}C$, and $\delta^{15}N$, placed in clean plastic bags, and stored on top of a towel in a cooler of ice for transport back to the lab. Archived macrophyte samples collected in 2019 were included in analyses to broaden the scope of the stable isotope and C:N:P assessment.

2.2. Sample analysis

Water samples for enterococci, *E. coli*, NH_4^+, NO_x, SRP, TN, and TP were analyzed by the National Environmental Laboratory Accreditation Program (NELAP) certified Lee County Environmental Laboratory, Fort Myers, FL

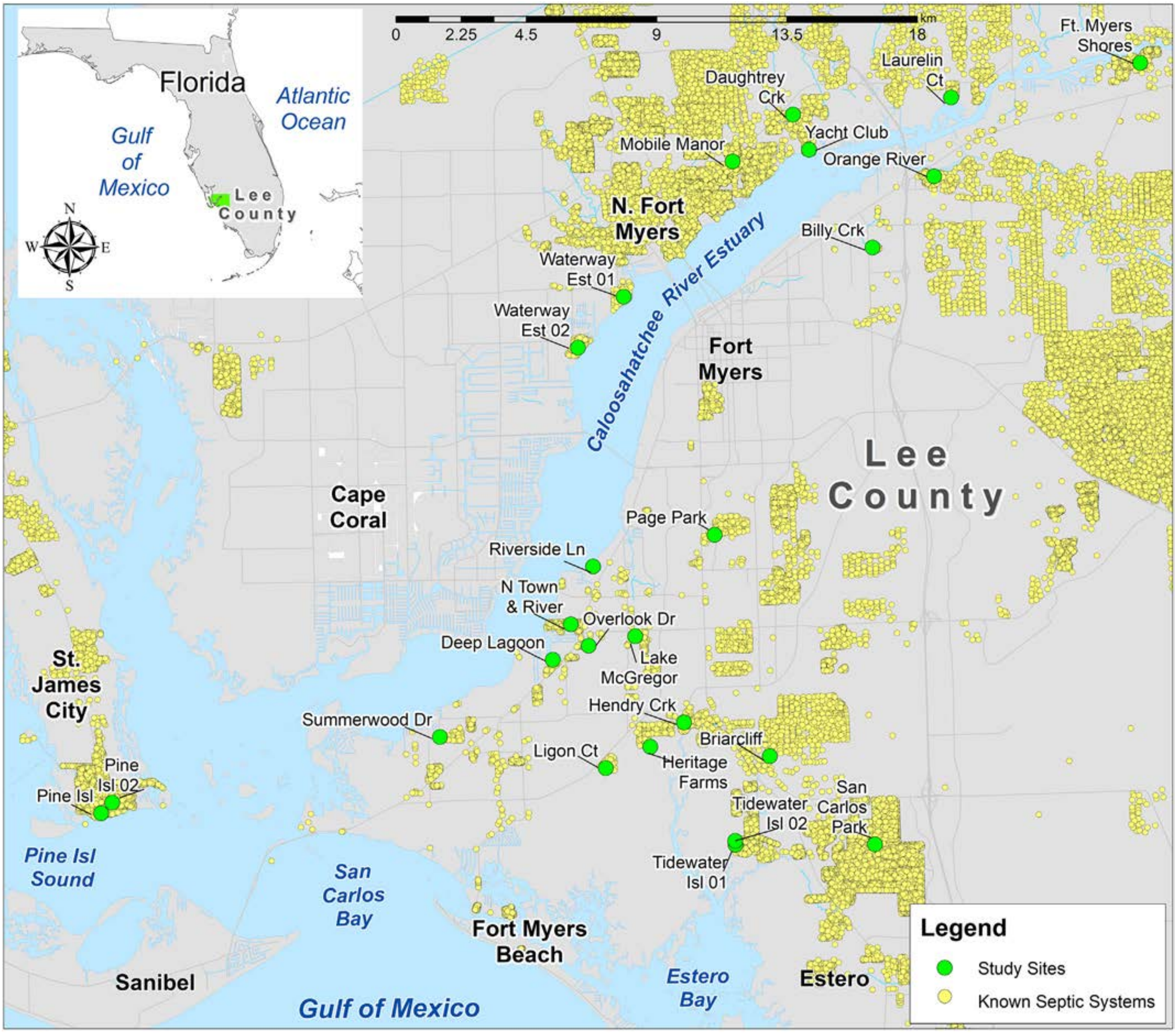

Fig. 1. Surface water sites (green circles) and known septic systems (yellow dots) in Lee County, Florida.

using the following standard methods: Enterolert™ for enterococci (ASTM D6503-19), Colilert-18™ for *E. coli* (ISO standard 9308-2:2012), EPA Method 350.1 for NH_4^+, EPA Method 353.2 for NO_x, EPA Method 365.1 for SRP and TP, and EPA Method 351.2 for TKN. Dissolved inorganic nitrogen (DIN) was calculated as $NH_4^+ + NO_x$. Total Nitrogen (TN) was calculated as TKN + NO_x. δ^{15}N-NO_3^- and δ^{18}O-NO_3^- samples were analyzed by Beta Analytic Inc., Miami, FL using the reduction of NO_x to nitrous oxide followed by continuous flow mass spectrometry (Altabet et al., 2019; Casciotti et al., 2002). Only two of five samples from Pine Island, Pine Island 02, and San Carlos Park were analyzed for δ^{15}N-NO_3^- and δ^{18}O-NO_3^- because NO_x concentrations were below the minimum required for analysis (2.86 μM). No samples from Summerwood Drive were assessed for δ^{15}N-NO_3 due to low NO_x concentrations and Ligon Court was not assessed for δ^{15}N-NO_3^- due to no overlapping dates. HF183 samples were analyzed by LuminUltra Technologies using a Bio-Rad QX200 Droplet Digital PCR System (Cao et al., 2015) and quantified for HF183 by Poisson Distribution Analysis (Sivaganesan et al., 2011). Sucralose, acetaminophen, carbamazepine, ibuprofen, and naproxen samples were analyzed by the NELAP certified Florida Department of Environmental Protection (FDEP) Laboratory, Tallahassee, FL using high performance liquid chromatography coupled with thermospray mass spectrometry and an ultraviolet detector (USEPA Method 8321B). For method detection limits see Tables 2-4.

POM samples were vacuum filtered onto pre-combusted 0.45-μm glass fiber filters, dried at ~60 °C, and halved. One half was analyzed at the University of Georgia's Stable Isotope Ecology Laboratory for %C, %N, δ^{13}C, and δ^{15}N by Isotope Ratio Mass Spectrometer (Thermo Delta V Environmental Analysis) coupled to a Carlo Erba NA1500 CHN Elemental Analyzer via a Thermo Conflo III Interface. The other half was analyzed at the University of Missouri's Soil and Plant Testing Laboratory for %P by Inductively Coupled Plasma Atomic Emission Spectroscopy (Viso and Zachariadis, 2018). Macrophyte tissue were rinsed briefly in DI water, cleaned, dried, and finely ground with mortar and pestle, then analyzed similarly to POM samples.

2.3. Data analysis

Water quality parameters were assigned thresholds to provide context (see "threshold" in Tables 2-4). The FDEP Ten Percent Threshold Value (TPTV) for Peninsular streams (FDEP 62-302) was the threshold for TN (> 110 μM) and

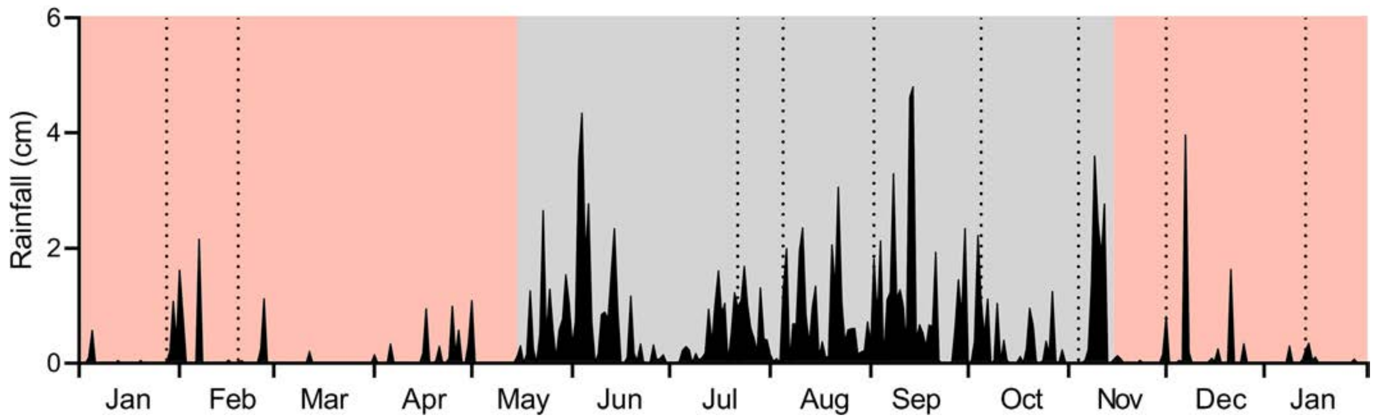

Fig. 2. Daily precipitation (mean) across fourteen weather stations within Lee County, FL from January 2020 to January 2021. Dashed bars represent sampling events. Red and grey shaded areas represent dry and wet seasons, respectively.

TP (> 3.9 μM), while the Class III water TPTV was used for enterococci (> 130 MPN/100 mL) and *E. coli* (> 410 MPN/100 mL). For $\delta^{15}N-NO_3^-$, values > +3 ‰ indicated a human/animal waste NO_3^- source and $\delta^{15}N-NO_3^-$ values < +2 ‰ indicated a synthetic fertilizer source. $\delta^{15}N-NO_3^-$ values between +2 to 7 ‰ could also indicate mixed NO_3^- sources and involved the consideration of additional data. For $\delta^{18}O-NO_3^-$, the threshold was defined as < +15 ‰ to indicate human/animal waste, soil organics, and/or reduced ammonia fertilizer sources. $\delta^{13}C$ values <-25 ‰ were considered to have a terrestrial or C_3 plant C source, while $\delta^{13}C$ values > -25 ‰ were attributed to more of a C_4 or marine C source (Peterson and Fry, 1987). $\delta^{15}N$ values of POM and macrophytes were interpreted as having a human/animal waste N source if > +3.0 ‰ (Costanzo et al., 2001). DIN:SRP, TDN:TDP, and POM C:N:P data were compared to the Redfield ratio (106:16:1; Redfield, 1958), while macrophyte C:N:P were compared to a modified ratio, where C:*N* > 13 and N:P < 30 represents N-limitation, and C:P > 350 and N:P > 30 represents P-limitation (Lapointe et al., 2021). For HF183, the threshold was 525 copies/100 mL (Boehm and Soller, 2020). For other parameters, thresholds were derived from statewide 2010–2021 water quality data for Florida from the FDEP Watershed Information Network database and considered "high" if > the 3rd quartile value.

For nutrient and bacterial parameters, half of the method detection limit was substituted for data below detection limits for calculation of means, while raw values were used in non-parametric analyses (Helsel, 2005). The following replacements were made for NH_4^+: 23 out of 192 samples and for NO_X: 22 out of 192 samples. For chemical tracers, any results below the minimum detection limit were considered as non-detects (Silvanima et al., 2018) and replaced with zeros for analyses. Estimated values and data between the detection limit and the practical quantitation limit were included in data analyses (Helsel, 2005). *t*-tests or, if parametric assumptions were not met, the non-parametric Mann-Whitney *U* tests were used to determine if parameters varied significantly between seasons. Log transformation was attempted for all parameters having <15 % non-detects before non-parametric statistics were employed. The non-parametric Spearman's rank-order correlation was used to assess monotonic associations between variables. Only significant correlations with r > 0.30 are described and correlations between mathematically related variables are not discussed. Differences were considered significant at p < 0.05. Analyses were conducted in SPSS v.28. Values are presented as mean ± SE unless noted.

3. Results

3.1. Rainfall

Rainfall during the study period was seasonally variable (Fig. 2). A cumulative total of 157 cm of rainfall was recorded from January 1, 2020, to January 31, 2021. A wet season from approximately May 15 to November 15, 2020, was evident, with some anomalous rainfall events occurring outside of this window. A cumulative total of 134 cm of rainfall was recorded during the wet season with a daily mean of 0.74 cm, whereas a cumulative total of 23.0 cm of rainfall was recorded during the dry season with a daily mean of 0.10 cm. The cumulative rainfall 10 days prior to the wet season sampling events were 7.57, 4.17, 5.97, 7.87, and 2.03 cm for July, August, September, October, and November, respectively. Dry seasons persisted from January 1 to May 14, 2020, and from November 16, 2020, to January 31, 2021. The cumulative rainfall 10 days prior to the dry season sampling events were 0.05, 0.08, 0.51, and 0.43 cm for January (2020), February, December, and January (2021), respectively.

3.2. Environmental parameters

Surface water measurements for environmental parameters, including pH, salinity, DO, and temperature are displayed in Table 1. Mean pH ranged from 7.05 ± 0.12 to 7.80 ± 0.06 and was significantly higher in the dry season (7.55 ± 0.03) than the wet season (7.43 ± 0.03; *t*-test, p = 0.004). Mean salinity ranged from 0.3 ± 0.02 to 25.3 ± 2.3 PSU with no significant difference between the dry season (4.7 ± 0.7 PSU) and wet season (4.0 ± 0.8; t-test, p = 0.51 PSU). Mean DO concentrations ranged from 1.11 ± 0.15 to 6.58 ± 0.40 mg/L and were significantly higher in the dry season (4.74 ± 0.20 mg/L) than the wet season (3.29 ± 0.17 mg/L; t-test, p < 0.001). Mean temperature ranged from 20.7 ± 1.7 °C to 27.4 ± 1.7 °C and was significantly lower in the dry season (20.7 ± 0.3 °C) than the wet season (27.90 ± 0.3 °C; t-test, p < 0.001).

3.3. Nutrient concentrations and stable isotopes

High concentrations of nutrients were often observed (Table 2). NH_4^+ was generally elevated, ranging from below detection to 95.4 μM with a mean of 9.45 ± 1.0 μM and a median of 4.79 μM. High concentrations of NH_4^+ (> 4.3 μM) were detected in 55 % of samples and at 96 % of sites. NO_x concentrations ranged from below detection to 34.8 μM with a mean of 7.90 ± 0.51 μM and a median of 6.07 μM. High concentrations of NO_x (> 15 μM) were detected in 16 % of samples and at 52 % of sites. SRP concentrations ranged from below detection to 10.3 μM with a mean of 1.98 ± 0.14 μM and a median of 1.45 μM. High concentrations of SRP (> 2.6 μM) were detected in 27 % of samples and at 56 % of sites. DIN:SRP ranged from 0.40 to 240 with a mean of 26.4 ± 3.1 and a median of 9.6. DIN:SRP > 30 were detected in 24 % of samples and at 44 % of sites. TN concentrations ranged from 27.9 to 243 μM with a mean of 79.2 ± 2.5 μM and a median of 71.1 μM. Concentrations of TN > 110 μM were detected in 12 % of samples and at 36 % of sites. TP ranged from 0.48 to 12.3 μM with a mean of 3.54 ± 0.18 μM and a median of 2.94 μM. Concentrations of TP > 3.9 μM

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Table 1
Site information including waterbody type, watershed, and environmental data measured during collections, including pH, salinity, dissolved oxygen (DO), and temperature (mean ± standard error). Riverside Lane is highlighted to indicate there are no nearby septic systems.

Site	Waterbody Type	Watershed	Septic Systems Within 300m	Salinity (PSU)	pH	DO (mg/L)	Temperature (°C)
Billy Creek	creek	Caloosahatchee	23	0.3 ± 0.0	7.51 ± 0.09	3.25 ± 0.29	24.6 ± 2.22
Briarcliff	canal	Hendry Creek	7	0.3 ± 0.0	7.53 ± 0.04	4.10 ± 0.49	26.0 ± 1.60
Daughtrey Creek	ditch	Caloosahatchee	53	0.8 ± 0.3	7.62 ± 0.05	3.39 ± 0.48	24.0 ± 1.61
Deep Lagoon	canal	Caloosahatchee	39	10.8 ± 2.6	7.64 ± 0.09	5.79 ± 0.58	24.9 ± 1.94
Fort Myers Shores	canal	Caloosahatchee	86	0.8 ± 0.3	7.38 ± 0.08	4.47 ± 0.40	23.4 ± 1.57
Hendry Creek	creek	Hendry Creek	53	3.8 ± 0.9	7.41 ± 0.03	2.38 ± 0.36	23.7 ± 1.55
Heritage Farms	ditch	Caloosahatchee	15	0.7 ± 0.0	7.47 ± 0.04	2.17 ± 0.32	23.5 ± 1.60
Lake McGregor	ditch	Caloosahatchee	20	0.4 ± 0.0	7.39 ± 0.12	1.86 ± 0.40	22.4 ± 1.68
Laurelin Court	canal	Caloosahatchee	43	1.1 ± 0.5	7.47 ± 0.05	4.61 ± 0.58	24.4 ± 1.90
Ligon Court	ditch	Hendry Creek	18	1.3 ± 0.1	7.32 ± 0.07	3.15 ± 1.06	20.7 ± 3.54
Mobile Manor	ditch	Caloosahatchee	153	1.0 ± 0.4	7.80 ± 0.06	2.81 ± 0.49	24.4 ± 1.42
North Town & River	canal	Caloosahatchee	65	10.8 ± 2.7	7.75 ± 0.06	6.58 ± 0.40	24.7 ± 1.90
Orange River	ditch	Caloosahatchee	87	1.1 ± 0.4	7.64 ± 0.04	3.32 ± 0.43	27.4 ± 1.69
Overlook Drive	ditch	Caloosahatchee	36	0.5 ± 0.0	7.27 ± 0.18	4.25 ± 0.49	22.3 ± 2.13
Page Park	ditch	Caloosahatchee	42	0.6 ± 0.0	7.54 ± 0.09	4.81 ± 0.68	24.7 ± 1.53
Pine Island	canal	Caloosahatchee	106	25.3 ± 2.3	7.05 ± 0.12	4.42 ± 0.67	24.9 ± 1.82
Pine Island 02	canal	Caloosahatchee	116	23.4 ± 3.3	7.42 ± 0.12	2.48 ± 0.66	26.2 ± 2.30
Riverside Lane	canal	Caloosahatchee	0	7.2 ± 2.2	7.66 ± 0.07	6.16 ± 0.53	25.2 ± 1.85
San Carlos Park	ditch	Hendry Creek	152	0.3 ± 0.0	7.41 ± 0.06	1.11 ± 0.15	26.2 ± 1.60
Summerwood Drive	ditch	Caloosahatchee	11	0.6 ± 0.0	7.18 ± 0.14	2.48 ± 0.58	21.4 ± 1.68
Tidewater Island 01	canal	Hendry Creek	59	6.6 ± 1.6	7.49 ± 0.05	4.57 ± 0.49	24.1 ± 1.69
Tidewater Island 02	canal	Hendry Creek	47	6.5 ± 1.5	7.65 ± 0.05	4.63 ± 0.62	23.5 ± 1.94
Waterway Estates 01	canal	Caloosahatchee	54	4.7 ± 1.5	7.37 ± 0.06	4.81 ± 0.55	24.6 ± 1.77
Waterway Estates 02	canal	Caloosahatchee	70	4.8 ± 1.5	7.65 ± 0.08	6.08 ± 0.66	24.5 ± 1.89
Yacht Club	canal	Caloosahatchee	62	1.5 ± 0.7	7.56 ± 0.06	5.37 ± 0.52	24.9 ± 1.80

were detected in 35 % of samples and at 64 % of sites. TN:TP ranged from 4.84 to 115 with a mean of 31.9 ± 1.4 and a median of 26.6. TN:TP > 30 were observed in 41 % of samples and at 36 % of sites. NH_4^+, SRP, TN, and TP concentrations were significantly (Mann-Whitney *U* tests, all $p < 0.001$) higher in the wet season the dry season. Significant seasonal differences were not observed in NO_x concentrations, DIN:SRP, or TN:TP (Mann-Whitney U tests, all $p > 0.05$).

Surface water $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were sometimes useful in discriminating NO_3^- sources, though many samples fell into the mixed source range (Fig. 3a). $\delta^{15}N\text{-}NO_3^-$ values ranged from −6.48 to +14.5 ‰ with a mean of +4.64 ± 0.34 ‰ and a median of +4.38 ‰. $\delta^{15}N\text{-}NO_3^-$ values > +3.00 ‰ were detected in 74 % of the samples with high enough NO_x concentrations to be analyzed (92 out of 191 samples) and at 44 % of sites. $\delta^{15}N\text{-}NO_3^-$ values between +2 and 7 ‰ were detected in 66 % of samples and at 84 % of sites (Fig. 3b). $\delta^{15}N\text{-}NO_3^-$ values <2 ‰ were observed in 16 % of samples and at 36 % of sites. $\delta^{18}O\text{-}NO_3^-$ values ranged from −22.9 to 24.2 ‰ with a mean of +6.53 ± 0.58 ‰ and a median of +6.63 ‰. There were no sites with mean $\delta^{18}O\text{-}NO_3^-$ values in the range of synthetic NO_3^- fertilizer or precipitation (Fig. 3a). Significant seasonal differences were not observed in $\delta^{15}N\text{-}NO_3^-$ or $\delta^{18}O\text{-}NO_3^-$ values (Mann-Whitney *U* tests, both $p > 0.05$).

POM chemical properties provided insight into available nutrient sources (Table 3). POM $\delta^{13}C$ values ranged from −39.2 to −18.0 ‰ with a mean of −28.9 ± 0.25 ‰ and a median of −28.8 ‰. POM $\delta^{13}C$ values > −25 ‰ were detected in 10 % of samples and at 36 % of sites. POM $\delta^{15}N$ values ranged from −3.07 to +16.0 ‰ with a mean of

Table 2
Dissolved nutrients and nutrient ratios by site in Lee County, FL (mean ± standard error), including ammonium (NH_4^+), nitrate + nitrite (NO_x), soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN) to SRP ratio (DIN:SRP), total nitrogen (TN), total phosphorus (TP), and TN to TP ratio (TN:TP). Thresholds are displayed to provide context for measured values and values in bold font exceed threshold values. Riverside Lane is highlighted to indicate that there are no nearby septic systems.

Site	NH_4^+ (μM)	NO_x (μM)	SRP (μM)	DIN:SRP	TN (μM)	TP (μM)	TN:TP
Method Detection Limit	1.00	0.71	0.13	N/A	7.14	0.19	N/A
Threshold	*>4.3*	*>15.1*	*>2.60*	*>30.0*	*>110*	*>3.90*	*>30.0*
Billy Creek	**16.8 ± 3.9**	**17.1 ± 3.7**	**4.10 ± 0.60**	10.7 ± 2.7	93 ± 6	**7.51 ± 0.95**	14.19 ± 2.2
Briarcliff	**4.6 ± 0.8**	6.5 ± 1.4	0.09 ± 0.03	**140 ± 24**	63 ± 2	1.02 ± 0.11	**67.35 ± 7.8**
Daughtrey Creek	**4.5 ± 1.1**	6.3 ± 1.4	**4.50 ± 0.88**	3.0 ± 0.8	69 ± 5	**6.17 ± 0.84**	12.72 ± 1.8
Deep Lagoon	2.8 ± 0.8	5.9 ± 2.6	1.68 ± 0.24	4.3 ± 1.1	68 ± 8	2.55 ± 0.29	27.18 ± 2.2
Ft Myers Shores	2.6 ± 0.9	10.0 ± 2.1	1.80 ± 0.51	9.0 ± 1.6	69 ± 7	3.03 ± 0.61	27.89 ± 4.8
Hendry Creek	**6.9 ± 0.8**	5.3 ± 0.9	0.77 ± 0.15	**30.6 ± 16**	65 ± 1	2.05 ± 0.14	**32.48 ± 1.9**
Heritage Farms	**25.7 ± 3.5**	13.9 ± 1.8	0.81 ± 0.24	**82.7 ± 19**	**113 ± 5**	2.09 ± 0.28	**58.77 ± 5.9**
Lake McGregor	**9.2 ± 2.4**	6.5 ± 2.8	1.05 ± 0.25	16.5 ± 4.4	61 ± 4	2.39 ± 0.34	32.44 ± 7.7
Laurelin Court	**4.5 ± 1.0**	11.3 ± 1.7	**3.05 ± 0.68**	6.4 ± 1.2	91 ± 6	**5.05 ± 0.70**	19.86 ± 2.3
Ligon Court	**43.5 ± 22**	5.0 ± 2.6	0.84 ± 0.23	**59.9 ± 21.6**	**195 ± 29**	**3.96 ± 1.22**	**59.6 ± 17**
Mobile Manor	3.0 ± 0.4	12.0 ± 2.1	**4.47 ± 1.1**	5.1 ± 1.2	59 ± 3	**7.06 ± 0.89**	9.03 ± 0.94
North Town & River	1.8 ± 0.5	7.4 ± 3.3	1.97 ± 0.31	4.2 ± 1.3	71 ± 8	2.86 ± 0.35	25.85 ± 2.3
Orange River	3.8 ± 0.7	14.4 ± 1.8	**3.36 ± 0.52**	6.3 ± 0.9	89 ± 2	**4.68 ± 0.57**	20.89 ± 2.2
Overlook Drive	**25.8 ± 6.0**	7.4 ± 1.4	1.55 ± 0.48	**54.5 ± 27**	**156 ± 13**	**5.93 ± 0.62**	28.46 ± 3.2
Page Park	**6.4 ± 0.8**	5.4 ± 1.2	0.34 ± 0.07	**44.3 ± 8.7**	43 ± 2	1.12 ± 0.16	**43.19 ± 6.1**
Pine Island	4.0 ± 1.7	2.2 ± 0.8	0.68 ± 0.15	9.3 ± 2.2	61 ± 5	1.61 ± 0.14	**38.63 ± 2.8**
Pine Island 02	**7.4 ± 3.9**	2.6 ± 1.2	0.99 ± 0.27	8.3 ± 2.4	71 ± 7	2.38 ± 0.47	31.63 ± 2.6
Riverside Lane	3.3 ± 1.5	10.1 ± 3.9	2.41 ± 0.41	5.2 ± 1.7	78 ± 10	**3.69 ± 0.53**	21.99 ± 2.0
San Carlos Park	**12.3 ± 3.3**	8.9 ± 3.5	0.38 ± 0.08	**74.0 ± 14**	51 ± 3	1.15 ± 0.14	**49.98 ± 7.3**
Summerwood Drive	**42.6 ± 12**	0.6 ± 0.2	**3.18 ± 0.62**	14.7 ± 4.4	**114 ± 14**	**5.06 ± 0.57**	22.92 ± 2.6
Tidewater Island 01	4.2 ± 0.6	3.9 ± 0.8	0.38 ± 0.12	29.7 ± 5.7	55 ± 4	1.00 ± 0.10	**59.93 ± 7.1**
Tidewater Island 02	3.4 ± 0.7	4.0 ± 0.9	0.21 ± 0.05	**38.3 ± 3.3**	63 ± 8	1.54 ± 0.48	**50.55 ± 6.5**
Waterway Estates 01	**8.6 ± 2.5**	10.7 ± 3.1	**4.10 ± 0.79**	4.6 ± 0.9	87 ± 8	**5.78 ± 1.03**	17.52 ± 2.5
Waterway Estates 02	**5.2 ± 1.4**	9.7 ± 3.5	**2.97 ± 0.52**	4.4 ± 1.1	83 ± 10	**4.55 ± 0.76**	20.58 ± 2.8
Yacht Club	3.2 ± 0.9	7.0 ± 1.1	**2.76 ± 0.51**	4.3 ± 0.9	79 ± 4	**4.20 ± 0.55**	20.54 ± 2.2

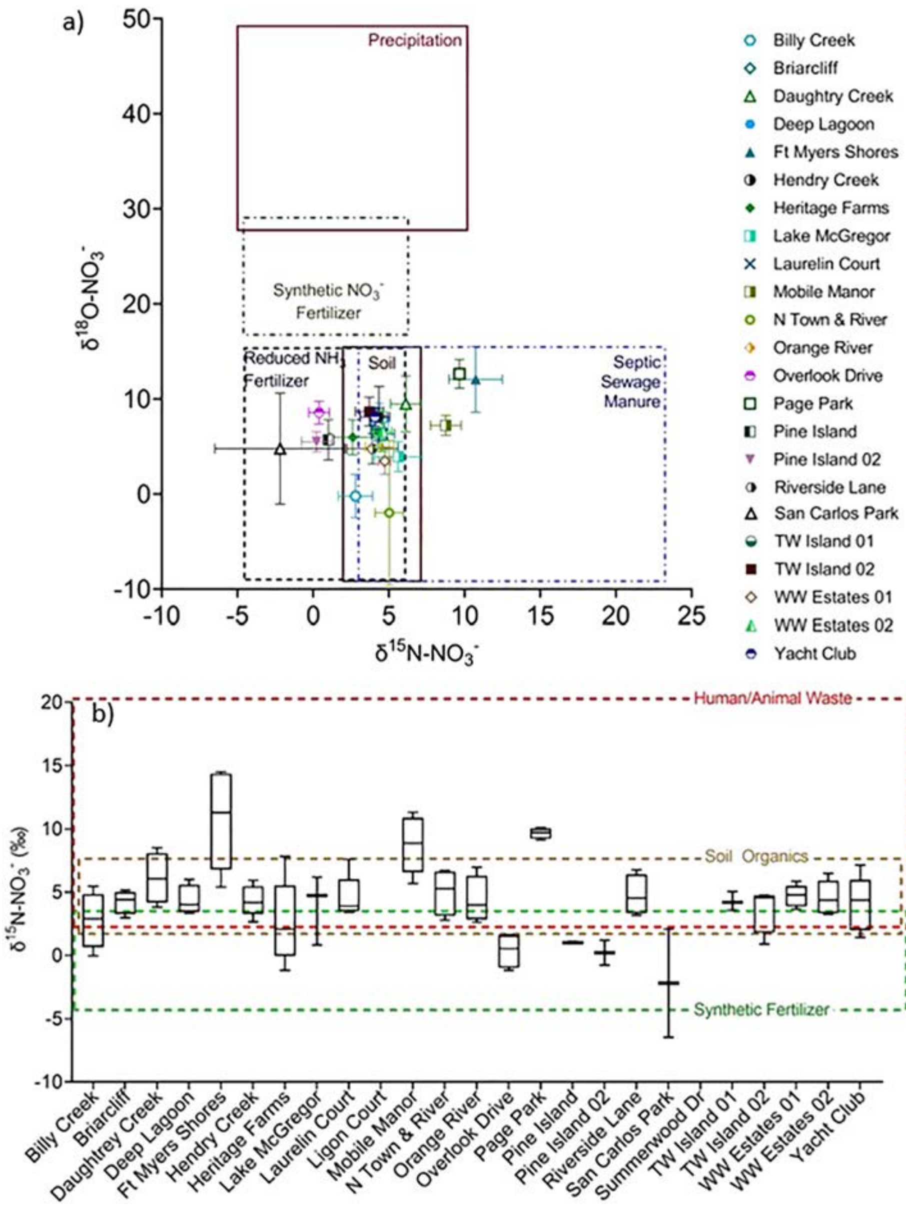

Fig. 3. a) Dual stable nitrogen isotope values of nitrate (δ^{15}N- NO_3^- and δ^{18}O-NO_3^-, mean ± SE) and b) stable nitrogen isotope values of nitrate (δ^{15}N-NO_3) observed by site in Lee County, FL. The colored boxes in panels represent the ranges for potential contributing sources of nitrate (NO_3^-).

+4.02 ± 0.22 ‰ and a median of +4.07 ‰. POM δ^{15}N values > +3.0 ‰ were detected in 67 % of samples and at 68 % of sites. POM N:P ranged from 1.83 to 148 with a mean of 15.1 ± 0.89 and a median of 14.9. POM N:P > 30 was observed in 2 % of samples and at 8 % of sites, while POM N:P < 15 was observed in 51 % of samples and at 92 % of sites. POM C:N ranged from 2.39 to 27.7 with a mean of 9.23 ± 0.24 and a median of 8.56. POM C:*N* < 6.6 was observed in 15 % of samples and at 60 % of sites. POM C:P ranged from 21.4 to 1083 with a mean of 124 ± 6.2 and a median of 114. POM C:P < 106 were observed in 41 % of samples and at 88 % of sites. There were no significant seasonal differences observed for any POM chemical properties (Mann-Whitney *U* tests, all p > 0.05).

Macrophyte chemical composition was also useful for understanding sources and availability of nutrients (Table 4, Fig. 4). Macrophyte δ^{13}C values at study sites ranged from −37.6 to −11.7 ‰ with a mean of −24.5 ± 0.88 ‰ and a median of −22.1 ‰. Macrophyte δ^{13}C values < −25 ‰ were observed at six study sites (Table 4). Beach macrophytes had slightly more enriched δ^{13}C values with a mean of −19.4 ± 1.6 ‰ and a median of −21.0 ‰. Macrophyte δ^{15}N values at study sites ranged from +1.66 to +18.4 with a mean of +7.66 ± 0.42 ‰ and a median of +8.74 ‰. Macrophyte δ^{15}N values > +3.0 ‰ were observed at all nine study sites that were sampled (Table 4). Beach macrophytes had slightly more depleted δ^{15}N values with a mean of +3.59 ± 0.47 ‰ and a median of +4.42 ‰. Macrophyte N:P at study sites ranged from 12.4 to 71.1 with a mean of 28.0 ± 1.6 and a median of 24.1. Three study sites had a mean N:P > 30 (Table 4). Beach macrophytes had slightly lower N:P with a mean of 25.7 ± 4.3 and a median of −23.3. Macrophyte C:N ranged from 11.4 to 27.7 at study sites with a mean of 17.0 ± 0.56 and a median of 15.0. Two study sites had mean C:*N* < 13. Beach macrophytes had slightly higher C:N with a mean of 18.6 ± 3.4 and a median of 13.7. Macrophyte C:P ranged from 165 to 1700 at study sites with a mean of 516 ± 47 and a median of 356. Two study sites had mean C:P < 350 (Table 4). Beach macrophytes had slightly lower C:P with a mean of 464 ± 95 and a median of 248.

For sites where macrophytes were collected in both seasons, some significant differences were observed. At Page Park in the dry season, the algal mat had significantly higher (Mann-Whitney *U* tests, all p < 0.05) δ^{13}C (−16.6 ± 0.46 ‰), C:N (26.8 ± 0.42), C:P (1,013 ± 8.4), and N:P (37.8 ± 0.28) compared to the wet season (−20.0 ± 0.99 ‰, 17.9 ±

Table 3
Chemical properties of particulate organic matter (POM), a proxy for phytoplankton, by site in Lee County, FL (mean ± standard error), including stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), as well as elemental ratios of nitrogen to phosphorus (N:P), carbon to nitrogen (C:N), and carbon to phosphorus (C:P). Thresholds are displayed to provide context for measured values and values in bold font exceed threshold values. Riverside Lane is highlighted to indicate that there are no nearby septic systems.

Site	POM $\delta^{13}C$ (‰)	POM $\delta^{15}N$ (‰)	POM N:P	POM C:N	POM C:P
Threshold	*>-25*	*>+3.00*	*>30*	*<6.6*	*<106*
Billy Creek	-27.5 ± 0.2	+2.54 ± 0.7	6.9 ± 2.7	13.2 ± 0.8	**91.3 ± 37**
Briarcliff	-33.5 ± 0.7	+2.46 ± 0.7	17.6 ± 0.8	8.00 ± 0.4	140 ± 8
Daughtrey Creek	-29.5 ± 1.2	+2.62 ± 1.0	11.0 ± 1.4	9.87 ± 1.0	**102 ± 9**
Deep Lagoon	-29.0 ± 0.7	**+5.95 ± 0.8**	14.0 ± 2.9	7.42 ± 0.9	**88.4 ± 15**
Fort Myers Shores	-31.3 ± 1.1	**+6.89 ± 1.8**	14.4 ± 0.7	8.89 ± 0.9	126 ± 12
Hendry Creek	-32.4 ± 0.5	+2.76 ± 0.5	17.2 ± 1.5	7.73 ± 0.5	133 ± 15
Heritage Farms	-33.4 ± 0.6	+1.41 ± 0.5	17.1 ± 1.3	8.00 ± 0.4	133 ± 7
Lake McGregor	-27.6 ± 0.3	**+5.32 ± 0.8**	7.28 ± 1.1	9.95 ± 1.0	**67.2 ± 7**
Laurelin Court	-28.8 ± 0.4	**+6.75 ± 1.5**	16.6 ± 1.4	11.0 ± 0.9	176 ± 11
Ligon Court	**-24.2 ± 0.7**	**+4.30 ± 1.0**	**68.7 ± 40**	**6.39 ± 2.1**	448 ± 318
Mobile Manor	-26.6 ± 0.5	**+4.41 ± 1.1**	5.40 ± 0.8	12.6 ± 1.1	**62.2 ± 5**
North Town & River	-28.1 ± 0.3	**+8.17 ± 1.5**	14.7 ± 2.4	7.66 ± 0.9	106 ± 16
Orange River	-29.0 ± 0.7	**+5.59 ± 1.0**	15.5 ± 1.4	9.73 ± 0.7	151 ± 18
Overlook Drive	-26.6 ± 0.4	**+3.63 ± 0.6**	19.3 ± 0.9	8.32 ± 0.3	160 ± 9
Page Park	-26.8 ± 0.5	**+3.80 ± 0.6**	12.5 ± 1.1	10.5 ± 1.3	117 ± 14
Pine Island	**-20.7 ± 0.5**	**+4.04 ± 1.0**	15.4 ± 1.8	8.47 ± 0.5	127 ± 15
Pine Island 02	**-23.2 ± 0.8**	+1.58 ± 0.8	15.2 ± 1.9	7.86 ± 0.4	117 ± 12
Riverside Lane	-26.9 ± 0.4	**+5.17 ± 0.9**	11.8 ± 1.7	8.37 ± 1.0	**89.4 ± 8**
San Carlos Park	-28.6 ± 0.2	+2.40 ± 1.2	7.31 ± 1.1	15.0 ± 2.9	**94.3 ± 9**
Summerwood Drive	-30.3 ± 1.2	**+3.39 ± 0.8**	15.2 ± 1.1	8.19 ± 0.7	120 ± 10
Tidewater Island 01	-30.8 ± 0.6	+2.65 ± 0.7	15.6 ± 1.7	8.62 ± 0.8	133 ± 19
Tidewater Island 02	-32.1 ± 1.1	**+3.16 ± 0.8**	20.6 ± 4.1	7.95 ± 0.6	159 ± 30
Waterway Estates 01	-29.7 ± 0.4	**+4.17 ± 0.5**	16.8 ± 1.7	7.93 ± 0.6	119 ± 10
Waterway Estates 02	-28.9 ± 0.7	**+5.68 ± 0.5**	16.7 ± 1.6	7.08 ± 0.2	**102 ± 10**
Yacht Club	-31.2 ± 0.8	**+4.80 ± 0.7**	16.3 ± 1.4	8.25 ± 0.7	129 ± 8

1.4, 602 ± 58, and 33.4 ± 1.2, respectively). At Page Park the algal mat $\delta^{15}N$ was not significantly variable by season (Mann-Whitney, $p = 0.262$). Conversely, at San Carlos Park seasonal differences in *Pistia stratiotes* $\delta^{15}N$ were observed (Mann-Whitney *U* test, $p = 0.005$) with significantly higher values in the dry season (+10.7 ± 1.8 ‰) than in the wet season (+4.88 ± 0.36 ‰). No significant seasonal differences were observed in $\delta^{13}C$, C:N, C:P, or N:P (Mann-Whitney *U* tests, all $p > 0.05$) of *P. stratiotes* from San Carlos Park.

Table 4
Chemical composition of macrophytes, including aquatic plants and algae, collected in Lee County at study sites and additional beach sites shown by site and species (mean ± standard error), including stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), as well as elemental ratios of nitrogen to phosphorus (N:P), carbon to nitrogen (C:N), and carbon to phosphorus (C:P). Thresholds are displayed to provide context for measured values and values in bold font exceed threshold values.

Site	Species	n	$\delta^{13}C$	$\delta^{15}N$	N:P	C:N	C:P
Threshold			>-25	>+3.00	>30	<13	<350
Study Sites		*69*	*-24.5 ± 0.04*	***7.66 ± 0.03***	*28.0 ± 0.05*	*17.0 ± 0.03*	***516 ± 0.29***
Briarcliff	algal mat	3	**-17.4 ± 0.21**	**4.64 ± 0.08**	**58.6 ± 0.78**	25.3 ± 0.23	**1,477 ± 3.5**
Heritage Farms		9	**-22.5 ± 0.27**	**10.75 ± 0.16**	25.6 ± 0.30	16.0 ± 0.18	**420 ± 1.5**
	Pistia stratiotes	3	-30.3 ± 0.13	**13.37 ± 0.18**	19.9 ± 0.18	14.2 ± 0.13	282 ± 0.6
	algal mat	6	**-18.6 ± 0.20**	**9.44 ± 0.09**	28.5 ± 0.45	16.9 ± 0.28	**489 ± 2.2**
Lake McGregor		9	-37.1 ± 0.07	**8.53 ± 0.12**	19.1 ± 0.20	**12.4 ± 0.11**	240 ± 0.88
	Cladophora prolifera	3	-36.8 ± 0.27	**9.55 ± 0.21**	23.4 ± 0.32	13.8 ± 0.20	322 ± 1.0
	Hydrilla verticillata	6	-37.3 ± 0.07	**8.02 ± 0.16**	17.0 ± 0.17	**11.8 ± 0.09**	199 ± 0.55
Mobile Manor	*Vallisneria americana*	2	-31.4 ± 0.22	**11.05 ± 0.12**	14.8 ± 0.70	**12.0 ± 0.29**	177 ± 2.1
Overlook Drive	algal mat	4	**-12.0 ± 0.16**	**5.85 ± 0.16**	27.8 ± 0.83	18.2 ± 0.34	**505 ± 3.5**
Page Park	algal mat	9	**-18.8 ± 0.18**	**7.81 ± 0.15**	**34.8 ± 0.20**	20.9 ± 0.25	**738 ± 1.7**
Pine Island		9	**-20.7 ± 0.11**	**3.20 ± 0.12**	**45.4 ± 0.46**	20.9 ± 0.27	**965 ± 2.5**
	Ulva lactuca	3	**-20.2 ± 0.15**	1.79 ± 0.12	28.9 ± 0.43	24.9 ± 0.23	**719 ± 1.7**
	Acanthophora sp.	3	**-20.0 ± 0.15**	**3.79 ± 0.11**	**40.3 ± 0.46**	**12.9 ± 0.11**	**520 ± 1.8**
	Gracilaria tikvahiae	3	**-21.9 ± 0.25**	**4.01 ± 0.18**	**67.1 ± 0.62**	24.8 ± 0.45	**1,655 ± 2.2**
San Carlos Park	*Pistia stratiotes*	15	-29.1 ± 0.06	**8.39 ± 0.15**	21.9 ± 0.12	15.9 ± 0.08	**350 ± 0.57**
Summerwood Drive		9	**-22.2 ± 0.24**	**7.89 ± 0.18**	17.6 ± 0.27	14.5 ± 0.16	254 ± 0.99
	Pistia stratiotes	3	-28.3 ± 0.12	**8.96 ± 0.10**	13.8 ± 0.27	**12.8 ± 0.16**	176 ± 0.86
	algal mat	6	**-19.2 ± 0.19**	**7.35 ± 0.30**	19.6 ± 0.42	15.4 ± 0.23	293 ± 1.4
Beach Sites		*9*	***-19.4 ± 0.24***	***3.59 ± 0.13***	*25.7 ± 0.40*	*18.6 ± 0.35*	***464 ± 1.9***
Captiva Island	algal mat	3	**-13.5 ± 0.13**	1.71 ± 0.09	**34.7 ± 1.51**	**10.1 ± 0.09**	349 ± 4.8
Fort Myers Beach		6	**-22.3 ± 0.23**	**4.52 ± 0.06**	21.2 ± 0.37	22.9 ± 0.53	**522 ± 3.0**
	Hydropuntia secunda	3	**-23.9 ± 0.10**	**4.46 ± 0.13**	16.9 ± 0.39	13.8 ± 0.20	232 ± 1.2
	Ulva lactuca	3	**-20.7 ± 0.29**	**4.59 ± 0.12**	25.5 ± 0.37	32.0 ± 0.32	**812 ± 1.3**
Overall		*78*	***-23.9 ± 0.03***	***7.19 ± 0.02***	*27.7 ± 0.05*	*17.2 ± 0.03*	***510 ± 0.25***

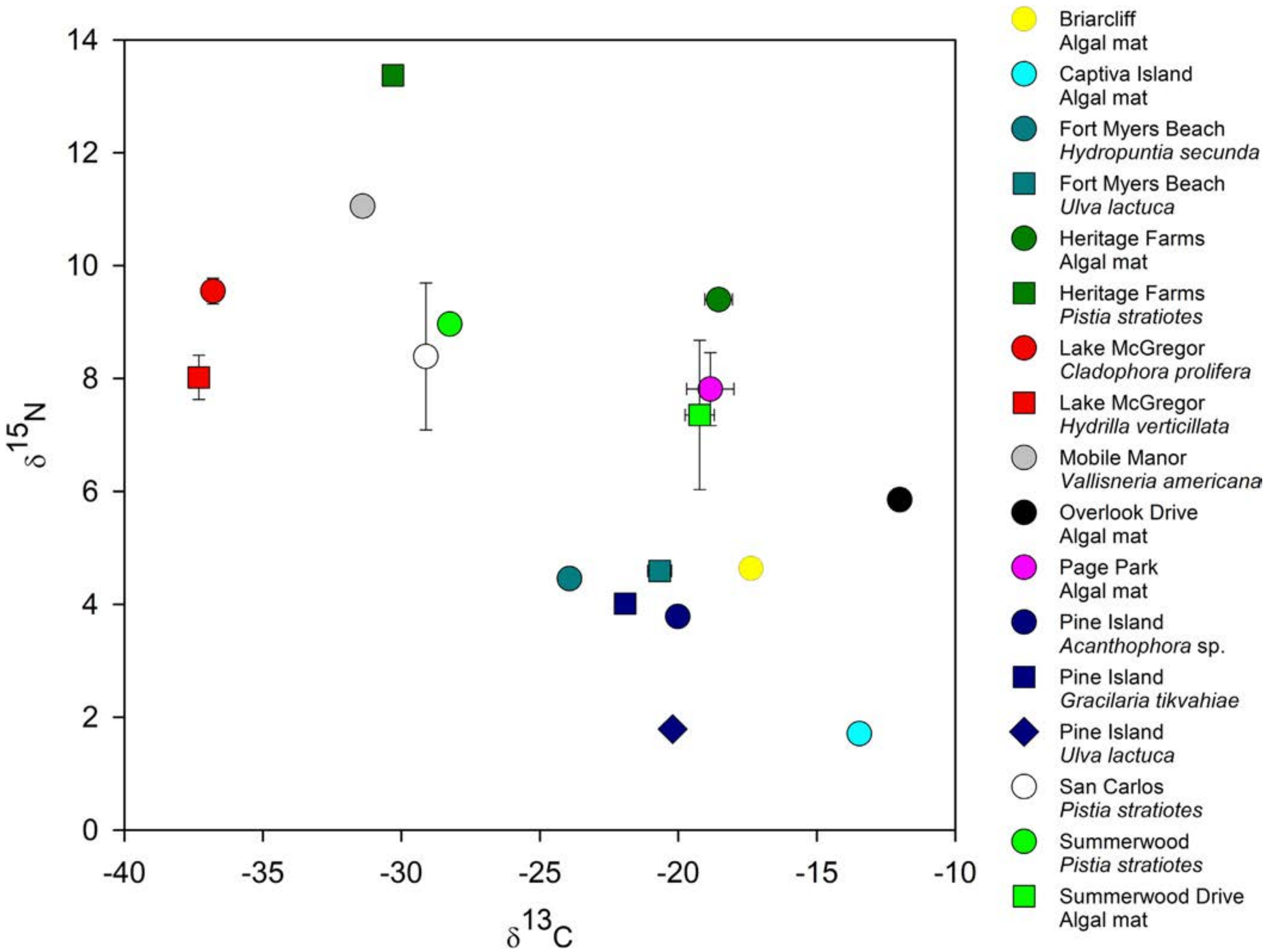

Fig. 4. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes of macrophytes collected during 2019 to 2020 at both study sites and additional beach sites throughout Lee County, FL.

3.4. Bacteria

Elevated fecal indicator bacteria were observed year-round (Table 5). HF183 ranged from 0 to 23,700 copies/100 mL with a mean of 363 ± 139 copies/100 mL and a median of 12.6 copies/100 mL. HF183 was detected in 50 % of all samples with concentrations >525 copies/100 mL detected in 10 % of samples and at 32 % of sites. Enterococci ranged from below to above detection limits with a mean of 789 ± 85 MPN/100 mL and a median of 276 MPN/100 mL. Enterococci concentrations >130 MPN/100 mL were detected in 63 % of samples and at 96 % of sites. *E. coli* concentrations ranged from below to above detection limits with a mean of 487 ± 61 MPN/100 mL and a median of 172 MPN/100 mL. *E. coli* concentrations >410 MPN/100 mL were detected in 31 % of samples and at 68 % of sites. Significant seasonal differences were not observed in HF183, enterococci, or *E. coli* concentrations (Mann-Whitney *U* tests, all $p > 0.05$).

3.5. Chemical tracers

Chemical tracers of human waste were commonly detected in surface water regardless of season (Table 5). Sucralose ranged from 0 to 5900 ng/L with a mean of 727 ± 72 ng/L and a median of 415 ng/L. Sucralose was detected in 99.5 % of samples with concentrations >380 ng/L detected in 54 % of samples and at 80 % of sites. Carbamazepine concentrations ranged from 0 to 18 ng/L with a mean of 1.37 ± 0.21 ng/L and a median below the limits of detection (< 0.8 ng/L). Carbamazepine was detected in 41 % of samples and at 72 % of sites. Some pharmaceuticals were detected less regularly in Lee County surface water (Table 3). Acetaminophen was only detected in four samples across four sites, while ibuprofen was only detected in three samples across two sites, and naproxen was only detected once. Seasonal increases in rainfall did not appear to affect the concentrations of human waste tracers in Lee County surface water, as significant seasonal differences were not observed in sucralose (Mann-Whitney *U* test, $p = 0.691$) or carbamazepine (Mann-Whitney *U* test, $p = 0.376$) concentrations.

3.6. Spearman correlations

There were many significant correlations observed (Fig. 5). NH_4^+ was positively correlated with rainfall ($r = 0.32$, $p < 0.001$), color ($r = 0.30$, $p < 0.001$), enterococci ($r = 0.48$, $p < 0.001$), and sucralose ($r = 0.31$, $p < 0.001$), but negatively correlated with pH ($r = -0.43$, $p < 0.001$), salinity ($r = -0.31$, $p < 0.001$), DO ($r = -0.49$, $p < 0.001$), $\delta^{15}N\text{-}NO_3^-$ ($r = -0.46$, $p < 0.001$), and POM $\delta^{15}N$ ($r = -0.48$, $p < 0.001$). NO_x was positively correlated with color ($r = 0.54$, $p < 0.001$), SRP ($r = 0.35$, $p < 0.001$), TP ($r = 0.30$, $p < 0.001$), and POM C:N ($r = 0.41$, $p < 0.001$), but negatively correlated with salinity ($r = -0.46$, $p < 0.001$), and $\delta^{18}O\text{-}NO_3^-$ ($r = -0.64$, $p < 0.001$). SRP was positively correlated with color ($r = 0.44$, $p < 0.001$) and TN ($r = 0.45$, $p < 0.001$), but negatively correlated with $\delta^{15}N\text{-}NO_3^-$ ($r = -0.31$, $p = 0.003$). DIN:SRP

Table 5
Bacterial and chemical tracers by site in Lee County, FL (mean concentration ± standard error). Thresholds are displayed to provide context for measured values. Values in bold font exceed threshold values and Riverside Lane is highlighted to indicate that there are no nearby septic systems. BDL = indicates concentrations that were below detection limits.

Site Name	HF183 (copies/100mL)	Enterococci (MPN/100mL)	*Escherichia coli* (MPN/100mL)	Sucralose (ng/L)	Acetaminophen (ng/L)	Carbamazepine (ng/L)	Ibuprofen (ng/L)	Naproxen (ng/L)
Method Detection Limit	3	2	2	10	8	0.8	20	8
Threshold	*525*	*130*	*410*	*380*	*8.00*	*1.00*	*20.0*	*8.00*
Billy Creek	220 ± 128	**3752 ± 790**	**2945 ± 708**	158 ± 27	BDL	BDL	BDL	BDL
Briarcliff	30 ± 28	54 ± 21	43 ± 18	**438 ± 68**	2.63	BDL	BDL	BDL
Daughtrey Creek	21 ± 16	**438 ± 168**	**430 ± 140**	262 ± 30	1.11	0.4 ± 0.3	BDL	BDL
Deep Lagoon	16 ± 14	62 ± 36	49 ± 19	**489 ± 68**	BDL	0.4 ± 0.2	BDL	BDL
Ft Myers Shores	65 ± 20	**1162 ± 431**	**793 ± 286**	182 ± 48	BDL	BDL	BDL	BDL
Hendry Creek	277 ± 178	**310 ± 82**	**443 ± 105**	**504 ± 72**	1.14	0.8 ± 0.3	BDL	BDL
Heritage Farms	**680 ± 214**	**659 ± 97**	106 ± 24	**3613 ± 207**	BDL	**3.2 ± 0.3**	BDL	5.25
Lake McGregor	187 ± 64	**1979 ± 462**	357 ± 56	172 ± 45	4.13	BDL	BDL	BDL
Laurelin Court	BDL	**303 ± 61**	153 ± 22	301 ± 64	BDL	0.1 ± 0.1	BDL	BDL
Ligon Court	BDL	**3227 ± 403**	**641 ± 282**	**1900 ± 400**	BDL	**4.4 ± 0.8**	BDL	BDL
Mobile Manor	125 ± 60	**1119 ± 301**	**796 ± 170**	**1120 ± 184**	BDL	**4.0 ± 0.7**	BDL	BDL
North Town & River	BDL	51 ± 19	32 ± 14	**458 ± 80**	BDL	0.2 ± 0.1	BDL	BDL
Orange River	112 ± 29	**1025 ± 397**	**699 ± 231**	277 ± 54	BDL	0.5 ± 0.2	BDL	BDL
Overlook Drive	98 ± 37	**2049 ± 322**	257 ± 50	**3725 ± 361**	BDL	**4.6 ± 0.6**	BDL	BDL
Page Park	39 ± 18	**1012 ± 467**	**903 ± 248**	**564 ± 57**	BDL	**12.2 ± 1.4**	BDL	BDL
Pine Island	92 ± 41	100 ± 68	**625 ± 441**	121 ± 17	BDL	BDL	BDL	BDL
Pine Island 02	18 ± 17	**201 ± 190**	**774 ± 715**	160 ± 15	BDL	BDL	BDL	BDL
Riverside Lane	56 ± 44	75 ± 41	54 ± 16	**504 ± 91**	BDL	0.1 ± 0.1	BDL	BDL
San Carlos Park	**1903 ± 412**	**670 ± 196**	244 ± 54	**424 ± 66**	BDL	**1.0 ± 0.4**	BDL	BDL
Summerwood Drive	**1455 ± 1322**	**1029 ± 392**	222 ± 55	**919 ± 50**	BDL	BDL	**23.4 ± 15**	BDL
Tidewater Island 01	19 ± 9	124 ± 54	126 ± 29	**589 ± 70**	BDL	**1.4 ± 0.3**	BDL	BDL
Tidewater Island 02	**2993 ± 2958**	**194 ± 108**	**625 ± 225**	**656 ± 82**	BDL	**1.1 ± 0.3**	BDL	BDL
Waterway Estates 01	236 ± 196	**667 ± 242**	**528 ± 254**	**466 ± 90**	BDL	0.8 ± 0.3	BDL	BDL
Waterway Estates 02	18 ± 7	**709 ± 208**	394 ± 125	**443 ± 84**	BDL	0.2 ± 0.2	7.3	BDL
Yacht Club	26 ± 16	44 ± 12	49 ± 9	221 ± 28	BDL	0.1 ± 0.1	BDL	BDL

was positively correlated with sucralose ($r = 0.32$, $p < 0.001$) and carbamazepine ($r = 0.33$, $p < 0.001$), but negatively correlated with salinity ($r = -0.30$, $p < 0.001$) and POM $\delta^{15}N$ ($r = -0.39$, $p < 0.001$). TN was positively correlated with color ($r = 0.64$, $p < 0.001$), enterococci ($r = 0.31$, $p < 0.001$), and TP ($r = 0.60$, $p < 0.001$), but negatively correlated with $\delta^{15}N\text{-}NO_3^-$ ($r = -0.53$, $p < 0.001$). $\delta^{15}N\text{-}NO_3^-$ was positively correlated with pH ($r = 0.38$, $p < 0.001$) and POM $\delta^{15}N$ ($r = 0.34$, $p = 0.003$). POM $\delta^{15}N$ was positively correlated with DO ($r = 0.33$, $p < 0.001$), but negatively correlated with POM C:N ($r = -0.31$, $p < 0.001$). POM $\delta^{13}C$ was positively correlated with POM C:N ($r = 0.33$, $p < 0.001$). POM C:N was also negatively correlated with salinity ($r = -0.58$, $p < 0.001$), DO ($r = -0.31$, $p < 0.001$), and enterococci ($r = -0.32$, $p < 0.001$). POM N:P was positively correlated with salinity ($r = 0.31$, $p < 0.001$). Enterococci was also positively correlated with *E. coli* ($r = 0.70$, $p < 0.001$), HF183 ($r = 0.33$, $p < 0.001$), and POM C:N ($r = 0.30$, $p < 0.001$), but negatively correlated with salinity ($r = -0.47$, $p < 0.001$) and DO ($r = -0.42$, $p < 0.001$). Additionally, *E. coli* and HF183 were negatively correlated with DO ($r = -0.30$, $p < 0.001$ and $r = -0.39$, $p < 0.001$, respectively). Sucralose and carbamazepine were also positively correlated ($r = 0.45$, $p < 0.001$).

4. Discussion

Multiple lines of evidence indicated human waste contamination in surface water at sites throughout Lee County, including high NH_4^+ and fecal bacteria concentrations, enriched $\delta^{15}N\text{-}NO_3^-$ and POM $\delta^{15}N$ values, the presence of HF183, and detectable concentrations of human waste chemical tracers. Many of these indicators were not seasonally variable, demonstrating the consistent influence of human waste contamination in the study area. For many of these sites, septic systems are a likely source of this contamination. To preserve human, economic, and ecological health, infrastructure improvements and smart urban planning will be required to mitigate the observed risk from human fecal contamination.

4.1. Nutrients and stable isotopes

Elevated NH_4^+ concentrations were frequently observed and were higher in the wet season. NH_4^+ was the dominant form of reactive N at many sites. The positive correlation of NH_4^+ with enterococci, *E. coli*, and HF183, suggests a human waste source, such as septic systems. Septic systems in areas with high water tables and porous sandy soils, such as Lee County, facilitate the rapid transport of NH_4^+ enriched groundwater to surface water (LeBlanc, 1985; Ptacek, 1998), especially in locations with high densities of septic systems and/or high water tables (Lapointe et al., 1990, 2012, 2017; Herren et al., 2021; Brewton et al., 2022). As many primary producers preferentially uptake NH_4^+ over other N sources (Blomqvist et al., 1994; Beversdorf et al., 2015), these inputs can play a critical role in the development of HABs. Some residential sites with relatively low septic system density had higher NH_4^+ than sites with higher densities, demonstrating that septic system density alone is not necessarily a predictor of poor water quality. In some cases, a single dysfunctional or poorly sited septic system can be the cause significant contamination. Additionally, upstream sources of human waste may also exist that contribute to water quality issues, such as leaky sewer pipes. At other sites with high NH_4^+, reduced NH_3 fertilizer application at adjacent golf courses could be another potential NH_4^+ source. Estuarine sites generally had lower NH_4^+ concentrations than freshwater sites, which may be attributed to dilution with the high volume and tidal mixing in the Caloosahatchee River Estuary, rather than reduced N loading.

NO_x concentrations rarely exceeded the 15.1 μM threshold, but still represent a significant fraction of the observed N at many sites. NO_x concentrations were frequently lower than NH_4^+, indicating incomplete nitrification. This may suggest that effluent from septic systems is not receiving sufficient treatment before reaching the adjacent surface water. This has previously been observed in the North Fort Myers area of Lee County where NO_x was lower than NH_4^+ in groundwater monitoring wells and adjacent surface water sites with septic systems and high water tables (Brewton et al., 2022). Thus, it may be that high water tables and/or lack of necessary organic carbon source in the sandy soils are also preventing nitrification of septic system NH_4^+ at other locations throughout Lee County. This is supported by other studies conducted in this region that found similarly high NH_4^+ (Lapointe and Bedford, 2007; Dixon et al., 2014; Heil et al., 2014).

Surface water $\delta^{15}N\text{-}NO_3^-$ values identified multiple sources of NO_x, including human/animal waste, soil organics, and reduced NH_3 fertilizers. Due to overlapping source values and variability in published interpretations, $\delta^{15}N\text{-}NO_3^-$ data must be analyzed in the context of other data to obtain a holistic picture of pollution sources (Carrey et al., 2021). Sites with

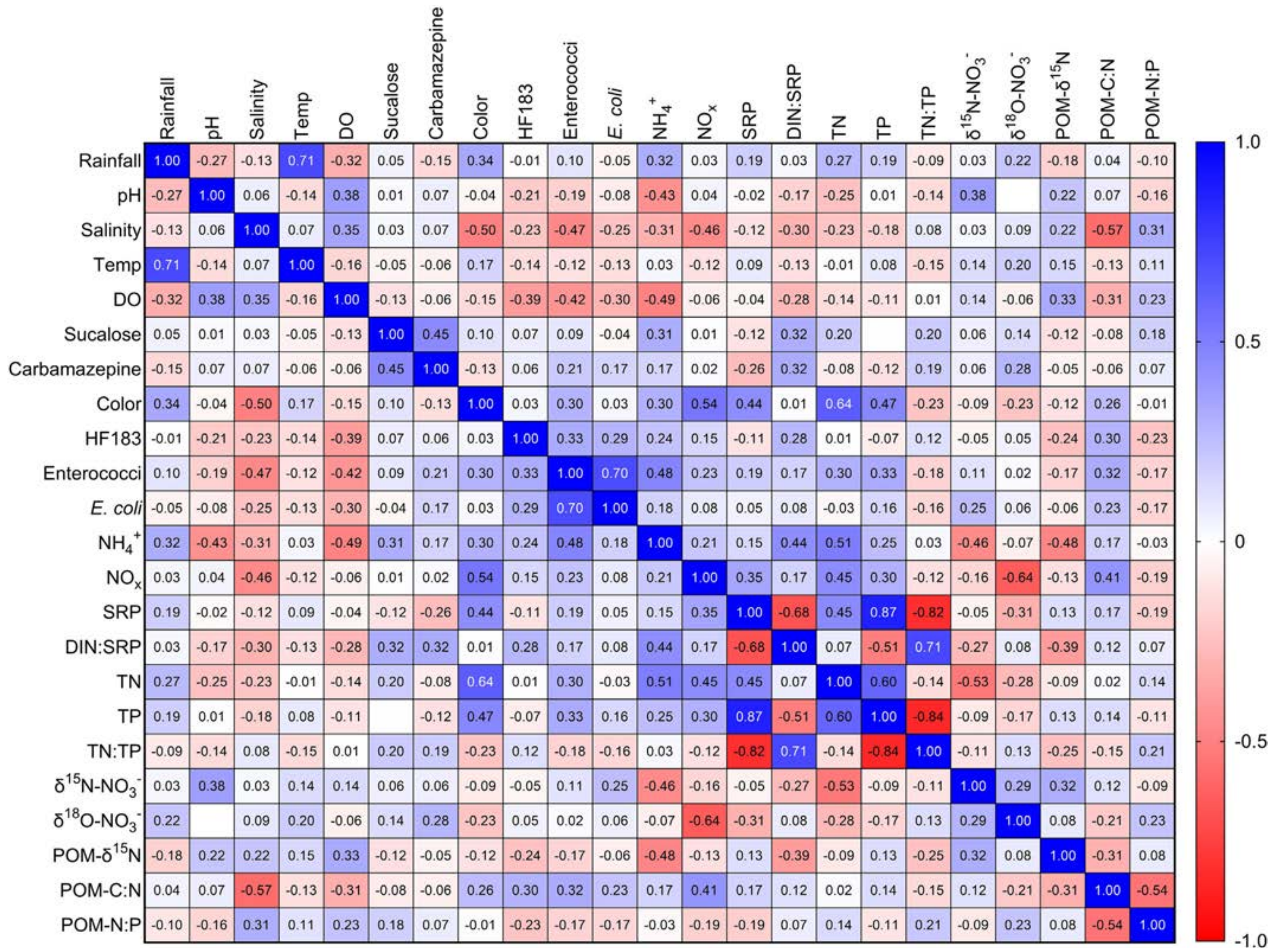

Fig. 5. Spearman Rank correlation coefficients (r) of water quality parameters collected throughout Lee County, FL with positive correlations indicated by blue shading and negative by red shading. Blank white cells indicate r values <0.01.

high δ^{15}N-NO_3^- values combined with detections of HF183, sucralose, and carbamazepine clearly indicated that human waste is likely a primary source of NO_x (Zhang et al., 2019). Many of the sites with more moderate δ^{15}N-NO_3^- values also had chemical tracer detections, indicating that human waste could be a contributing NO_x source, though soil organics and fertilizers remain additional possibilities. Interestingly, surface water δ^{15}N-NO_3^- values in this study were slightly lower (+4.64 ‰) than what has been observed near septic systems in Florida (+6.99 ‰), while the sucralose concentrations were similar (727 ng/L this study vs. 684 ng/L; Herren et al., 2021). These differences reinforce that there is site specific variability and that data must be assessed holistically during pollution source tracking efforts. The occasional detection of negative δ^{15}N-NO_3^- values suggested that synthetic fertilizers sometimes contribute to surface water NO_x loading, but they are usually not the dominant NO_x source in the study area. As no δ^{18}O-NO_3^- values were observed in this study in the range of precipitation or synthetic NO_3^- fertilizer (Fig. 3a), fertilizers are probably not important sources of NO_x in the study area.

Most POM samples and all macrophytes had enriched δ^{15}N values within the range of human/animal waste influence (> +3.0 ‰; Costanzo et al., 2001). The mean δ^{15}N value for macrophytes (+7.99 ‰; Table 4) in this study is less enriched than that recently reported for freshwater canals draining into the Indian River Lagoon, FL (+9.69 ‰; Herren et al., 2021); however, both values are representative of a human/animal waste N source. Aside from human waste inputs, animal waste such as from dogs or cats, can also contribute similarly enriched N to study sites; however, the inclusion of human waste tracers in concert with the δ^{15}N data allows for source confirmation. Despite this confirmation, domestic animal waste can certainly contribute to water quality issues, particularly in urban environments, and efforts should be made to encourage residents in urban areas to clean up after their pets.

Depleted δ^{13}C values in POM (< −25 ‰) revealed that the primary C source for most sites was C_3 plants and therefore likely terrestrial in origin. By contrast, POM originating from C_4 plants or marine organic matter is relatively more enriched (−13 to −23 ‰; Peterson and Fry, 1987; Lapointe et al., 2021). Pine Island and Pine Island 02 were the most marine influenced sites sampled during this study. Accordingly, these two sites had the highest mean δ^{13}C values (−18 to −26 ‰). Ligon Court also had somewhat enriched δ^{13}C values (−23 to −26 ‰) which may be explained by high relative abundance of St. Augustine grass (*Stenotaphrum secundatum*), which is a C_4 plant and therefore naturally enriched in δ^{13}C (about −13 ‰; Peterson and Fry, 1987).

Generally, TN concentrations did not exceed surface water standards for Peninsular streams (118 μM), but many do exceed the standard for the downstream Lower Caloosahatchee Tidal Segments 1 and 2 (35.7 μM). Thus, the study sites that drain into the Caloosahatchee River are a contributing TN source to the Caloosahatchee Estuary. Septic systems are likely a contributing TN source to the study sites; however, septic system densities at sites that exceeded the TN standards for Peninsular streams are not high when compared to other sites with lower TN concentrations. For example, San Carlos Park and Mobile Manor have relatively high septic system densities (152 and 153 per 300 m^2, respectively; see Supplement, Figs. S1–23) but did not exceed the TN standard. Improperly functioning septic systems directly adjacent to the Heritage Farms, Ligon Court, and Overlook Drive sites may account for this discrepancy as average TN concentrations for septic system effluent can range as high as 8429 μM (118 mg/L; Lapointe et al., 2022). Stormwater runoff is another potential source of TN. In Florida, TN concentrations in urban stormwater runoff typically averages 70 to 210 μM (Badruzzaman et al., 2012; Jani et al., 2020). Additionally, high TN concentrations were adjacent to golf courses where fertilizers and/or reuse water are applied. Monthly average TN concentrations in reclaimed water from the Fiesta Village Wastewater Treatment Plant that services this area ranges from 43 to 93 μM (Lee County Utilities). Although nutrient uptake by turfgrass greatly reduces the amount of N that reaches surface water through stormwater runoff (80 to 90 % uptake; Hochmuth et al., 2009; Shaddox and Unruh, 2018), improperly applied fertilizer and reuse water could also contribute to the high TN concentrations.

P concentrations were elevated throughout the study area. Septic system effluent is P rich (Corbett et al., 2002) and although adsorption may reduce the amount of P that reaches groundwater and adjacent surface water, high water tables and porous sandy soils may facilitate rapid transport of unadsorbed P (Herren et al., 2021). Further, high densities of septic systems may cause the soil to transition from a P sink to a P source over time as the soil becomes P saturated (Robertson, 2008). A significant portion of P detected in this study may originate from phosphate bearing geological formations in the Caloosahatchee River watershed (Odum, 1953). Wastewater treatment plant effluent represents another potential P source. In the study area, the Fiesta Village Wastewater Treatment Plant uses alum treatment for P removal, resulting in average TP concentrations <3.2 μM in final effluent, however, extended periods of high flow can intermittently yield P concentrations up to 8.4 μM (Lee County Utilities).

Nutrient loading from human waste transported through groundwater can result in stoichiometric shifts because of adsorption of SRP, which can elevate N:P (Lapointe et al., 1990). The highest DIN:SRP were observed in urban drainage ditches and canals, while lower DIN:SRP were observed in estuarine canals located directly on the Caloosahatchee River Estuary. The mean DIN:SRP (26.4:1) and TDN:TDP (31.9:1) were above the Redfield ratio (16:1) and septic system influent (~14; Lapointe et al., 2022) but below levels (30:1) indicating P limitation. Similarly, the mean N:P and C:P values of POM (15:1, 124:1) were close to Redfield proportions and balanced phytoplankton growth. The higher mean N:P and C:P in macrophytes (25:1, 455:1) suggest these plants could experience mild P-limitation. The general lack of strong P-limitation is expected as the geology in Lee County is P-rich (Odum, 1953).

The relatively high C:N of POM and macrophytes support previous research showing that N is generally the limiting nutrient for primary production in Lee County (Lapointe and Bedford, 2007). The mean C:N of POM (9.2:1) and macrophytes (17.0:1) were both higher than the Redfield ratio (6.6:1), suggesting N limited growth, especially in the macrophytes. Macrophytes sampled along a transect from the Caloosahatchee River to offshore coastal reefs in 2004 reported a mean C:N of 13.7, lower than the present study and indicating only weak N-limitation (Lapointe and Bedford, 2007).

4.2. Bacteria

Septic systems likely contribute to high bacterial counts observed at some of the sites assessed in this study, but additional sources may also exist, such as animal feces, decaying vegetation, reuse water, or leaking sewage infrastructure. Fecal indicator bacteria may also increase in the environment where P concentrations are high (Mallin and Cahoon, 2020). Therefore, enterococci and *E. coli* concentrations may not be directly indicative of loading; rather, populations may be sustained by P inputs that allow them to persist and grow in the environment. This study suggests a similar relationship exists in Lee County surface waters, as enterococci and *E. coli* were positively correlated with TP.

Lower fecal bacteria counts were observed at more saline sites. Negative correlations between salinity and fecal indicator bacteria have previously been reported (Lapointe et al., 2012). In the presence of ultraviolet radiation, *E. coli* survival rates decline sharply at salinities >10 PSU (Chan and Killick, 1995). This aligns with the negative correlation between salinity and enterococci or *E. coli* concentrations, suggesting that low bacterial counts observed at marine influenced sites may be explained by survival rates rather than decreased loading. Dilution from flushing may also reduce bacterial concentrations in tidally influenced areas (Mill et al., 2006).

The presence of HF183 with no significant seasonal effects indicated year-round human waste contamination at many sites in Lee County. HF183 was positively correlated with enterococci and *E. coli*, suggesting that a significant portion of these bacteria originate from human waste. Most sites had sporadic detections of HF183 in high concentrations, but Heritage Farms and San Carlos Park had consistently high concentrations. San Carlos Park is in a residential neighborhood with high densities of septic systems, while septic densities at Heritage Farms were much lower (15 septic system within 300 m; see Supplement, Figs. S7 and S18). This also supports the idea that septic system density alone may not always be an accurate predictor of potential water quality impacts. Other site-specific considerations may be required, such as distance to water from individual septic systems, age and maintenance of the septic system, or soil conditions. The highest concentrations of HF183 were detected at Tidewater Island 02 (23,700 copies/100 mL) and Summerwood Drive (10,700 copies/mL). While these were isolated events and may not represent a chronic issue, these extremely high detections demonstrate that periodic human waste inputs occur at these locations. Similar sporadic discharges may occur in other locations but are more difficult to detect than continuous inputs. Like fecal bacteria, a negative correlation between HF183 and salinity may be explained by higher dilution at sites with tidal flushing or natural degradation rather than decreased loading from human waste.

Following the passage of Hurricane Ian through the Lee County study area in late-September 2022, an abnormal increase in *Vibrio vulnificus* infections (~28 cases in 2022) and deaths (8 in 2022) occurred in people exposed to surface waters (https://www.floridahealth.gov/diseases-and-conditions/vibrio-infections/vibrio-vulnificus/). The presence of as little as 1 % of human wastewater in warm coastal waters can lead to increased virulence of *V. vulnificus* and 100 to 1000-fold higher concentrations, resulting in a higher chance of infection (Conrad and Harwood, 2022). Positive correlations have also been observed between *V. vulnificus* and fecal bacteria (Blackwell and Oliver, 2008). Thus, minimizing human waste inputs into Lee County surface waters should help to reduce these occurrences.

4.3. Chemical tracers

Ubiquitous detection of sucralose demonstrated widespread human waste contamination. This aligns with a survey of Florida surface water that estimated 57 to 89 % of canals, streams, and rivers would have detectable amounts of sucralose, which positively correlated with urban land use (Silvanima et al., 2018). In this study, sucralose concentrations were generally high and positively correlated with NH_4^+. Previous studies in Florida have observed a similar relationship between sucralose and NH_4^+, which has been attributed to non-functioning, poorly sited septic systems (Lapointe et al., 2017; Herren et al., 2021; Brewton et al., 2022). Sucralose contamination is attributable to high densities of septic systems at some sites (e.g., Mobile Manor), while others have multiple potential sources, including reclaimed water application, municipal sewer infrastructure, and upstream wastewater treatment plant effluent (Supplement, Figs. S1–23). Interestingly, in this study the “reference” site that did not have septic systems nearby (Riverside Lane) had sucralose concentrations up to 850 ng/L. This is most likely reflective of background concentrations within the Caloosahatchee River Estuary, as other sites within the estuary exhibited similar concentrations. Another possible source could be from boats emptying waste within the canal, but this is unlikely given the low concentrations of HF183, bacteria, and other chemical tracers at the site.

The ubiquitous detection of sucralose in this study demonstrates the difficulty of identifying unimpacted reference sites within highly urbanized watersheds.

High concentrations of sucralose were occasionally observed. The highest (up to 5900 ng/L) and most consistent sucralose concentrations exceeded previously reported concentrations in Lee County surface water (1200 ng/L; Brewton et al., 2022). In laboratory toxicity studies, concentrations of >1000 mg/L sucralose was required to reach a lethal endpoint for invertebrates and fishes; however, environmentally relevant concentrations may produce non-lethal effects (Lewis and Tzilivakis, 2021). For example, mysid shrimp (*Daphnia magna*) exposed to ≥5000 ng/L sucralose concentrations showed altered swimming height and speed and expressed biomarkers of neurotoxicity and oxidative stress (Eriksson Wiklund et al., 2012, 2014). Additionally, gammarid amphipods (*Gammarus* spp.) experienced a longer time to reach food and shelter (Eriksson Wiklund et al., 2012). Further, common carp (*Cyprinus carpio*) experienced oxidative damage to their gills, muscle, brain, and liver at concentrations ≥50 ng/L (Saucedo-Vence et al., 2017). These sublethal effects of sucralose exposure suggest that there could be negative ecological implications to continued exposure and that minimizing inputs of sucralose to surface water may be beneficial.

Pharmaceutical tracers of human waste were also detected in many surface waters. Previous work in Lee County observed carbamazepine to be ubiquitously present in surface water of North Fort Myers at low concentrations with higher concentrations observed in groundwater near septic systems (Brewton et al., 2022). The highest surface water carbamazepine concentration observed in that study (28 ng/L) exceeded the highest concentration of this study (12 ng/L), illustrating within county differences. Like a previous study that found no meaningful correlation ($r = 0.07$) between surface water carbamazepine concentrations and fecal bacteria (Sauvé et al., 2012), weak positive correlations were observed in this study between carbamazepine and enterococci ($r = 0.21$) or *E. coli* ($r = 0.17$; Fig. 3). Thus, while in Lee County carbamazepine was not an accurate predictor of fecal bacteria, when detected it provided additional evidence of a human waste source. The occasional presence of the pain relievers acetaminophen, ibuprofen, and naproxen also supplied evidence of human waste contamination at some sites.

4.4. Ecological effects

As noted above, land-based nutrient loading, such as the high NH_4^+ concentrations observed in this study, can provide favorable conditions for the development of certain HABs (Lapointe and Bedford, 2007; Lapointe et al., 2012, 2015). This is supported by blooms of the blue-green algae, *Microcystis aeruginosa,* that occurred in the Caloosahatchee River Estuary in 2005, 2016, and 2018 (Lapointe et al., 2006; Lapointe et al., 2020; Brewton et al., 2022). Additionally, red tide (*Karenia brevis)* blooms are known to be stimulated by NH_4^+ inputs (Doig and Martin, 1974). This was demonstrated after the stormwater runoff from hurricanes Charley, Frances, and Jeanne caused significant N enrichment of the Caloosahatchee River and coastal waters, including a six-fold increase in coastal NH_4^+ concentrations (Lapointe and Bedford, 2007) that fueled a large *K. brevis* bloom. The bloom persisted in the coastal waters off Southwest Florida for much of 2005 and resulted in widespread hypoxia and fish mortalities (Lapointe and Bedford, 2007; Yentsch et al., 2008). Similarly, after Hurricane Ian in 2022, a red tide rapidly developed in Lee County coastal areas and the Gulf of Mexico. Although the blooms were patchy and impacts varied temporally and by beach, from September 2022 through March 2023 red tide was present along Lee County's coast at concentrations that have caused fish kills and respiratory irritation (see https://www.flickr.com/photos/myfwc/sets/72157635398013168/). The relationship between NH_4^+ loading from storm events and recurring HABs illustrates the importance of reducing local N inputs.

Nutrient loading from fertilizers has been considered as a significant contributor to water quality issues and HABs in Florida. Consequently, policymakers have imposed residential fertilizer ordinances throughout the state (Hochmuth et al., 2009). Unfortunately, in some coastal areas these ordinances have not yielded the desired nutrient loading reductions, and water quality and HABs continue to worsen (Lapointe et al., 2020; Krimsky et al., 2021). Human waste sources have been increasingly identified as another important factor contributing to HABs in Florida (Lapointe et al., 2012, 2015, 2021; Brewton et al., 2022). The present study finds that human waste from septic systems contributes to nutrient and bacteria loading in surface water throughout Lee County. This warrants close monitoring as these inputs may be a primary driver of HABs and hazardous conditions within the Caloosahatchee River Estuary and downstream coastal waters.

At estuarine canal sites located directly on the Caloosahatchee River Estuary, the influence of septic systems and other human waste sources was not as pronounced as in the smaller drainage ditches and canals and it may be that the methods used in this study were not sufficient to assess impacts at sites with higher flushing rates. Given the proximity to sensitive ecosystems, such as seagrass and mangroves, more investigation may be warranted at these locations. For example, seasonal collections of macrophytes, such as seagrasses and macroalgae, could provide insight into longer-term nutrient loading (Lapointe et al., 2015, 2020; Herren et al., 2021).

5. Conclusion

As urban development of coastal regions continues globally, it will be of paramount importance to understand how to plan for sustainable growth while protecting water quality for its intended uses. This study demonstrates that contamination from human waste can present a significant ecological and human health concern in urbanized watersheds. Further, this study shows that in medium to high density urban areas, the use of septic systems adjacent to waterbodies can result in widespread contamination of surface waters. In Lee County, this contamination has likely led to waterbody impairment and contributes to downstream issues, such as waterbody closures and the development and maintenance of HABs. Our results suggest that a master wastewater plan including replacement of septic systems with centralized sewer or other alternatives would reduce nutrient and bacterial loading in the Lee County study area. Sustainable population growth in coastal regions will require smart urban development with sufficient wastewater infrastructure and advanced nutrient removal capabilities, especially as these challenges will worsen with expected increases in temperature, groundwater levels, and extreme storm events.

CRediT authorship contribution statement

Kevin N. Tyre: data curation, methodology, formal analyses, data visualization, writing- original draft, writing- review and editing. **Rachel A. Brewton**: conceptualization, methodology, data visualization, project administration, writing- review and editing. **Lisa B. Kreiger**: conceptualization, data curation, methodology, writing- review and editing. **Brian E. Lapointe**: conceptualization, methodology, writing- review and editing, project administration.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.162716.

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Fertilizer restrictions are not sufficient to mitigate nutrient pollution and harmful algal blooms in the Indian River Lagoon, Florida

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A B S T R A C T

In Florida's Indian River Lagoon (IRL), anthropogenic eutrophication has resulted in harmful algal blooms and catastrophic seagrass losses. Hoping to improve water quality, policy makers enacted fertilizer bans, assuming that this would reduce the nitrogen (N) load. To assess the effectiveness of these bans, seawater and macroalgal samples were collected at 20 sites "pre" and ~ five-years "post" bans and analyzed to determine concentrations of dissolved nutrients and stable nitrogen isotope values ($\delta^{15}N$). Higher concentrations of ammonium and nitrate were observed post-ban and macroalgal $\delta^{15}N$ values increased. A comparison of nutrient concentrations and $\delta^{15}N$ between brown tide (*Aureoumbra lagunensis*) blooms indicated that the post-ban bloom was more strongly N-enriched with higher $\delta^{15}N$ values than the pre-ban bloom, which had depleted values in the range of fertilizers. These data indicate a primary role of human waste influence in the IRL, suggesting that current management actions have been insufficient at mitigating eutrophication.

1. Introduction

Eutrophication of coastal environments is a global issue with myriad accompanying ecological effects. Excess nutrient inputs, particularly nitrogen (N) and phosphorus (P), often result in increased harmful algal blooms (HABs), seagrass die-offs, hypoxia/anoxia, and fish kills. Sources of N and P commonly include stormwater runoff, fertilizer, animal or human waste, wastewater treatment plant effluent, and atmospheric deposition (Nixon, 1995; Howarth et al., 2000; Nixon, 2009; Hochmuth et al., 2011; Glibert and Burford, 2017). In the United States (US), recognition of water pollution as a growing problem led to the passage of the Federal Clean Water Act (FCWA) in 1972. The FCWA required states to identify impaired water bodies and establish total maximum daily loads (TMDLs) for pollutants. TMDL programs have since been established in every state to protect waters from excess N and P pollution.

Research in the urbanized areas of the northeastern US has shown that human waste is often the largest contributing N source driving eutrophication and HABs (Sham et al., 1995; McClelland et al., 1997; Valiela et al., 1997a; Valiela et al., 2000; Kinney and Valiela, 2011; Lloyd, 2014). In Waquoit Bay on the south shore of Cape Cod, Massachusetts, wastewater (primarily septic system effluent) accounted for 48 % of the N load to estuaries, compared to 29 % for atmospheric deposition and 16 % for fertilizer use (Sham et al., 1995; Valiela et al., 1997a). The predominance of human waste as a land-based N source was evidenced by the positive relationship between stable N isotope values ($\delta^{15}N$) of groundwater and the proportion of groundwater N coming from wastewater (McClelland et al., 1997). Waste from humans and animals has more enriched (higher) $\delta^{15}N$ values compared to fertilizers and atmospheric deposition and thus can be used to help discriminate among the major N source contributions (Valiela et al., 2000; Costanzo et al., 2001; Cole et al., 2004). Similarly, septic system effluent accounts for ~50 % of the N loads entering Great South Bay and Peconic Bay on Long Island, New York (Kinney and Valiela, 2011; Lloyd, 2014).

The Indian River Lagoon (IRL) on Florida's east-central Atlantic coast (Fig. 1) is a biologically diverse and ecologically important estuary (Tremain and Adams, 1995) that is experiencing eutrophication due to excess nutrient loading (Lapointe et al., 2015; Barile, 2018). As such, some segments of the IRL are classified as impaired under Section 303(d) of the United States Clean Water Act of 1972 for total nitrogen (TN) and total phosphorus (TP). Ecological results of this eutrophication include recurring macroalgal and phytoplankton HABs, including brown tides (*Aureoumbra lagunensis*), catastrophic seagrass losses, fish kills, and unusual mortality events (UMEs) of marine mammals, including the threatened Florida manatee (Gobler et al., 2013; Lapointe et al., 2015;

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Phlips et al., 2015; Lapointe et al., 2020; Herren et al., 2021; Lewis et al., 2021; Morris et al., 2021; Phlips et al., 2021; Allen et al., 2022; Landsberg et al., 2022). In recent decades, water managers, policy makers, and environmental activists have considered fertilizers to be the primary contributing N source (71 %) from residential land use to these impairments in the IRL (https://savetheirl.org/education/how-does-your-lawn-hurt-the-lagoon/).

Consequently, fertilizer restrictions have been implemented in counties and municipalities along the IRL to reduce nutrient inputs from urban and agricultural land uses to achieve TMDL targets for the IRL (https://befloridiannow.org/fertilizer-ordinances/). The Florida Watershed Restoration Act that was passed in 1999 provided legislation for the formal implementation of Florida's TMDL program using agricultural best management practices (BMPs). The Florida Department of Agriculture and Consumer Services (FDACS) is responsible for implementing BMP programs throughout the state. BMPs were intended to prevent nonpoint source pollutant discharges from agricultural lands, thereby improving the water quality of nearby surface water and groundwater. Farmers enrolled in BMP programs are required to limit N and P fertilizer application and to keep complete records of application.

In 2007, fertilizer regulations were extended to include urban turfgrass through the creation of the Urban Turf Fertilizer Rule (Florida Administrative Code 5E-1.003). This rule required all fertilizers sold in packages <50 lbs (22.7 kg) for turfgrass use to be labeled with appropriate nutrient content information and guidelines for application. Turfgrass fertilizer regulations were further extended in 2009 by codifying the Model Ordinance for Florida-Friendly Fertilizer Use on Urban Landscapes into state law (Florida Statute 403–9337). This statute requires counties and municipalities within impaired watersheds to adopt the BMPs outlined in the model ordinance. These requirements include the prohibition of fertilizer application within 10 ft. (~3.05 m) of any waterbody, prohibition of P fertilizer application unless a soil test indicates deficiency, and mandated use of slow-release fertilizers. Additionally, companies and contractors are required to complete a six-hour BMP training program in order to obtain a license from FDACS for commercial fertilizer application. The model ordinance also prohibits fertilizer application during the summer rainy season (June 1 to September 30), commonly called a "fertilizer blackout." In addition to

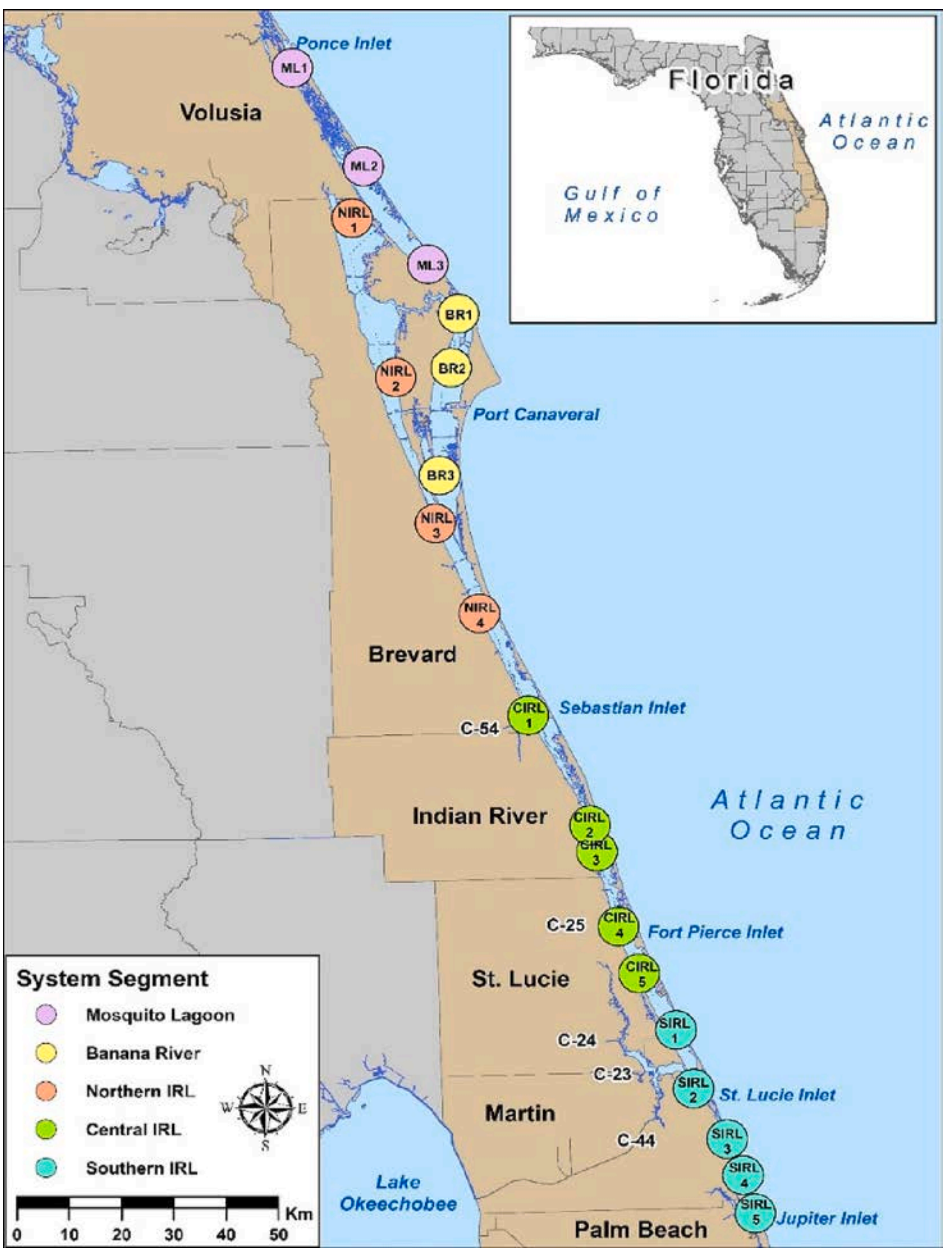

Fig. 1. The Indian River Lagoon (IRL), on Florida's east-central coast, showing sites by segment with the Mosquito Lagoon (ML; sites ML1–3) in purple, the Banana River (BR; sites BR1–3) in yellow, the northern IRL (NIRL; sites NIRL1–4) in light red, the central IRL (CIRL; sites CIRL1–5) in green, and the southern IRL (SIRL; site SIRL1–5) in teal, as well as associated counties, tidal inlets, and drainage canals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

fertilizer ordinances, voluntary BMPs have also been developed to minimize nutrient runoff from urban landscapes, such as the Florida-Friendly Landscaping™ (FFL) Program (Momol et al., 2021).

Due to the classification as an impaired waterbody, counties within IRL watersheds are required to comply with the BMPs outlined in the Florida-friendly model ordinance. Accordingly, Volusia, Brevard, Indian River, St. Lucie, Martin, and Palm Beach counties have all codified the state model ordinance into county law. St. Lucie county was the first to formally adopt the state model ordinance in March 2011. By April 2014, all remaining counties had also complied with the model ordinance adoption. From 2013 to 2015, counties along the IRL implemented multiple fertilizer bans ranging from the model ordinance (Volusia, Palm Beach) to more stringent ordinances (Brevard, Indian River, St. Lucie, and Martin).

In this study we sought to understand the effectiveness of these fertilizer application bans in reducing nutrient loading to the IRL. Was there an associated decrease in dissolved ambient nutrients or a change in the tissue nutrient and/or stable isotope values of phytoplankton or macroalgae that would suggest a shift in the available nutrients and stoichiometry fueling eutrophication in the IRL? This question was tested by comparing dissolved seawater nutrient concentrations and tissue nutrient and isotope data of brown tides and macroalgae collected pre- and ~ 5 years post-fertilizer bans.

2. Materials and methods

2.1. Study location

The study included five IRL segments spanning from Jupiter Inlet in the south to Ponce Inlet in the north. These segments included Mosquito Lagoon (ML), the Banana River (BR), the northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL) with a total of twenty sites (Fig. 1) that were previously described in Lapointe et al., 2015. The number of sites varied by segment with $n = 3$ in the ML, $n = 3$ in the BR, $n = 4$ in the NIRL, $n = 5$ in the CIRL, and $n = 5$ in the SIRL (Fig. 1).

2.2. Rainfall

Rainfall data for the duration of the project (April 2011 – August 2017) were obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (https://www.ncdc.noaa.gov/). All available stations were included for each county to obtain the best geographical and temporal coverage. Rainfall for all six counties bordering the IRL was combined and assessed as monthly rainfall averages ± SE.

2.3. Sampling

Sampling was conducted during wet and dry seasons from 2011 to 2017. During sampling events, a combination of calibrated YSI™ Models 63, 85, 1030, and ProODO handheld meters were used to determine salinity (PSU), temperature (°C), and pH.

2.4. Dissolved nutrient concentrations

Collections of seawater for dissolved nutrient and chlorophyll *a* analyses occurred in 2011–2012 (pre-ban) and 2016–2017 (post-ban) during wet and dry seasons. Accordingly, the sampling events were classified as dry pre-ban (June 2 – July 13, 2011), wet pre-ban (November 4 – January 11, 2012), wet post-ban (October 19 – December 1, 2016), and dry post-ban (March 8 – April 13, 2017). During each sampling event, triplicate ($n = 3$) seawater samples were collected ~0.25 m below the surface into 0.25 L high-density polyethylene (HDPE) bottles and submerged ice in a cooler until returned to the Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI-FAU) laboratory for processing. Sites adjacent to canals, tributaries, and inlets (NIRL4, CIRL1, CIRL2, CIRL3, CIRL4, SIRL2, and SIRL5 in Fig. 1) were sampled during an ebbing tide to account for tidal pumping of groundwater (Lapointe et al., 1990). The seawater samples were filtered through 0.7 μm GF/F filters and frozen at −20 °C until analysis, while filters were retained and frozen for estimation of chlorophyll *a* concentrations.

The filtered seawater samples were analyzed to determine dissolved nutrient concentrations at Nutrient Analytical Services at Chesapeake Biological Laboratory (NAS-CBL) on a Technicon Auto-Analyzer II for nitrate (NO_3^-), total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP), and total dissolved phosphorus (TDP) or a Technicon TRAACS 800 for ammonium (NH_4^+) and nitrite (NO_2^-). Detection limits were 0.21 μM for NH_4^+, 0.01 μM for NO_3^- and NO_2^- (combined as NO_x hereafter), 0.02 μM SRP, 2.06 μM for TDN, and 0.05 μM for TDP. The resulting data were used to characterize ambient dissolved inorganic nitrogen ($DIN = NH_4^+ + NO_x$), as well as DIN:SRP and TDN:TDP. At NAS-CBL, the filtered chlorophyll *a* samples were extracted in 90 % acetone and centrifuged. The samples were then measured fluorometrically for chlorophyll *a* concentrations before and after acidification with 5 % HCl with a Turner Designs TD700 fluorometer equipped with a daylight white lamp, 340–500 nm excitation filter and > 665 nm emission filter or with a Turner Designs Trilogy fluorometer.

2.5. Stable isotopes and elemental composition of macroalgae

At the same 20 sites, composite samples of the most abundant macroalgae were collected in 2011–2012 and 2016–2017 to characterize elemental composition (%C, %N, %P), molar ratios (C:N:P), and stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values. In the laboratory, the samples were cleaned, rinsed briefly in DI water, sorted into three composite replicates per species, dried for 48 h at 60 °C in a Fisher *Isotemp*® laboratory oven, ground with a mortar and pestle, and then split into two vials. Additionally, samples of organically-enriched sediment or "muck" (Sigua et al., 2000) collected from Turkey Creek in the NIRL on 12/15/2016, Scott's granular fertilizer, Milorganite biosolids fertilizer, and biosolids obtained from the Central District Wastewater Treatment Plant, Miami, FL on 04/13/2021 were processed as above. Samples collected in 2011–2012 were analyzed for $\delta^{13}C$, $\delta^{15}N$, %N, and %C at the University of California – Davis's Stable Isotope Facility and at NASL-CBL for %P as described in Lapointe et al. (2015). Samples collected in 2016–2021 were analyzed at a different lab than in 2011–2012 as described below. In 2016–2021, ground samples were shipped to University of Georgia's Center for Applied Isotope Studies Stable Isotope Ecology Laboratory (UGA-SIEL) for analysis. At UGA-SIEL, samples were split. One half was analyzed for $\delta^{13}C$, $\delta^{15}N$, %C, and %N on a Thermo Delta V IRMS coupled to a Carlo Erba NA1500 CHN-Combustion Analyzer via a Thermo Conflo III Interface (see http://sisbl.uga.edu/ratio.html#top; Thermo Scientific, 2007). National Institute of Standards and Technology reference materials 8549, 8558, 8568, and 8569 were used to routinely calibrate working standards prepared in the laboratory. The other half was analyzed for %P, where approximately 2 mg of dried tissue was weighed into crucibles, ashed at 500 °C for 4 h, and extracted with 0.2 mL of Aqua Regia acid (Allen et al., 1974; Jones et al., 1990). The acid extracts were then diluted 41:1 with deionized water for TP (as PO_4-P) analysis on an Alpkem 300 series analyzer. The resulting data were used to calculate molar ratios (C:N:P).

2.6. Brown tide sampling

Due to recurring brown tides (*Aureoumbra lagunensis*) during the study period, bloom locations were sampled on 8/7/2012–8/9/2012 in the NIRL (NIRL 1 and NIRL5) and ML (ML1–3), on 01/29/2016 in the NIRL (near NIRL2) and BR (near BR2–3), and on 10/23/2017 in the NIRL (NIRL3–4) and BR (BR3). During these sampling events seawater samples were collected as above for dissolved nutrient and chlorophyll *a*

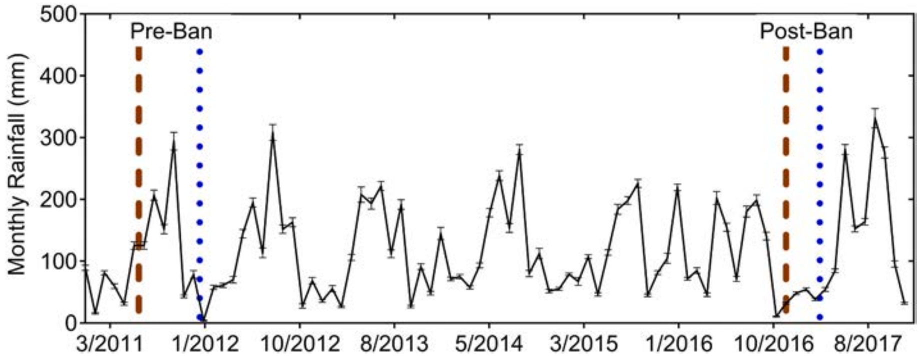

Fig. 2. Average monthly rainfall (mm) ± SE from January 1, 2011 – December 31, 2017 for the six counties that border the Indian River Lagoon, FL, USA, including (from north to south) Volusia, Brevard, Indian River, St. Lucie, Martin, and Palm Beach, showing approximate timing of sampling events during the dry (June 2011 and December 2016; brown dashed lines) and wet (December 2011 and March 2017; blue dashed lines) seasons. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analyses. Additionally, particulate organic matter (POM) was collected as a proxy for phytoplankton. For POM collections, surface water was collected with a clean secondary vessel and immediately coarse filtered through a 200 μm nylon sock filter into a 1 L HDPE bottle to remove detritus and microzooplankton, per Savoye et al. (2003). In the lab, the POM samples were vacuum filtered through pre-combusted 47 mm glass fiber filters. The filters were frozen at -20 °C until analysis and analyzed as above for $\delta^{13}C$, $\delta^{15}N$, %C, %N, and %P.

2.7. Statistical analyses

Data were compared to the Florida Department of Environmental Protection Numeric Nutrient Criteria for the IRL from St. Lucie Estuary to the southern border of Indian River County (Florida Administrative Code 62–302.532), which were 0.72 mg/L (51.4 μM) TDN, 0.07 mg/L (2.26 μM) TDP, and 4.7 μg/L chlorophyll *a*. While not applicable to the entire IRL, these criteria were chosen as they represented the largest segment of our study area with numerically defined target concentrations that could be used for comparison.

Variation in dissolved seawater nutrient concentrations and tissue nutrient and isotope data of brown tides and macroalgae were compared between IRL segment, season, and ban status. The data were analyzed in SPSS v. 27 and Minitab v. 19, while data visualizations were created in Prism v. 9 by Graphpad and ArcMap v. 10.6. Shapiro-Wilk normality test, Levene's test of equality of error variances, and Q-Q plots were used to assess data conformation to parametric assumptions. Data meeting parametric assumptions were analyzed with a multivariate analysis of variance (MANOVA) followed by univariate analysis of variance (ANOVA) and Tukey tests on significant differences. When parametric assumptions were not met, the non-parametric Kruskal-Wallis (3 or more groups) and Mann-Whitney *U* (2 groups) tests were used on non-transformed data. For all analyses, significance was considered at $p \leq 0.05$. Unless otherwise noted, data are presented as mean ± standard error (SE).

3. Results

3.1. Rainfall

Rainfall was variable throughout the study period (Fig. 2). Prior to the pre-ban dry season sampling, May 2011 was relatively dry with rainfall of 31.3 ± 20 mm. Rainfall increased leading up to the pre-ban wet season sampling with 294 ± 120 mm in October 2011. Prior to the post-ban wet season sampling, rainfall was elevated with 199 ± 82 mm in September 2016. Rainfall decreased leading up to the post-ban dry season sampling with 54.2 ± 28 mm in February 2017.

3.2. Environmental parameters

Environmental parameters were variable between season and lagoon segment. Mean salinity was 30.4 ± 0.85 PSU. Pre-ban salinity (33.1 ± 0.99 PSU) was significantly higher (Mann-Whitney *U* test, $p < 0.001$) than post-ban (26.8 ± 1.3 PSU). Salinity was variable by season (Mann-Whitney *U* test, $p < 0.001$) with more saline water in the dry season (33.9 ± 0.89 PSU) than in the wet (26.6 ± 1.3 PSU). There was also variability in salinity by lagoon segment (Kruskal-Wallis test, $p < 0.001$) with significantly higher values observed in the ML (36.5 ± 1.1 PSU) than in the BR (28.1 ± 1.5 PSU) and CIRL (27.4 ± 2.0 PSU), while the SIRL (32.8 ± 1.2 PSU) and NIRL (28.9 ± 2.2 PSU) had intermediate values. Mean pH was 8.10 ± 0.04. pH was significantly higher (Mann-Whitney *U* test, $p < 0.001$) pre-ban (8.09 ± 0.05) than post-ban (7.91 ± 0.04). There was seasonal variability observed in pH (Mann-Whitney *U* test, $p = 0.005$) with higher values in the dry season (8.10 ± 0.04) compared to the wet (7.88 ± 0.05). pH was not variable by lagoon segment (Kruskal-Wallis test, $p = 0.46$). Mean water temperature was 25.8 ± 0.44 °C. Significantly higher (Mann-Whitney *U* test, $p < 0.001$) water temperatures were observed pre-ban (27.4 ± 0.63 °C) compared to post-ban (23.9 ± 0.45 °C). Water temperature was variable by season (Mann-Whitney *U* test, $p < 0.001$) with higher temperatures in the dry season (28.0 ± 0.63 °C) than the wet season (23.3 ± 0.31 °C). Water temperature was not variable by lagoon segment (Kruskal-Wallis test, $p = 0.24$).

3.3. Dissolved nutrient concentrations

NH_4^+ concentrations were elevated throughout the study with a mean of 3.30 ± 0.40 μM. Though not significantly different (Mann-Whitney *U* test, $p = 0.45$), mean pre-ban NH_4^+ concentrations (2.62 ± 0.48 μM) were lower than post-ban (4.02 ± 0.66 μM). Significant differences were observed by segment (Kruskal-Wallis test, $p < 0.001$) with the BR having the highest NH_4^+ concentration (7.79 ± 1.9 μM), followed by the CIRL (4.64 ± 0.92 μM), NIRL (2.56 ± 0.51 μM), SIRL (1.29 ± 0.23 μM), and ML (1.06 ± 0.18 μM). Seasonal differences were also observed (Mann-Whitney *U* test, $p = 0.004$) with significantly higher mean NH_4^+ concentrations observed in the wet season (4.34 ± 0.63 μM) than the dry season (2.26 ± 0.49 μM).

Within segment differences were observed in NH_4^+ concentrations (Fig. 3a). In the ML, NH_4^+ concentrations were significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban during the wet season (0.33 ± 0.04 μM) than pre-ban (0.77 ± 0.11 μM). Conversely, in the BR during the wet season NH_4^+ concentrations post-ban (25.8 ± 2.9 μM) were significantly higher (Mann-Whitney *U* test, $p < 0.001$) than pre-ban (2.23 ± 0.90 μM). In the NIRL, NH_4^+ concentrations were generally higher post-ban, but significant differences (Mann-Whitney *U* test, $p < 0.001$) were only observed in the dry season (0.55 ± 0.54 μM pre-ban compared to 4.82 ± 1.4 μM post-ban). In the CIRL, wet season NH_4^+

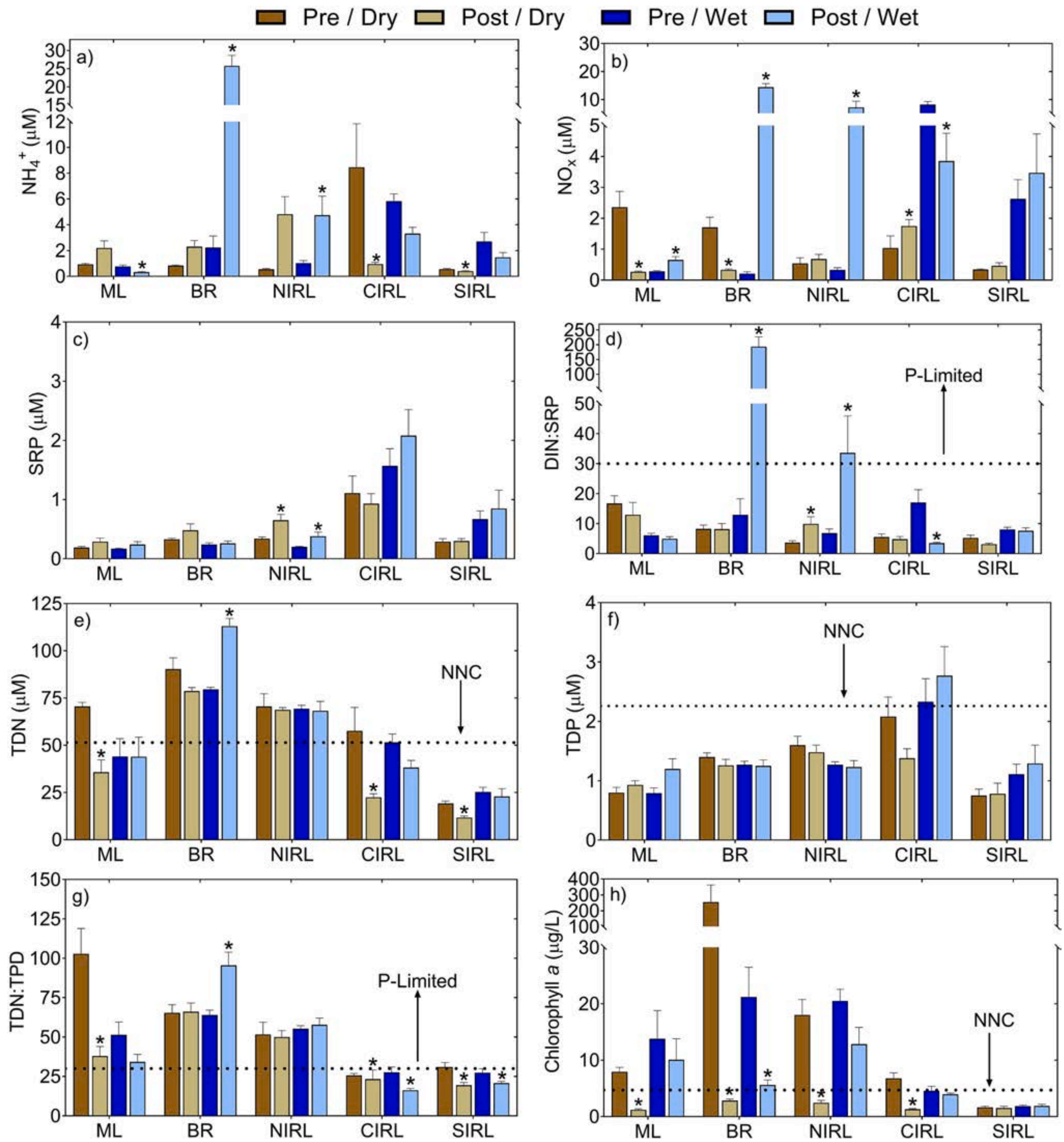

Fig. 3. Dissolved nutrient concentrations and other characteristics of surface water (mean ± SE) observed in the Indian River Lagoon (IRL), FL by lagoon segment are shown seasonally (dry / wet) and pre- and ~ five-years post- fertilizer ordinance implementation, including a) ammonium (NH_4^+), b) nitrate + nitrite (NO_x), c) soluble reactive phosphorus (SRP), d) DIN:SRP with a dotted line at 30, indicating P-limitation, e) total dissolved nitrogen (TDN) with a dotted line at 51.4 μM, indicating the IRL Numeric Nutrient Criteria (NNC), f) total dissolved phosphorus (TDP) with a dotted line at 2.26 μM, indicating the IRL NNC, g) TDN:TDP with a dotted line at 30, indicating P-limitation, and h) chlorophyll *a* concentrations with a dotted line at 4.7 μg/L indicating the IRL NNC; asterisks indicate seasonal sampling events that were significantly different from others; only within group differences are represented.

concentrations were significantly lower (Mann-Whitney *U* test, $p = 0.004$) post-ban (3.31 ± 0.49 μM) compared to pre-ban (5.84 ± 0.55 μM). In the SIRL, dry season NH_4^+ concentrations were significantly lower (Mann-Whitney *U* test, $p = 0.037$) post-ban (0.41 ± 0.04 μM) compared to pre-ban (0.56 ± 0.05 μM).

NO_x concentrations were also elevated throughout the study with a mean of 2.51 ± 0.27 μM. Post-ban NO_x concentrations (3.16 ± 0.46 μM) were significantly higher (Mann-Whitney *U* test, $p = 0.005$) than pre-ban (1.88 ± 0.27 μM). Significant differences were observed by segment (Kruskal-Wallis test, $p < 0.001$) with the BR having the highest NO_x concentration (4.17 ± 1.1 μM), followed by the CIRL (3.71 ± 0.51 μM), NIRL (2.00 ± 0.61 μM), SIRL (1.72 ± 0.39 μM), and ML (0.89 ± 0.19 μM). Seasonal differences were also observed (Mann-Whitney *U* test, $p < 0.001$) with significantly higher NO_x concentrations observed in the wet season (4.10 ± 0.49 μM) than the dry season (0.91 ± 0.09 μM).

Within segment differences were observed in NO_x concentrations

(Fig. 3b). In the ML, dry season NO_x concentrations were significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban (0.27 ± 0.02 μM) compared to pre-ban (2.36 ± 0.51 μM), while wet season NO_x concentrations were significantly higher (Mann-Whitney *U* test, $p = 0.002$) post-ban (0.65 ± 0.11 μM) compared to pre-ban (0.28 ± 0.03 μM). Similarly, in the BR dry season NO_x concentrations post-ban (0.33 ± 0.3 μM) were significantly lower (Mann-Whitney *U* test, $p < 0.001$) than pre-ban (1.71 ± 0.32 μM), while wet season NO_x concentrations were significantly higher (Mann-Whitney *U* test, $p < 0.001$) post-ban (14.4 ± 1.3 μM) as compared to pre-ban (0.21 ± 0.06 μM). In the NIRL, wet season NO_x concentrations were significantly higher (Mann-Whitney *U* test, $p < 0.001$) post-ban (7.23 ± 2.2 μM) compared to pre-ban (0.33 ± 0.07 μM). Interestingly, in the CIRL dry season NO_x concentrations were significantly higher (Mann-Whitney *U* test, $p = 0.004$) post-ban (1.75 ± 0.21 μM) compared to pre-ban (1.04 ± 0.39 μM), while wet season NO_x concentrations were significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban (3.85 ± 0.90 μM) compared to pre-ban (8.21 ± 1.1 μM). In the SIRL, significant differences were not observed in NO_x concentrations by season and ban status (Mann-Whitney *U* tests, both $p > 0.97$).

SRP concentrations averaged 0.64 ± 0.05 μM. Post-ban SRP concentrations (0.72 ± 0.09 μM) were not significantly different (Mann-Whitney *U* test, $p = 0.06$) than pre-ban (0.56 ± 0.07 μM). Significant differences were observed by segment (Kruskal-Wallis test, $p < 0.001$) with the CIRL having the highest SRP concentration (1.42 ± 0.16 μM), followed by the SIRL (0.53 ± 0.09 μM), NIRL (0.38 ± 0.04 μM), BR (0.33 ± 0.03 μM), and ML (0.22 ± 0.02 μM). Seasonal differences in SRP concentrations were not observed (Mann-Whitney *U* test, $p = 0.515$).

There were few within segment differences observed in SRP concentrations by season and ban status (Fig. 3c). In the NIRL, during both seasons SRP concentrations were significantly higher (Mann-Whitney *U* tests, both $p < 0.007$) post-ban (overall mean 0.52 ± 0.7 μM) compared to pre-ban (overall mean 0.27 ± 0.02 μM). However, in the ML, BR, CIRL, and SIRL there were no significant differences in SRP concentrations by season and ban status (Mann-Whitney *U* tests, all $p > 0.19$).

DIN:SRP were variable with a mean of 15.8 ± 2.6. There were no significant differences in DIN:SRP (Mann-Whitney *U* test, $p = 0.80$) by ban status. Significant differences were observed by segment (Kruskal-Wallis test, $p = 0.007$) with the BR having the highest DIN:SRP (55.7 ± 16), followed by the NIRL (12.6 ± 3.2), ML (10.2 ± 1.5), CIRL (7.71 ± 1.3), and SIRL (6.02 ± 0.46). Seasonal differences were also observed (Mann-Whitney *U* test, $p = 0.001$) with significantly higher DIN:SRP observed in the wet season (24.4 ± 5.1) than the dry season (7.07 ± 0.06).

Some within segment differences were observed in DIN:SRP (Fig. 3d). In the BR wet season DIN:SRP post-ban (193 ± 33) were significantly higher (Mann-Whitney *U* test, $p < 0.001$) than pre-ban (13.0 ± 5.3), while dry season DIN:SRP were not significantly different (Mann-Whitney *U* test, $p = 0.931$). In the NIRL, DIN:SRP were significantly higher (Mann-Whitney *U* test, both $p < 0.004$) post-ban in both the wet (33.6 ± 12) and dry (9.93 ± 2.4) seasons compared to pre-ban (6.84 ± 1.4 and 3.633 ± 0.68, respectively). In the CIRL wet season DIN:SRP were significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban (3.49 ± 0.30) compared to pre-ban (17.0 ± 4.3). In the ML and SIRL there were no significant differences in DIN:SRP by season and ban status (Mann-Whitney *U* test, all $p > 0.26$).

TDN concentrations were elevated throughout the study with an overall mean of 50.7 ± 2.0 μM, which is just below the IRL NNC. Mean post-ban TDN concentrations (46.0 ± 2.9 μM) were significantly lower (Mann-Whitney *U* test, $p = 0.007$) than pre-ban (55.3 ± 2.7 μM), which exceeded the target. Significant differences were observed by segment (Kruskal-Wallis test, $p < 0.001$) with the BR having the highest TDN concentration (90.4 ± 3.0 μM), followed by the NIRL (69.3 ± 2.2 μM), ML (48.60 ± 4.3 μM), CIRL (42.5 ± 3.8 μM), and SIRL (19.8 ± 1.4 μM). Seasonal differences were not observed in TDN (Mann-Whitney *U* test, $p = 0.14$).

Within segment differences were observed in TDN concentrations (Fig. 3e). In the ML, dry season TDN concentrations were significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban (35.7 ± 6.5 μM) compared to pre-ban (70.6 ± 2.1 μM). In the BR wet season TDN concentrations were significantly higher (Mann-Whitney *U* test, $p < 0.001$) post-ban (113 ± 4.1 μM) than pre-ban (79.7 ± 0.99 μM). In the CIRL and SIRL, dry season TDN concentrations were significantly lower (Mann-Whitney *U* test, both $p < 0.001$) post-ban (22.5 ± 1.9 μM and 11.7 ± 0.78 μM, respectively) compared to pre-ban (57.7 ± 12 μM and 19.2 ± 1.3 μM, respectively). In the NIRL there were no significant differences in TDN concentrations by season and ban status (Mann-Whitney *U* test, all $p > 0.43$).

TDP concentrations were variable with a mean of 1.39 ± 0.06 μM, which did not exceed the target. There were no significant differences in TDP concentrations by ban status (Mann-Whitney *U* test, $p = 0.61$). Significant differences were observed by segment (Kruskal-Wallis test, $p < 0.001$) with the CIRL having the highest TDP concentration (2.14 ± 0.19 μM), followed by the NIRL (1.40 ± 0.06 μM), BR (1.29 ± 0.04 μM), SIRL (0.98 ± 0.10 μM), and ML (0.93 ± 0.06 μM). Seasonal differences were not observed in TDP (Mann-Whitney *U* test, $p = 0.16$). No within segment differences were observed in TDP concentrations (Fig. 3f; Mann-Whitney *U* test, all $p > 0.22$).

TDN:TDP were variable with a mean of 42.4 ± 1.8. There were significant differences in TDN:TDP by ban status (Mann-Whitney *U* test, $p = 0.001$) with higher ratios pre-ban (46.3 ± 2.6) as opposed to post-ban (38.3 ± 2.5). Additionally, there were significant differences observed in TDN:TDP by segment (Kruskal-Wallis test, $p < 0.001$) with the BR having the highest TDN:TDP (72.7 ± 3.6), followed by the ML (56.6 ± 6.6), NIRL (53.7 ± 2.5), SIRL (24.6 ± 1.2), and CIRL (23.2 ± 1.2). Seasonal differences were not observed in TDN:TDP (Mann-Whitney *U* test, $p = 0.94$).

Some within segment differences were observed in TDN:TDP (Fig. 3g). In the ML, dry season TDN:TDP post-ban (37.9 ± 6.1) were significantly lower (Mann-Whitney *U* test, $p < 0.001$) than pre-ban (103 ± 16), while in the BR, wet season TDN:TDP post-ban (95.5 ± 8.4) were significantly higher (Mann-Whitney *U* test, $p = 0.003$) than pre-ban (64.0 ± 3.1). In the CIRL and SIRL, TDN:TDP were significantly lower (Mann-Whitney *U* tests, all $p < 0.05$) post-ban in both the wet (16.3 ± 1.2 and 20.7 ± 1.2, respectively) and dry (23.3 ± 5.8 and 19.4 ± 1.7, respectively) seasons compared to the pre-ban wet (27.6 ± 3.3 and 27.4 ± 2.6, respectively) and dry (25.7 ± 1.2 and 31.0 ± 2.9, respectively) seasons. In the NIRL there were no significant differences in TDN:TDP by season and ban status (Mann-Whitney *U* test, all $p > 0.26$).

Chlorophyll *a* concentrations were elevated throughout the study with a mean of 16.1 ± 4.8 μg/L, exceeding the target concentration (4.7 μg/L). Mean post-ban chlorophyll *a* concentrations (27.6 ± 9.2 μg/L) were significantly higher (Mann-Whitney *U* test, $p < 0.001$) than pre-ban (4.08 ± 0.53 μg/L). Significant differences were observed by segment (Kruskal-Wallis test, $p < 0.001$) with the BR having the highest chlorophyll *a* concentration (71.1 ± 31 μg/L), followed by the NIRL (14.1 ± 1.5 μg/L), ML (8.24 ± 1.7 μg/L), CIRL (4.15 ± 0.4 μg/L), and SIRL (1.72 ± 0.13 μg/L). Seasonal differences were also observed in chlorophyll *a* (Mann-Whitney *U* test, $p = 0.006$) with higher concentrations in the dry season (23.3 ± 9.5 μg/L) than the wet (8.95 ± 0.93 μg/L).

Within segment differences were observed in chlorophyll *a* concentrations (Fig. 3h). In the ML, dry season chlorophyll *a* concentrations were significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban (1.19 ± 0.18 μg/L) compared to pre-ban (7.94 ± 0.78 μg/L). In the BR, chlorophyll *a* concentrations were significantly lower (Mann-Whitney *U* test, both $p \leq 0.003$) post-ban in both the dry (2.82 ± 0.28 μg/L) and wet (5.60 ± 0.89 μg/L) seasons compared to pre-ban (255 ± 107 μg/L and 21.2 ± 5.3 μg/L, respectively). In the NIRL, dry season chlorophyll *a* concentrations were significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban (2.43 ± 0.43 μg/L) compared to pre-ban (18.0 ± 2.7 μg/L). In the CIRL, dry season chlorophyll *a* concentrations were also significantly lower (Mann-Whitney *U* test, $p < 0.001$) post-ban (1.30 ±

Table 1
Multivariate analysis of variance (MANOVA) of macroalgae stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values by fertilizer ban status (pre or post), season (wet or dry), and Indian River Lagoon segment, including the Mosquito Lagoon (ML), the Banana River, the northern IRL, the central IRL, and the southern IRL, as well as subsequent univariate analysis of variance (ANOVA) on significant factors and interactions; significant factors considered at $p < 0.05$ are shown with *p*-values in bold.

Factor	df	MS	*F*	*P*
MANOVA				
Ban status	2, 429	–	0.189	**<0.001**
Season	2, 429	–	0.122	**<0.001**
Lagoon segment	8, 860	–	0.239	**<0.001**
Ban status * Season	2, 429	–	0.071	**<0.001**
Ban status * Segment	8, 860	–	0.220	**<0.001**
Segment * Season	8, 860	–	0.056	**0.002**
Ban status * Segment * Season	8, 860	–	0.077	**<0.001**
ANOVA $\delta^{15}N$				
Ban status	1	63.8	13.9	**<0.001**
Season	1	0.76	0.164	0.685
Lagoon segment	4	104	22.5	**<0.001**
Ban status * Season	1	146	31.7	**<0.001**
Ban status * Segment	4	102	22.2	**<0.001**
Segment * Season	4	14.2	3.09	**0.016**
Ban status * Segment * Season	4	5.42	1.18	0.319
ANOVA $\delta^{13}C$				
Ban status	1	1088	92.5	**<0.001**
Season	1	689	58.5	**<0.001**
Lagoon segment	4	92.7	7.88	**<0.001**
Ban status * Season	1	28.1	2.38	0.123
Ban status * Segment	4	59.0	5.01	**<0.001**
Segment * Season	4	40.4	3.43	**0.009**
Ban status * Segment * Season	4	88.4	7.51	**<0.001**

0.11 μg/L) compared to pre-ban (6.78 ± 0.98 μg/L). In the SIRL there were no significant differences in chlorophyll *a* concentrations by season and ban status (Mann-Whitney *U* test, all $p > 0.39$).

3.4. Stable isotopes and elemental composition of macroalgae

A total of 450 macroalgae samples were analyzed (see Tables S1 and 2), including 211 that were collected pre-ban and 239 collected post-ban. By lagoon segment, 54 macroalgae samples were collected from the ML, 66 from the BR, 111 from the NIRL, 119 from the CIRL, and 100 from the SIRL. During the wet season, 217 macroalgae samples were collected, while 233 were collected during the dry season.

Stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) were significantly variable by ban status (MANOVA, $p < 0.001$), season (MANOVA, $p < 0.001$), and lagoon segment (MANOVA, $p < 0.001$; Table 1). Additionally, there were significant two-way interactions of ban status x season (MANOVA, $p < 0.001$), ban status x lagoon segment (MANOVA, $p < 0.001$), and lagoon segment by season (MANOVA, $p = 0.002$). Finally, the three-way interaction of ban status x lagoon segment x season was also significant (MANOVA, $p < 0.001$; see Figs. 4 and 5).

Mean macroalgal $\delta^{13}C$ values were − 20.0 ± 0.20 ‰. Macroalgal $\delta^{13}C$ values were significantly (ANOVA, $p < 0.001$) more enriched pre-ban (−18.2 ± 0.26) than post-ban (−21.6 ± 0.26) and in the dry (−18.7 ± 0.25 ‰) compared to the wet (−21.5 ± 0.29 %) season. Variability in macroalgal $\delta^{13}C$ values by lagoon segment were also observed (ANOVA, $p < 0.001$) with significantly higher values in the BR (−18.7 ± 0.53 ‰), compared to the CIRL (−20.4 ± 0.32 ‰), SIRL (−20.1 ± 0.56 ‰), and ML (−21.4 ± 0.53 ‰), while the NIRL (−19.6 ± 0.33 ‰) was significantly higher than the ML. Pre-ban macroalgal $\delta^{13}C$ values were significantly more enriched in the BR (−16.0 ± 0.89 ‰), CIRL (−18.3 ± 0.26 ‰), and SIRL (−17.5 ± 0.71 ‰) than post-ban values for those segments (−20.5 ± 0.48 ‰, −22.20 ± 0.44 ‰, and − 22.7 ± 0.71 ‰, respectively). By segment and season, significantly more enriched macroalgal $\delta^{13}C$ values were observed during the dry season in the BR (−17.2 ± 0.64 ‰), NIRL (−17.4 ± 0.47 ‰), and CIRL (−19.1 ± 0.29 ‰), than in those segments during the wet season (−20.2 ± 0.78 ‰, −21.4 ± 0.28 ‰, and − 21.9 ± 0.54 ‰, respectively). There was also significant variability in macroalgal $\delta^{13}C$ observed by ban status, lagoon segment, and season (ANOVA, $p < 0.001$) with generally more depleted values post-ban (see Fig. 5a and Tables S1-S2).

Mean macroalgal $\delta^{15}N$ was +6.84 ± 0.13 ‰. Macroalgal $\delta^{15}N$ was significantly (ANOVA, $p < 0.001$) higher post-ban (+7.24 ± 0.20 ‰) than pre-ban (+6.39 ± 0.14 ‰). Seasonal differences were not observed in macroalgal $\delta^{15}N$ (ANOVA, $p = 0.69$). Variability in macroalgal $\delta^{15}N$ by lagoon segment was observed (ANOVA, $p < 0.001$) with significantly higher values in the BR (+8.49 ± 0.40 ‰), than in the CIRL (+7.15 ± 0.15 ‰), while the NIRL (+7.37 ± 0.30 ‰) was similar to both. All other segments were significantly higher than the SIRL (+5.70 ± 0.20 ‰) and ML (+5.16 ± 0.21 ‰). By ban status and segment, macroalgal $\delta^{15}N$ values in the BR post-ban (+10.3 ± 0.43 ‰) were significantly higher than all other segments pre or post-ban. The second highest macroalgal $\delta^{15}N$ values were observed in the NIRL post-ban (+8.26 ± 0.45 ‰), which was similar to the $\delta^{15}N$ values observed in the CIRL both pre- and post-ban (+7.16 ± 0.23 ‰ and + 7.13 ± 0.21 ‰, respectively). By ban and season, the post-ban dry season had significantly higher macroalgal $\delta^{15}N$ values (+7.76 ± 0.24 ‰) than all other seasons. Wet seasons for both pre-ban (+7.01 ± 0.27 ‰) and post-ban (+6.80 ± 0.29 ‰) were similar, while the pre-ban dry season had the lowest average macroalgal $\delta^{15}N$ values (+5.94 ± 0.13 ‰). By segment and season, the BR had the highest macroalgal $\delta^{15}N$ values in the dry season (+8.62 ± 0.64 ‰), followed by the BR wet season (+8.35 ± 0.49 ‰), the NIRL wet season (+7.97 ± 0.48 ‰), and the CIRL dry season (+7.50 ± 0.20 ‰), all of which were significantly higher than the SIRL and ML in both seasons (range + 4.83–5.79 ‰). The interaction of macroalgal $\delta^{15}N$ by ban status, lagoon segment, and season was not significant (ANOVA, $p = 0.319$; Table 1; Fig. 5b and Tables S1-S2).

Mean macroalgal C:N was 16.2 ± 0.39. Macroalgal C:N was significantly variable by ban status (Mann-Whitney *U* test, $p = 0.02$) with higher ratios observed pre-ban (16.9 ± 0.58) than post-ban (15.6 ± 0.52). There was significant variability observed in macroalgal C:N by season (Mann-Whitney *U* test, $p < 0.001$) with higher ratios observed in the dry season (17.3 ± 0.50) than the wet (15.1 ± 0.59). Macroalgal C:N was variable by segment (Kruskal-Wallis, $p < 0.001$) with significantly higher ratios in the SIRL (18.4 ± 0.99) and ML (17.1 ± 0.68) than the NIRL (15.5 ± 0.69) or CIRL (13.9 ± 0.49); the BR (17.4 ± 1.5) was also apparently higher than the NIRL or CIRL. Additionally, there was significant variability in macroalgal C:N within lagoon segment observed by ban status and season. Significantly higher macroalgal C:N post-ban were observed in the ML in the dry season and the CIRL and SIRL in the wet season, while significantly lower macroalgal C:N post-ban were observed in the NIRL in the dry season and the ML, BR, and NIRL in the wet season (see Fig. 5c and Tables S1-S2).

Mean macroalgal C:P was 589 ± 27. Macroalgal C:P was not significantly variable by ban status (Mann-Whitney *U* test, $p = 0.94$) or season (Mann-Whitney *U* test, $p = 0.75$). Macroalgal C:P was variable by segment (Kruskal-Wallis, $p < 0.001$) with significantly lower ratios in the CIRL (314 ± 13) than in the ML (761 ± 75), NIRL (748 ± 66), BR (702 ± 55), or SIRL (574 ± 67). There was also significant variability in macroalgal C:P within lagoon segment observed by ban status and season. Significantly higher macroalgal C:P post-ban were observed in the SIRL in the wet season, while significantly lower C:P post ban were observed in the ML, NIRL, and SIRL in the dry season and in the BR in the wet season (see Fig. 5d and Tables S1-S2).

Mean macroalgal N:P was 35.1 ± 1.0. Macroalgal N:P was not significantly variable by ban status (Mann-Whitney *U* test, $p = 0.56$). There was significant variability observed in macroalgal N:P by season (Mann-Whitney *U* test, $p < 0.001$) with higher ratios observed in the wet (38.4 ± 1.6) than the dry (32.0 ± 1.3) season. Macroalgal N:P was

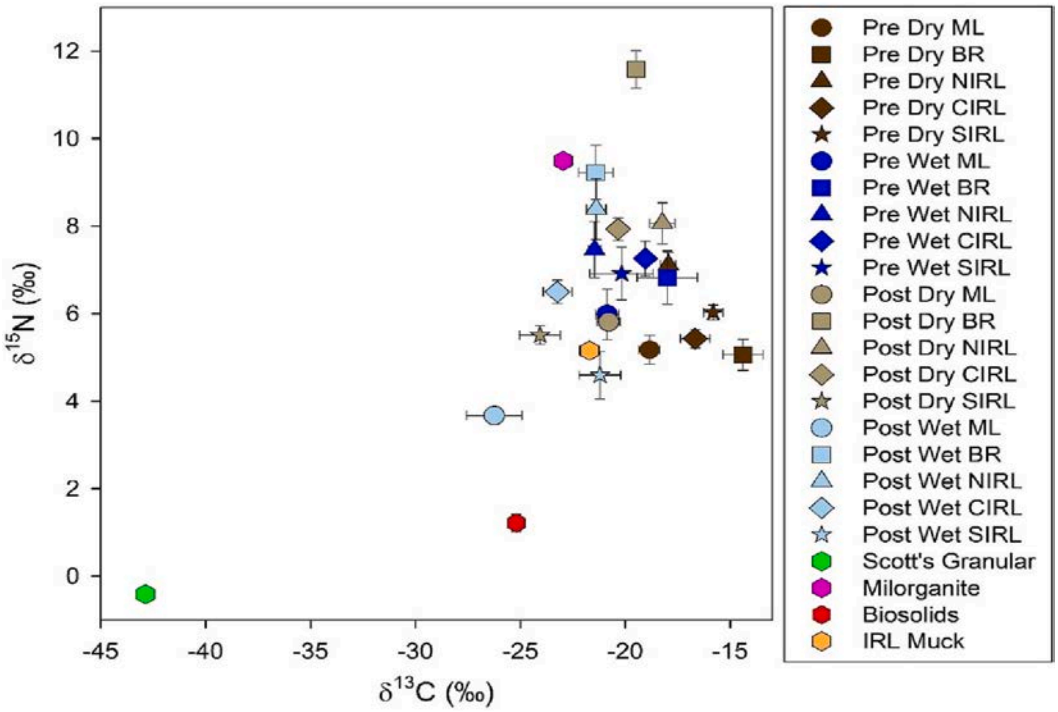

Fig. 4. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values (‰) of macroalgae collected in the Indian River Lagoon (IRL) before (2011–2012, "pre-ban") and ~ five-years after (2016–2017, "post-ban") the enactment of fertilizer bans shown by ban status (pre vs. post), season (wet or dry), and lagoon segment, including the Mosquito Lagoon (ML), Banana River (BR), northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL). Also showing values for Scott's granular fertilizer, Milorganite, biosolids, and IRL muck.

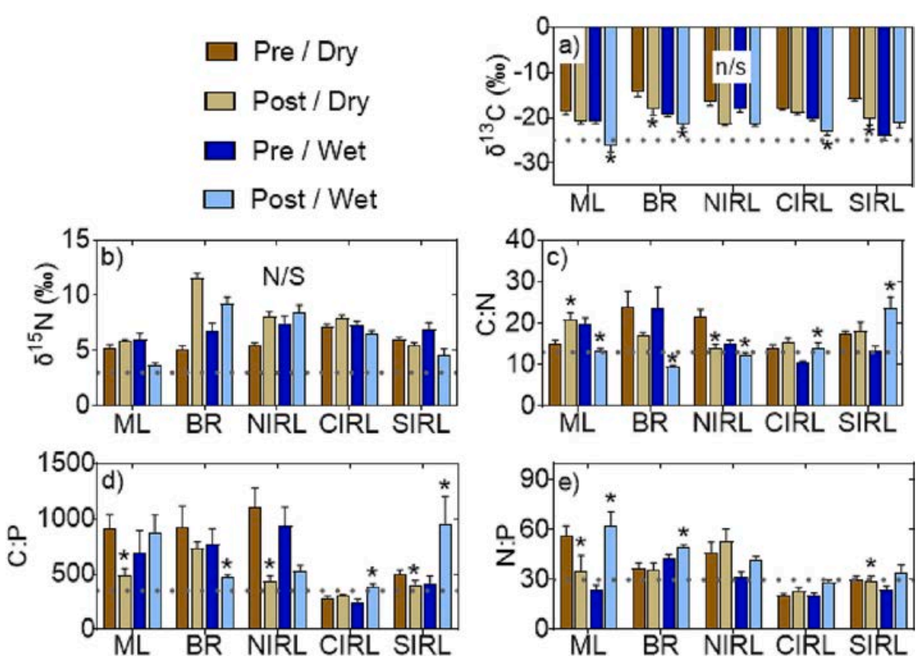

Fig. 5. Chemical properties of macroalgal tissue (mean ± SE) by season (wet/dry) from the Indian River Lagoon (IRL) before (2011–2012, pre) and ~ five-years after (2016–2017, post) the enactment of fertilizer bans shown by ban status (pre vs. post), season (wet or dry), and lagoon segment, including the Mosquito Lagoon (ML), Banana River (BR), northern IRL (NIRL), central IRL (CIRL), and southern IRL (SIRL), including a) stable C isotope values (δ^{13}C), b) stable N isotope values (δ^{15}N), c) carbon (C) to nitrogen (N) ratio (C:N), d) C to phosphorus (P) ratio (C:P), and e) N:P. Significant differences within segment and season by ban status as determined with ANOVA or Kruskal-Wallis tests and post-hoc Tukey or Mann-Whitney *U* tests are indicated by asterisks; only within group differences are represented.

variable by segment (Kruskal-Wallis, $p < 0.001$) with significantly higher ratios in the ML (45.8 ± 4.0), NIRL (43.4 ± 2.6), and BR (42.3 ± 1.5) than in the SIRL (29.4 ± 1.5) and CIRL (23.4 ± 0.83). There was also significant variability in macroalgal N:P within lagoon segment observed by ban status and season. Significantly lower macroalgal N:P was observed in the ML and SIRL in the dry season, while significantly higher macroalgal N:P was observed in the ML and BR in the wet season (see Fig. 5e and Tables S1-S2).

3.5. *Brown tides*

The mean NH_4^+ concentration during brown tide blooms was 1.82 ± 0.54 μM. NH_4^+ was significantly (Kruskal-Wallis, $p < 0.001$) lower pre-ban in 2012 (0.33 ± 0.02 μM) than post-ban in 2016 (0.52 ± 0.05 μM) or 2017 (6.48 ± 1.6 μM). The NIRL had significantly (Kruskal-Wallis, $p < 0.001$) higher NH_4^+ (3.53 ± 1.1 μM) than the BR (0.39 ± 0.06 μM) and ML (0.30 ± 0.02 μM). By segment and year, the NIRL in

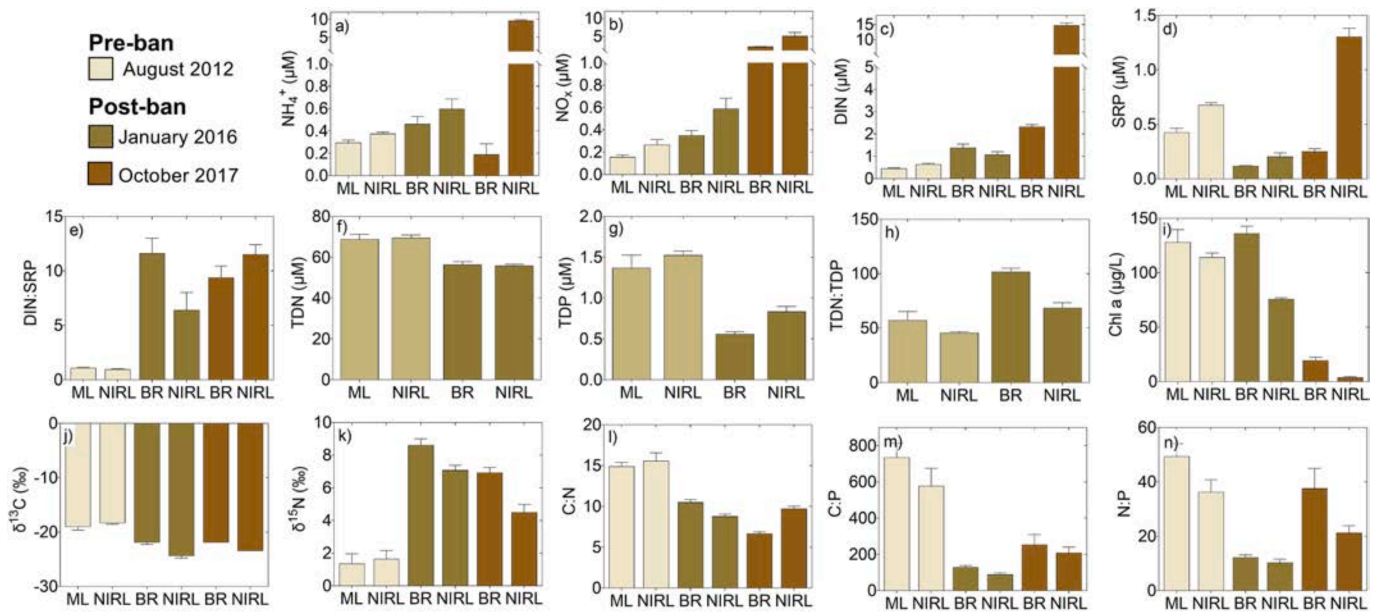

Fig. 6. Properties related to brown tide (*Aureoumbra lagunensis*) blooms pre-ban in August 2012 (light brown) and post-ban in January 2016 (medium brown) and October 2017 (dark brown) in the Indian River Lagoon (IRL) by lagoon segment, including the Mosquito Lagoon (ML), Banana River (BR), and northern IRL (NIRL) showing (mean ± SE) concentrations of a) ammonium (NH_4^+), b) nitrate + nitrite (NO_x), c) dissolved inorganic nitrogen (DIN), d) soluble reactive phosphorus (SRP), e) DIN to SRP ratio (DIN:SRP), f) total dissolved nitrogen (TDN), g) total dissolved phosphorus (TDP), h) TDN to TDP ratio (TDN:TDP), and i) chlorophyll *a*, as well as chemical properties of particulate organic matter (POM) a proxy for phytoplankton, including j) stable C isotope values ($\delta^{13}C$), k) stable N isotope values ($\delta^{15}N$), l) carbon (C) to nitrogen (N) ratio (C:N), m) C to phosphorus (P) ratio (C:P), and n) N:P. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2017 had the highest average NH_4^+ of the project (9.62 ± 0.28 μM; Fig. 6a).

The mean NO_x concentration during brown tide blooms was 1.20 ± 0.32 μM. By year, the NO_x concentrations were all significantly different (Kruskal-Wallis, $p < 0.001$). Pre-ban in 2012, average NO_x was lowest (0.20 ± 0.03 μM), followed by post-ban 2016 (0.45 ± 0.05 μM) and 2017 (4.13 ± 0.82 μM). There was significantly (Kruskal-Wallis, $p < 0.001$) higher NO_x concentrations in the NIRL (1.99 ± 0.62 μM) and BR (0.80 ± 0.24 μM) compared to the ML (0.16 ± 0.02 μM). By segment and year, the NIRL 2017 had the highest average NO_x of the project (5.12 ± 1.0 μM; Fig. 6b). Brown tide DIN concentrations are shown by sampling event in Fig. 6c.

The mean SRP concentration during brown tide blooms was 0.48 ± 0.07 μM. In 2016, SRP was significantly (Kruskal-Wallis, $p < 0.001$) lower (0.15 ± 0.02 μM) than in 2012 (0.53 ± 0.04 μM) or 2017 (0.95 ± 0.18 μM) with no patterns pre or post-ban. SRP concentrations were significantly (Kruskal-Wallis, $p < 0.001$) higher in the NIRL (0.73 ± 0.11 μM) and ML (0.43 ± 0.04 μM) than in the BR (0.15 ± 0.02 μM). By segment and year, the NIRL in 2017 had the highest SRP concentration of the project (1.31 ± 0.8 μM; Fig. 6d).

The mean DIN:SRP during brown tide blooms was 6.56 ± 0.86. DIN:SRP was significantly (Kruskal-Wallis, $p < 0.001$) lower pre-ban in 2012 (1.03 ± 0.05) compared to post-ban in 2016 (9.54 ± 1.2) and 2017 (10.8 ± 0.75). Overall, the BR had the highest average DIN:SRP (11.1 ± 1.1), followed by the NIRL (6.29 ± 1.2) and ML (1.09 ± 0.07), which was significantly lower than the BR (Kruskal-Wallis, $p < 0.001$). By segment and year variability in DIN:SRP were also observed (Fig. 6e).

The mean TDN concentration during brown tide blooms was 62.7 ± 1.5 μM. Pre-ban in 2012, TDN was significantly (Mann-Whitney *U* test, $p < 0.001$) higher (69.2 ± 1.5 μM) than post-ban in 2016 (56.2 ± 0.90 μM); TDN was not analyzed for in 2017. The ML had the highest TDN (62.8 ± 2.2 μM), followed by the NIRL (62.8 ± 2.2 μM) and BR (56.4 ± 1.5 μM), which was significantly lower than the ML (Kruskal-Wallis, $p = 0.007$). By segment and year, the NIRL in 2012 had the highest average TDN concentration of the project (69.6 ± 1.3 μM; Fig. 6f).

The mean TDP concentration during brown tide blooms was 1.05 ± 0.09 μM. Pre-ban in 2012, TDP was significantly (Mann-Whitney *U* test, $p < 0.001$) higher (1.43 ± 0.10 μM) than post-ban in 2016 (0.67 ± 0.05 μM); TDP was not analyzed for in 2017. TDP was significantly (Kruskal-Wallis, $p < 0.001$) lower in the BR (0.56 ± 0.03 μM) than in the ML (1.37 ± 0.16 μM) or NIRL (1.18 ± 0.11 μM). By segment and year, the NIRL in 2012 had the highest average TDP of the project (1.53 ± 0.05 μM; Fig. 6g).

The mean TDN:TDP during brown tide blooms was 70.6 ± 4.8. Post-ban in 2016, TDN:TDP was significantly (Mann-Whitney *U* test, $p < 0.001$) higher (88.6 ± 5.1) than pre-ban in 2012 (52.7 ± 4.9). The BR had significantly (Kruskal-Wallis, $p < 0.001$) higher TDN:TDP (102 ± 3.2) than the ML (57.4 ± 7.9) and NIRL (57.2 ± 4.2). By segment and year, variability in TDN:TDP were also observed (Fig. 6h).

The mean chlorophyll *a* concentration during brown tide blooms was 92.3 ± 8.6 μg/L. In 2017, chlorophyll *a* was significantly (Kruskal-Wallis, $p < 0.001$) lower (9.07 ± 2.8 μg/L), than in 2016 (112 ± 8.8 μg/L) and 2012 (123 ± 7.2 μg/L) with no patterns pre or post-ban. Chlorophyll *a* concentrations in the ML (128 ± 12 μg/L) and BR (107 ± 16 μg/L) were significantly (Kruskal-Wallis, $p < 0.001$) higher than in the NIRL (64.6 ± 11.2 μg/L). By segment and year, the BR had the highest average chlorophyll *a* concentration of the project in 2016 (136 +/- 6 μg/L; Fig. 6i).

The mean POM $\delta^{13}C$ value during the brown tide blooms was −21.3 ± 0.43 ‰. Pre-ban in 2012, POM $\delta^{13}C$ values (−18.8 ± 0.37 ‰) were significantly higher (Kruskal-Wallis test, $p < 0.001$) than post-ban in 2016 (−22.9 ± 0.40 ‰) or 2017 (−23.0 ± 0.81 ‰). The ML had significantly (Kruskal-Wallis test, $p < 0.001$) higher POM $\delta^{13}C$ values (−19.2 ± 0.61 ‰) than in the BR (−21.9 ± 0.26 ‰) or NIRL (−22.1 ± 0.75 ‰). By segment and year, the NIRL had the highest POM $\delta^{13}C$ values of the project in 2012 (−18.4 ± 0.17 ‰; Fig. 6j).

The mean POM $\delta^{15}N$ value during brown tide blooms was +4.87 ± 0.52 ‰. Pre-ban in 2012, POM $\delta^{15}N$ values (+1.47 ± 0.40 ‰) were significantly lower (Kruskal-Wallis test, $p < 0.001$) than post-ban in 2016 (+8.00 ± 0.32 ‰) or 2017 (+5.31 ± 0.52 ‰). The BR had significantly (Kruskal-Wallis test, $p < 0.001$) higher POM $\delta^{15}N$ values (+8.20 ± 0.36 ‰) than in the NIRL (+4.41 ± 0.59 ‰) or ML (+1.36 ± 0.60 ‰). By segment and year, the BR had the highest POM $\delta^{15}N$ values of the project in 2016 (+8.62 ± 0.38 ‰; Fig. 6k).

The mean POM C:N during brown tide blooms was 11.6 ± 0.52. Pre-ban in 2012, POM C:N (15.2 ± 0.47) was significantly higher (Kruskal-Wallis test, $p < 0.001$) than post-ban in 2016 (9.82 ± 0.32) or 2017 (8.73 ± 0.54). The ML had significantly (Kruskal-Wallis test, $p = 0.002$) higher POM C:N (14.9 ± 0.49) than in the NIRL (11.4 ± 0.80) or BR (9.55 ± 0.56). By segment and year, the NIRL had the highest POM C:N of the project in 2012 (15.6 ± 0.97; Fig. 6l).

The mean POM C:P during brown tide blooms was 354 ± 48. Pre-ban in 2012, POM C:P (673 ± 62) was significantly higher (Kruskal-Wallis test, $p < 0.001$) than post-ban in 2016 (113 ± 8.3) or 2017 (225 ± 27). The ML had significantly (Kruskal-Wallis test, $p < 0.001$) higher POM C:P (736 ± 77) than in the NIRL (292 ± 60) or BR (160 ± 21). By segment and year, the ML had the highest POM C:P of the project in 2012 (736 ± 77; Fig. 6m).

The mean POM N:P during brown tide blooms was 27.6 ± 2.8. POM N:P in 2012 (44.2 ± 3.6) and 2017 (26.7 ± 3.9) were significantly higher (Kruskal-Wallis test, $p < 0.001$) than 2016 (8.73 ± 0.54) with no patterns pre or post ban. The ML had significantly (Kruskal-Wallis test, $p = 0.002$) higher POM N:P (49.4 ± 4.6) than in the NIRL (22.6 ± 3.1) or BR (18.6 ± 3.7). By segment and year, the ML had the highest POM N:P of the project in 2012 (49.4 ± 4.6; Fig. 6n).

4. Discussion

4.1. Nutrient dynamics of brown tides and macroalgae

Following five years of mandatory wet season fertilizer blackouts along the IRL, water quality and HABs have worsened in the NIRL and BR, leading to catastrophic seagrass die-offs (Lapointe et al., 2020; Morris et al., 2022) and starvation of manatees (Allen et al., 2022; Landsberg et al., 2022). Our comparative pre- versus post-ban nutrient data indicate that the wet season fertilizer blackouts were not as effective as policy makers had hoped. The worsening trend in the northern segments was evidenced by significant increases in post-wet season NH_4^+, NO_3^-, TDN, and SRP compared to the pre-ban wet seasons, with very high TDN concentrations of up to 113 μM in the BR. However, the overall mean IRL TDN concentration decreased significantly to 50.7 μM, which is just below the IRL target of 51.4 μM, contributing to a slight decrease in the TDN:TDP (46.3 to 38.3) but with notably high values in the BR (72.7). These findings suggest that the increasing concentrations of dissolved inorganic N and P observed in some segments of the IRL following ~five years of fertilizer bans would support the worsening trend of algal blooms. For example, dramatic spikes in NH_4^+ and NO_3^- occurred during the post-ban wet season (Fig. 3 a,b) in the BR and NIRL where severe brown tides occurred in 2012–2013, 2015–2016, and 2017-2018 (Phlips et al., 2021). This is important as northeastern brown tides (*Aureococcus anophagefferens*) from Long Island Sound are favored in environments with moderate concentrations of NH_4^+ (Taylor et al., 2006), such as the BR and NIRL during the post-ban sampling. Additionally, brown tides proliferate when DON dominates the N pool (Gobler and Sañudo-Wilhelmy, 2001; Gobler et al., 2013). These conditions existed in the NIRL and BR during this study where DON represented ~90 % of the N.

The spikes in NH_4^+ and NO_3^- co-occurred with spikes in the DIN:SRP ratio to high values well above 30, indicating strong P limitation of the brown tides. This shift toward stronger P-limitation would favor blooms of *Aureoumbra lagunensis*, which also formed dense blooms in the Laguna Madre, Texas at very high N:P ratios (~140:1) (Rhudy et al., 1999). We observed similarly high N:P >100 in the BR in the wet season, illustrating how small-celled brown tides can sustain blooms by scavenging nutrients at low concentrations and skewed N:P ratios (Sunda et al., 2006; Gobler et al., 2013). Our results underscore the conclusions of Wurch et al. (2019) that P limitation plays a key role in the dynamics of brown tides (Sun et al., 2012), especially relating to bloom decline.

Increased nutrient availability to the brown tides post-fertilizer bans was also evidenced by changes in POM elemental composition and stoichiometry. The significantly higher C:N of the brown tide in 2012 (15.2) compared to 2016 (9.82) indicates greater N enrichment post-fertilizer bans. Post-ban P enrichment also occurred, evidenced by a six-fold decrease in C:P from 2012 (673) to 2016 (113). The resulting decrease in the POM N:P ratio from pre-ban in 2012 (44.2) to post-ban in 2016 (8.73) and 2017 (26.7) reflects increasing P availability and a lower degree of P-limitation in the more recent brown tides.

A change in N source supporting blooms of *A. lagunensis* post-fertilizer bans was evidenced by a significant increase in POM $\delta^{15}N$ values between 2012 and 2016/2017. The increase in POM $\delta^{15}N$ values from < +3 ‰ pre-ban to > +5–8 ‰ post-ban is significant and reflects a shift in N sources from relatively depleted $\delta^{15}N$ values, such as atmospheric deposition, synthetic fertilizers and/or biosolids (see Fig. 4) to more enriched sources, such as human or animal waste (+3–24 ‰) (Heaton, 1986; Costanzo et al., 2001; Savage, 2005; Hinkle et al., 2008). This increase may reflect decreased fertilizer N loading as a result of the bans, which would lead to enrichment of the $\delta^{15}N$ values. The highest $\delta^{15}N$ values (+8.62 ‰) occurred in the BR during the 2016 brown tide and closely matched values for partially treated wastewater (+8 ‰) (Costanzo et al., 2001; Lapointe et al., 2005; Savage, 2005), which would be expected in this highly urbanized area with aging wastewater collection systems and secondary treatment without N removal. Slightly lower, but still enriched, POM $\delta^{15}N$ values for the overall mean brown tide (+4.87 ‰) and the NIRL brown tide (+4.41 ‰) are similar to values for septic system effluent (+4.9 ‰) (Hinkle et al., 2008), which has been widely observed in macroalgae throughout the IRL (Lapointe et al., 2015; Barile, 2018). The volatilization and microbial processing (nitrification) of ammonium from septic effluent would enrich the DIN $\delta^{15}N$ value to levels observed in the POM during this study (Lapointe et al., 2017).

Changes in macroalgal elemental composition further reflect the increases in nutrient availability following the fertilizer bans. A decrease in the C:N of macroalgae from 23.8 to 13.0 in the BR and 18.2 to 12.9 in the NIRL suggest N enrichment post- fertilizer bans in these segments where brown tides and seagrass die-off have been most intense and problematic. The C:P decreased from 823 to 684 in the ML, 858 to 593 in BR, and 1020 to 490 in the NIRL, compared to increases that were observed in the more southern segments. This pattern is similar to that of *A. lagunensis* and indicates reduced P limitation in the three northern segments – ML, BR, and NIRL- since 2011 during the period when recurrent brown tides and other phytoplankton HABs have worsened.

Similar to the brown tides, macroalgae isotopes further demonstrated changes in N sources during the study period. Macroalgal $\delta^{15}N$ values increased significantly from pre-ban to post-ban in the BR (from +5.84 to +10.31 ‰) and NIRL (from +6.45 to +8.26 ‰). This enrichment suggests a decrease in nutrient sources with depleted $\delta^{15}N$ values (i.e., fertilizers and atmospheric deposition), which could be a result of decreased fertilizer application following the bans and/or an increase in nutrient sources with more enriched stable isotope values (human or animal waste) that could be attributed to population growth or aging infrastructure. The increased NH_4^+ combined with more enriched $\delta^{15}N$ levels in the BR and NIRL (from +2 to +5 ‰ enrichment) indicates that there could be greater inputs of human and animal waste at these locations. There was a lack of strong seasonal pattern in $\delta^{15}N$ values potentially indicating continuous loading from human waste. Similar year round enrichment of $\delta^{15}N$ values without distinct seasonal patterns has been observed in other human waste contaminated watersheds in Florida (Tyre et al., 2023). These high macroalgal tissue $\delta^{15}N$ values between +8.26 to +10.3 ‰ are similar to values reported for macroalgae in close proximity to sewage outfalls in Moreton Bay, Australia (Costanzo et al., 2001), Himmerfjardin, Sweden (Savage, 2005), and southeast Florida (Lapointe et al., 2005), all indicating a strong presence of human waste N. These high $\delta^{15}N$ values are typical of processed human waste whereby microbial processing and volatilization of ammonium results in fractionation and $\delta^{15}N$ enrichment. Further, concentrations of the artificial sweetener sucralose collected at the BR and NIRL sites during 2022–2023 (249 ng/L; Lapointe, *unpublished data*) were above the 2013–2014 average IRL concentration (55 ng/L) (Herren et al., 2021) providing more evidence of the high inputs of human waste at these locations.

In contrast to the BR and NIRL, water quality in the more southerly IRL segments were either stable or improved slightly over the study period. In the CIRL and SIRL, there was no change in NH_4^+, while NO_3^- and chlorophyll *a* declined at most sites. The C:N ratio of macroalgae increased in the CIRL and SIRL, indicating greater N-limitation, whereas the $\delta^{15}N$ values of macroalgae decreased. These segments have a greater degree of tidal flushing through the Sebastian, Fort Pierce, St. Lucie, and Jupiter inlets, and also have had active septic-to-sewer programs, such as in the City of Port St. Lucie, Martin County, and the Jupiter/Tequesta area. In Brevard County, which borders much of the NIRL and BR, Tetra Tech estimates hooking up ~1500 OSTDS within 55 yards of water would remove 408,863 lbs. / N / yr, an order of magnitude higher than the reduction expected from fertilizer ordinances (45,896 lbs. / N / yr) (TetraTech and Closewaters, 2016; TetraTech, 2023).

4.2. Management implications

The deteriorating conditions in the IRL demonstrate the urgent need for more comprehensive mitigation actions. To this end, Brevard County has a multi-faceted *Save Our Indian River Lagoon Project Plan* in place with plans to reduce nutrient loading from a variety of sources including fertilizer, grass clippings, reclaimed water from wastewater treatment facilities, sprayfields, rapid infiltration basins, package plants, sewer laterals, septic systems, and stormwater (TetraTech, 2023). While fertilizer ordinances have been effective at reducing nutrient inputs and chlorophyll *a* in some locations throughout Florida, they are “not likely to be a standalone solution to preventing nutrient pollution in downstream water bodies” (Smidt et al., 2022). Additionally, recent studies have shown that reduced domestic fertilizer applications during the wet season blackouts had insignificant effects in Brevard County (Krimsky et al., 2021), as well as in Florida lakes (Smidt et al., 2022). The lack of significant nutrient reduction following fertilizer bans may also relate to legacy nutrients from previous land uses and fertilizer applications (Krimsky et al., 2021; Smidt et al., 2022).

Fertilizers and septic systems are the two major sources of N to Florida waters, followed by reclaimed water and atmospheric deposition (Badruzzaman et al., 2012). Recent estimates for residential fertilizer contributions to the IRL are much lower than the originally defined contribution of 71 % (https://savetheirl.org/education/how-does-your-lawn-hurt-the-lagoon/). In the *Save Our Indian River Lagoon Project Plan* the estimated amount of TN loading per year from residential fertilizers was 81,644 pounds (37,033 kg/yr), while septic systems within 55 yards (~50 m) of the IRL represented 299,590 pounds per year (185,457 kg/yr) (TetraTech, 2023). Thus, these current N loading estimates represent a 21 % contribution from residential fertilizers compared to 79 % from septic systems. These loading estimates are similar to those reported in other septic system impacted urbanized estuaries (Sham et al., 1995; Valiela et al., 1997a). However, the initial overestimation of N contributions from residential fertilizers applications led to broad public support and the passage of numerous fertilizer ordinances along the IRL during the study period. This ultimately diverted attention, efforts, and funds that potentially could have been more effective if allocated to reducing human waste impacts.

Summertime wet season fertilizer blackouts could also have unintended negative consequences for the IRL. First, winter fertilizer bans are more effective at reducing N and chlorophyll *a* concentrations in Florida lakes than summer bans that had no detectable impact (Smidt et al., 2022). A similar lack of effect for decreased N runoff during summer fertilizer bans was reported in Brevard County (Krimsky et al., 2021). This may be because nutrient uptake is higher in the summer months or because turfgrass growth potential wanes in the winter in response to cooler shorter days, leading to decreased uptake of applied nutrients (Barton and Colmer, 2006; Hochmuth et al., 2011). Additionally, healthy, well irrigated, urban turf has a high capacity for inorganic N retention and can act as a nutrient sink in the soil/landscape system rather than a nutrient source (Hochmuth et al., 2009, 2011; Lusk et al., 2018). As such, in a review of irrigation and nutrient management, N losses from turfgrass were $<$ 5 % of applied N when irrigation was optimized and with application of fertilizer that matched the plants requirements (Barton and Colmer, 2006). For Florida turfgrass, it was found that even steep slopes with intense irrigation rates resulted in $<$0.1 % of applied N in runoff (Shaddox and Unruh, 2018). This is likely because turfgrasses are very efficient at absorbing N and leach little when healthy, though improperly fertilized, slow-growing turfgrass may leach more (Erickson et al., 2001; Hochmuth et al., 2009, 2011; Sartain, 2015). However, fertilizer application may affect the composition of the N in runoff with estimates of attributing ~16–64 % for NO_3^- and NH_4^+ based fertilizers, demonstrating that fertilizers are a variable, but important component of urban stormwater runoff (Yang and Toor, 2016, 2017; Krimsky et al., 2021).

Given the importance of irrigation and turf nutrition in managing a healthy soil/landscape during the summer wet season when maximum stormwater runoff occurs, it is not surprising that there have been no detectable widespread water quality improvements in the IRL that support fertilizer restrictions as a panacea. In fact, for Brevard County, the blackouts correlate closely with a worsening trend in nutrients and HABs. Thus, a reassessment of these policies might be prudent. “Fine-tuning” fertilizer recommendations for various conditions, such as established vs. new turf, to optimize nutrient management may result in reduced losses to downstream waters (Lusk et al., 2018). Further, resource managers could adopt a multi-tiered outreach approach where residents are instructed in the importance of not over-fertilizing combined with the necessity to maintain healthy vegetation to minimize nutrient runoff. For example, the University of Florida's Florida-Friendly Landscaping (FFL) Program has nine principles: right plant, right place; water efficiently; fertilize appropriately; mulch; attract wildlife; manage yard pests responsibly; recycle yard waste; reduce stormwater runoff; and protect the waterfront (Momol et al., 2021). An examination of homeowners' knowledge and perception of sustainable landscaping practices found that the more a Florida homeowner knows about sustainable landscape programs, such as Florida Friendly Landscapes, the more likely they are to participate (Zhang et al., 2021). To this end, Brevard County currently has a planned outreach program including the topics of fertilizer application, grass clippings, and excess irrigation, as well as maintenance of stormwater ponds, septic systems, and sewer laterals (TetraTech, 2023), which may help promote literacy on these issues.

Other activities in Brevard County intended to improve conditions could also have contributed to the worsening trend of nutrients, HABs, and seagrass die-off in specific locations. During the period of study from March–October 2016, a remedial environmental dredging project (Fig. 7A) removed 180,000 m^3 of sediment at Turkey Creek (Palm Bay) (Cox et al., 2018) in the IRL near our NIRL4 site. High concentrations of NH_4^+ (5.40 μM), NO_3^- (9.38 μM), and SRP (0.739 μM) occurred at NIRL4 during the dredging, a period when enriched $\delta^{15}N$ values of red drift macroalgae (Fig. 7B,F; +9.14 ‰) and brown tides (Fig. 7E; +3.68 ‰) also occurred. During this bloom, *Gracilaria tikvahiae* with depleted pigment content (a symptom of N-limitation) was observed at Turkey Creek (Fig. 7C), possibly due to competition for N with the brown tide (Lapointe and Ryther, 1979). Turkey Creek drains a highly urbanized watershed around Palm Bay and includes thousands of conventional septic systems. A common symptom of groundwater-borne septic effluent discharge into creeks and tributaries of the IRL is the buildup of organic-rich muck, which can have the appearance of “black

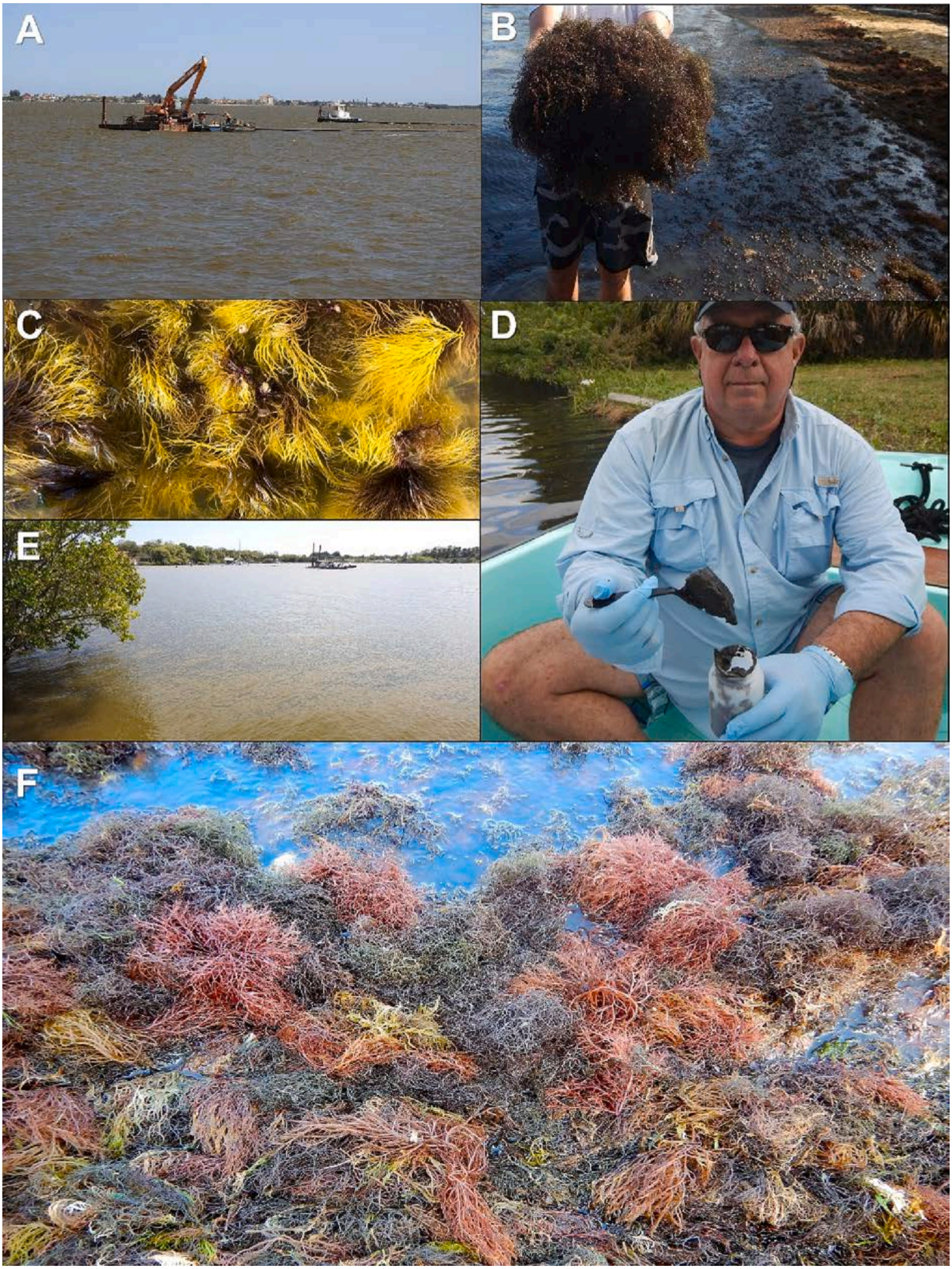

Fig. 7. Muck removal, macroalgal blooms, and brown tides at Turkey Creek, an Indian River Lagoon (IRL) tributary. A) A dredge boat used for muck removal in the IRL outside of Turkey Creek. B) Nitrogen (N) enrichment of *Gracilaria tikvahiae* at Castaway Point Park near Turkey Creek evidenced by the dark pigmentation. C) Depleted pigment content in *G. tikvahiae* from Castaway Point Park near Turkey Creek, showing evidence of N limitation (Lapointe and Ryther, 1979), possibly due to competition with the brown tide *Aureoumbra lagunensis*. D) B. Lapointe collecting muck samples in Turkey Creek. E) The brown tide and a dredge boat in Turkey Creek. F) Drift algal community at Castaway Point Park near Turkey Creek. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mayonnaise" (Fig. 7D). A study in Jupiter, FL, in the southern end of the IRL, found very high concentrations of coprostanol (1000 to 6000 μg/kg) in the Jupiter Creek muck (Lapointe and Krupa, 1995); coprostanol is a fecal sterol and indicator of human waste buildup in sediments (Hatcher and McGillivary, 1979). Muck samples collected from Turkey Creek on 12/15/2016 (Fig. 7D) had $\delta^{15}N$ values averaging +5.2 ‰ (Fig. 4), very close to the values for septic system effluent (+4.9 ‰) (Hinkle et al., 2008). These observations and data suggest that groundwater-borne septic system effluent has contributed to the buildup of muck in Turkey Creek, similar to what was reported for Jupiter Creek (Lapointe and Krupa, 1995). The relationship of elevated nutrient concentrations and $\delta^{15}N$ values in macroalgae and brown tides at Turkey Creek during the dredging operations suggest that nutrients from human waste buildup in the muck were resuspended into the water column where they could support macroalgal blooms and brown tides. Six-years post-dredging in 2022, ambient nutrient concentrations at Turkey Creek remained high with concentrations similar to those during the study period ($NH_4^+ = 5.61$ μM, $NO_X = 8.17$ μM, SRP = 0.45 μM). However, the muck removal may help facilitate seagrass recovery in this location if the sediment conditions and water column nutrients become more favorable.

5. Conclusions

It is clear from the data presented here and the worsening conditions in the NIRL and BR that additional efforts and thoughtful reassessment of current policies are urgently needed to reduce nutrient loading to the IRL. This is especially important given the long residence time of water in the northern segments (ML, BR, and NIRL) (Bilskie et al., 2019) that has been a physical factor favoring phytoplankton bloom development with increasing nutrient loading (Valiela et al., 1997b; Burkholder et al., 2007; Phlips et al., 2015, 2021). Our $\delta^{15}N$ data strongly suggest that N contributions from human waste are the largest N source to the NIRL and BR, similar to that reported for estuaries on Cape Cod and Long Island (Sham et al., 1995; McClelland et al., 1997; Valiela et al., 1997a; Valiela et al., 2000; Kinney and Valiela, 2011; Lloyd, 2014). Currently, more Brevard County funds are being allocated to muck removal projects ($193,100,693) than improving wastewater infrastructure ($160,604,114; https://www.brevardfl.gov/SaveOurLagoon). This study suggests it may be prudent to prioritize reducing human waste nutrient inputs into the IRL, prior to mitigating the impacts of internal nutrient sources, when possible. Our findings also re-affirm the need to address the root cause(s) of environmental problems if management actions are to be cost-effective and successful in the long term. Community based efforts, such as fertilizer restrictions, are important and can engage local populations in environmental protection efforts (Krimsky et al., 2021). For example, small-scale seagrass planting efforts, such as what has been done in Brevard County, can also create a positive feedback loop, allowing the community to feel invested in the recovery of IRL seagrass populations (Virnstein, 2021). While this community engagement fosters a connection with the natural environment, it can also detract from other societal-scale issues affecting water quality, such as nutrient pollution and other contaminants resulting from inadequate wastewater infrastructure in a rapidly growing urban environment.

CRediT authorship contribution statement

Brian E. Lapointe: conceptualization, methodology, writing- original draft, writing- review and editing, project administration. Rachel A. Brewton: conceptualization, methodology, formal analyses, data visualization, project administration, writing- review and editing. Lynn Wilking: data curation, methodology, writing- review and editing. Laura Herren: conceptualization, data curation, methodology, writing- review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.115041.

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Effectiveness of the Timing of SEASONAL FERTILIZER RESTRICTIONS on URBAN LANDSCAPES

Specific Appropriation 146
Final Report

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University of Florida | December 22, 2023

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LIST OF ABBREVIATIONS

BMPs = Best Management Practices

C = Carbon

FDEP = Florida Department of Environmental Protection

FFL = Florida-Friendly Landscaping™

HOA = Homeowners Association

IFAS = Institute of Food and Agricultural Sciences, University of Florida

IRL = Indian River Lagoon

lb = pound

n = sample size of the population

N = Nitrogen

NH_4^+ = Ammonium

NO_3^- = Nitrate

NO_3–N = Nitrate-nitrogen

O = Oxygen

P = Phosphorus

PON = Particulate organic N

UF/IFAS = University of Florida, Institute of Food and Agricultural Sciences

EXECUTIVE SUMMARY

Increasing inputs of nitrogen (N) and phosphorus (P) in aquatic systems are primary contributors to the decline in water quality across Florida. While both natural occurrences and human activities contribute to the transport of N and P into Florida's surface and groundwater, N and P inputs via landscape fertilizers have been a particular focus for local communities.

Since May 2000, at least 36 counties and 98 additional municipalities have established official urban landscape fertilizer ordinances, also called “fertilizer bans”, fertilizer “blackout” periods, “restrictive periods”, or “restricted season”. There is concern over whether fertilizer ordinances are effective at reducing pollution or providing ecological benefits, particularly given their ubiquity throughout the state and the variety of different ordinance types. This concern led to a fertilizer ordinance development moratorium until July 1, 2024, through the Florida Senate - 2023, Bill No. SB 2502, Lines 2455 – 2460. Via this legislation, and from funds in the Specific Appropriation 146, the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) shall “…evaluate the effectiveness of the timing of seasonal fertilizer restrictions on urban landscapes toward achieving nutrient target objectives for waterbodies statewide.”

Therefore, the purpose of this document is to provide a literature review of the most recent and relevant studies linking fertilization of urban landscapes with nutrient export to the environment, and potential subsequent water quality concerns in Florida. As a specific objective, this document discusses the effectiveness of seasonal fertilizer restrictions in decreasing nutrient contributions to aquatic ecosystems. Finally, this report summarizes the published studies, details recommendations regarding the current fertilizer ordinances, and identifies areas where further research is required to understand the effect of fertilizer ordinances on Florida’s waterbodies.

The literature review summarizes and relates studies performed mainly in Florida, with some additional studies from other states when deemed relevant. The following are the main points analyzed:

- Studies relating nutrient export from urban landscapes, the resulting water quality, and the environmental, public health, economic, and social impacts of nutrient-driven water quality responses.

- The history and evolution of fertilizer ordinances in an attempt to counteract the detrimental effects of nutrient pollution on Florida waterbodies.
- The sources of nutrients to waterbodies, where it is clear that fertilizers are not the only source of the nutrients N and P. Additional sources include septic systems; reclaimed water, stormwater pond, and wastewater treatment plant outflows; atmospheric deposition (via lightning, rain, and dry deposition of fine particulates); organic materials such as grass clippings and other yard waste, compost, and animal wastes; erosion and weathering of soils or geologic materials. The main sources are then analyzed separately, with a special section regarding fertilizers and their nutrient exports from the landscape.
- The few studies performed in Florida regarding the efficacy of fertilizer ordinances are detailed and analyzed.
- Finally, human behavior related to the awareness and compliance of fertilizer ordinances is analyzed.

This document ends with a summary and recommendations. A key takeaway message of the recent studies is that there is no single source of urban nutrients to Florida's waterbodies, and that the task then becomes to place the various sources in context with each other to learn which sources might be the most important in a given location and time.

There have been six studies (five published as peer-reviewed scientific articles) on the efficacy of fertilizer restrictions in Florida. These six studies are detailed and analyzed. Except for one study, they were performed in small or specific areas and analyzed fertilizer ordinance impacts in relatively short time scales (less than 5 years). Varied and sometimes contradictory findings were reported by these studies, which can be attributed to the fact that not all ordinances exert the same influence on the water quality parameters analyzed. In addition, because there are many sources of nutrients in waterbodies, it is often difficult in short term or small scale studies to resolve the effect of fertilizer ordinances from other potential causes of altered nutrient inputs into a waterbody, whether due to natural or human causes. Furthermore, waterbodies can respond in a variety of different ways to increasing nutrient inputs. There are natural processes occurring within waterbodies that can temporarily or permanently remove nutrients, reducing their impact on other water quality metrics and potentially obscuring changes in watershed nutrient management.

The difficulty in pin-pointing specific causes of water quality responses is exacerbated when studies are conducted at small scales, over limited time frames, and without a comprehensive measure of watershed hydrology and water quality parameters. Moreover, the divergence in the effectiveness of fertilizer restrictions depends on various factors, including the specific regulations in place, the (lack of) enforcement, education, or awareness of those regulations, the willingness of the local community to comply, and the ecological and environmental conditions of the area. Thus, the existing evidence to date does not conclusively indicate that fertilizer ordinances are effective in solving water quality problems. This is not necessarily indicative of the ordinances not achieving their intended goals; rather, the current lack of comprehensive evidence makes it challenging to assess the ecological impact of these ordinances.

Further studies are necessary to identify the relative contribution of different sources of nutrients into waterbodies. Additional studies that include the timing, type, and amount of fertilizer applied in urban/suburban areas and their ecological impact on waterbodies are necessary. At the same time, these studies should address the knowledge of and the compliance with the local ordinances. These additional studies would facilitate the development of site-specific strategies to reduce N and P contributions from different sources to waterbodies and make better decisions regarding future regulations and public funding to remediate this ongoing problem statewide.

BACKGROUND

Increasing inputs of nitrogen (N) and phosphorus (P) into aquatic systems are primary contributors to the decline in water quality across Florida. While both natural occurrences and human activities contribute to the transport of N and P from terrestrial environments into Florida's surface waters and groundwater, N and P inputs from landscape fertilizers have been a particular focus for local communities. (Note: the terms “landscape” and “urban landscape” are used interchangeably in this document to mean the installed or existing plant material that is maintained around urban/suburban built structures for aesthetic or functional purposes.)

Turfgrass is the dominant plant material in urban and suburban landscapes and is a prominent feature of urban watersheds. Milesi et al. (2005) estimated that turfgrass covered 1.9% of the total continental U.S. surface area in 2001 and 6.8% of Florida’s surface area. Since 2001, the amount of housing in Florida has increased from 7,477,001 units (US Census Bureau, 2023a) to 10,257,426 units in 2022 (US Census Bureau, 2023b), a 37% increase. Therefore, the area of turf and landscapes has likely increased with housing increases.

Urban soils, whether native or disturbed/mixed due to the urban development process, often lack sufficient N and occasionally lack sufficient P to support the healthy growth and desired quality of turfgrass and other landscape plants. To overcome this nutritional deficiency, fertilizer is commonly applied. However, if not done correctly, fertilization associated with high rainfall and/or excess irrigation can mobilize excess nutrients to the environment, potentially impairing water quality (Carey et al., 2012).

In Florida, the first municipal fertilizer regulation was adopted by St. Johns County in May 2000. Since then, at least 36 counties and 98 additional municipalities have established official urban landscape fertilizer ordinances (FFL, 2023a). In general, these fertilizer ordinances include application standards, enforcement, exemptions, and applicator training. Fertilizer application restrictions are also called “fertilizer bans”, fertilizer “blackout” periods, “restrictive periods” or “restricted season”. These restrictions are often seasonal or encompass periods during the year when the application of fertilizers containing N or P or both on urban landscapes is prohibited. The premise behind these ordinances is that N and P associated with the fertilizer product may be leached through the soil profile into groundwater or directly runoff from the soil during the rainy season, or that excess N and P not taken up by plants during the dormant season (in the case of winter seasonal restrictions) may be susceptible to leaching or runoff losses and transported to waterbodies.

As an artifact of their local implementation, fertilizer ordinances vary across the state in their timing and other regulatory requirements. Due to these varied ordinances and regulations, there is concern over whether certain ordinances are more or less effective than others. This concern led to a fertilizer ordinance development moratorium through the Florida Senate – 2023, Bill No. SB 2502, Line 2455, Pg. 86, Section 85, which established: "In order to implement Specific Appropriation 146 of the 2023-2024 General Appropriations Act, a county or municipal government may not adopt or amend a fertilizer management ordinance, pursuant to s. 403.9337, Florida Statutes, which provides for a prohibited application period not in existence on June 30, 2023. This section expires July 1, 2024."

Via this legislation, and using funds from Specific Appropriation 146, the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) should "…evaluate the effectiveness of the timing of seasonal fertilizer restrictions on urban landscapes toward achieving nutrient target objectives for waterbodies statewide. IFAS must submit a final report, including results and recommendations by December 31, 2023, to the chair of the Senate Appropriations Committee and the chair of the House Appropriations Committee."

Therefore, the main purpose of this document is to provide a literature review of the most recent and relevant studies linking fertilization of urban landscapes with nutrient export to the environment, and potential subsequent water quality concerns in Florida. As a specific objective, this document discusses the effectiveness of seasonal fertilizer restrictions in decreasing nutrient contributions to aquatic ecosystems. Finally, based on the published studies, this report details recommendations regarding the current fertilizer ordinances and identifies areas where further research is required to understand the effect of fertilizer ordinances on Florida's waterbodies.

LITERATURE REVIEW

Nutrients and water quality

Nutrients such as N and P are naturally occurring elements that form the building blocks of life and are essential for plant growth. However, excess nutrient inputs into waterbodies can cause degradation and are often responsible for the proliferation of plants, algae, and cyanobacteria (blue-green algae) within waterbodies through a process known as eutrophication. Eutrophication impairs ecosystem health and the beneficial services provided by healthy waterbodies. Additionally, eutrophication contributes to harmful algal blooms (including cyanobacterial blooms and brown tides), reduced water clarity, seagrass die-offs, decreased or depleted oxygen levels (hypoxia/anoxia), fish and wildlife die-offs, and loss of biodiversity (including species important for commerce and recreation) (Gobler et al., 2013; Lapointe et al., 2015; Phlips et al., 2015; Lapointe et al., 2020; Herren et al., 2021; Morris et al., 2021; Phlips et al., 2021; Allen et al., 2022; Landsberg et al., 2022; Lapointe et al., 2023). In addition to these environmental effects, elevated nutrients may impair the use of water for drinking, industry, agriculture, tourism and recreation, and other purposes leading to public health, social, and economic impacts (Abbott et al., 2021; Dodds et al., 2006).

Fertilizer ordinances

In an attempt to counteract the detrimental effects of nutrient pollution on Florida waterbodies, almost all of Florida's highly urbanized counties and/or municipalities have implemented fertilizer ordinances (Figure 1). These ordinances typically apply to every residential landscape, restricting the use of N and P fertilizers during specific times (e.g., summer or winter), with exemptions for agricultural properties, vegetable gardens, golf courses, and athletic fields (FFL, 2023b).

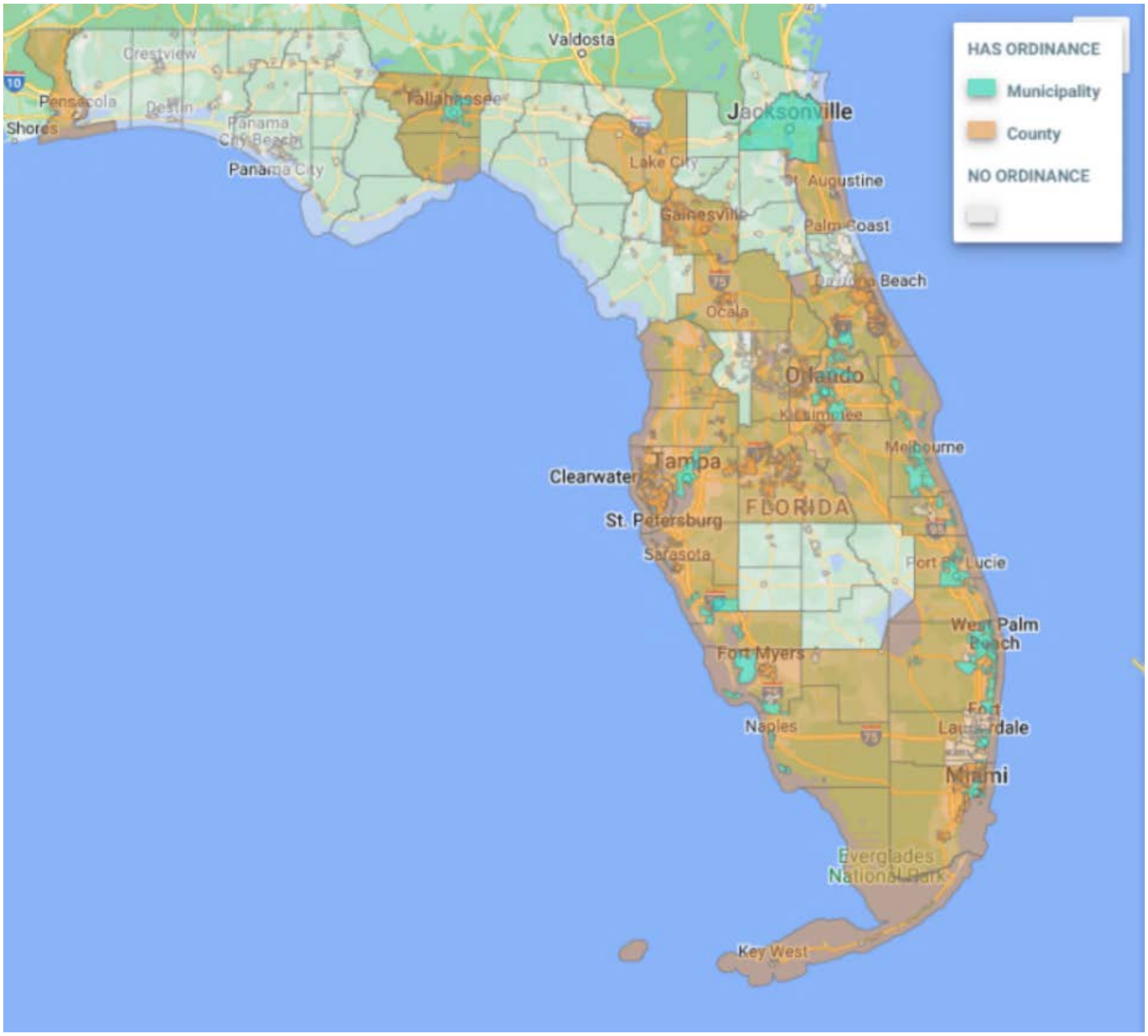

Figure 1. Florida fertilizer ordinances. (Source: FFL, 2023a.)

The implementation of fertilizer ordinances was started by St. Johns County in May 2000 (Ordinance NO. 2000-60) and in September 2003 the Florida Department of Environmental Protection (FDEP) published the “Guidelines for Model Ordinance Language for Protection of Water Quality and Quantity Using Florida Friendly Lawns and Landscapes”. However, large-scale adoption of fertilizer ordinances did not occur until 2007, triggered by a 13-month long *Karenia brevis* red tide bloom along Florida’s southwest coast and the adoption of a model ordinance for fertilizer regulation by the Southwest Florida Regional Planning Council (Hartman et al., 2008).

Beyond residential ordinances, the Urban Turf Fertilizer Rule (Florida Administrative Code 5E-1.003) was implemented in December 2007. This rule established label language requirements for fertilizer products for urban turf or lawns, packaged in containers or bags such that the net weight is 49 pounds or less, and distributed for home and garden use. Furthermore, the rule required printed directions for use for N that adhered to UF/IFAS annual fertilization guidelines for established turfgrass lawns in north, central, and south Florida. The rule also required industry professionals to follow relevant Best Management Practices (BMPs) for the appropriate industry segment (e.g., green industry and golf).

In 2009, the Model Ordinance for Florida-Friendly Fertilizer Use on Urban Landscapes was adopted into state law (Florida Statute 403.9337) as a mechanism intended to reduce the export of nutrients from urban landscapes to Florida's groundwater and surface waters (FFL, 2023b). The statute encourages county and municipal governments to adopt the Model Ordinance or equivalent. Local governments located within the watershed of an impaired water body, shall, at a minimum, adopt FDEP's Model Ordinance or they may adopt additional or more stringent standards than the model ordinance if the following criteria are met:

- "The local government has demonstrated—as part of a comprehensive program to address nonpoint sources of nutrient pollution, which is science based, and economically and technically feasible—that additional or more stringent standards than the model ordinance are necessary in order to adequately address urban fertilizer contributions to nonpoint source nutrient loading to a water body."
- "The local government documents that it has considered all relevant scientific information, including input from the department, the institute, the Department of Agriculture and Consumer Services, and UF/IFAS, if provided, on the need for additional or more stringent provisions to address fertilizer use as a contributor to water quality degradation. All documentation must become part of the public record before adoption of the additional or more stringent criteria."

Key provisions of the FDEP's Model Ordinance include:

- Prohibition of fertilizer application containing N or P to turf or landscape plants during the "Prohibited Application Period, or to saturated soils";
- Surface water setbacks (within a certain distance of a waterbody);
- The use of deflector shields on application equipment;
- The use of slow-release fertilizers;
- Low maintenance zones;
- Soil testing before applying P fertilizer;
- Fertilizer nutrient content and application rates;
- Application rates and practices;
- Training requirements;
- Licensing of commercial applicators.

Current ordinances generally include these key provisions or variants. Furthermore, law SB 494, from June 2009 requires all commercial fertilizer applicators to have a Florida Department of Agriculture and Consumer Services (FDACS) fertilizer license by January 1, 2014. In addition, optional BMPs, like the Florida-Friendly Landscaping™ (FFL) Program, have been introduced in some ordinances to further curb nutrient leaching and/or runoff from urban settings (Momol et al., 2021).

As of May 2023, there were 36 counties and 98 additional municipalities in Florida that have enacted residential fertilizer ordinances (FFL, 2023a). Some of these county ordinances apply to unincorporated areas only, which are sometimes complemented by individual municipalities within a county. Among the 36 counties that have fertilizer ordinances, 18 have specific summer bans.

Sources of nutrients to waterbodies

Nitrogen and P are both fundamental nutrients required by every living organism on Earth. However, they are often in short supply and can be the limiting factor for the growth of microbes, plants, and animals within an ecosystem.

Florida-specific studies of the sources of N and P in urban watersheds are growing in number and spatial coverage. Badruzzaman et al. (2012) published one of the earliest summaries of nutrient sources and their transport to Florida waterbodies, based on a synthesis of a small number of peer-reviewed and grey literature studies. Since then, new research has expanded the evidence-base of how various N and P sources are contributing to waterbodies in the state. These new studies, which are largely peer-reviewed, are continuing to become more geographically inclusive of the state, and to date have focused on N and P contributions to watersheds.

From these studies and selected studies from outside of Florida, it is clear that fertilizers are not the only source of N and P. Other sources include septic systems; reclaimed water; stormwater pond and wastewater treatment plant outflows; atmospheric deposition (via lightning, rain, and dry deposition of fine particulates); organic materials such as grass clippings and other yard waste, compost, and animal wastes; erosion and weathering of soils or geologic materials (Nixon, 1995; Howarth et al., 2000; Nixon, 2009; Hochmuth et al., 2012; Badruzzaman et al., 2012; Lapointe et al., 2015; Glibert and Burford, 2017; Hobbie et al., 2017; Yang and Toor, 2017; Jani et al., 2020; Lusk et al., 2020; Reisinger et al., 2020; Krimsky et al., 2021; Lapointe et al., 2023; Lusk et al., 2023). Geologic weathering is a particularly important source of P in Florida, where there are naturally occurring P-rich geological formations throughout parts of the state (e.g., central Florida phosphate mines), providing naturally elevated P concentrations in surface water and groundwater ecosystems.

A key takeaway message of the recent studies is that there is no single source of urban nutrients to Florida's waterbodies, and that the task then becomes to place the various sources in context with each other to learn which sources might be the most important in a given location and time. Nutrient source tracking methods such as the use of stable isotopes have been the most common means of identifying nutrient sources. For example, the isotopic composition of N and oxygen (O) in NO_3^- (nitrate) have been used to infer the relative contributions of atmospheric deposition, fertilizers, pet wastes, and human wastewater (including septic systems) to stormwater runoff N in studies in the Indian River Lagoon and in coastal urban areas around Tampa Bay (Lapointe et al., 2015; Yang and Toor, 2017; Krimsky et al., 2021, Jani et al., 2020; LaPointe et al., 2023). In other studies, the isotopic composition of N and carbon (C) in organic materials have been used to estimate the contributions of materials such as grass clippings and leaf litter to the total N loads of stormwater in Tampa Bay (Lusk et al., 2020). Another promising source tracking method for waters impacted by human waste is the use of trace organic compounds such as sucralose, pharmaceuticals, hormones, and steroids, all of which are not typically fully removed by the wastewater treatment process and may be used as indicators of wastewater as a potential nutrient source in waterbodies. However, matching concentrations of wastewater tracers with potential nutrient impacts to receiving waters remains an important research need.

In the following discussion, we summarize findings of nutrient source studies conducted mainly over the last decade, with an emphasis on Florida-specific studies but also focusing on other locations, when doing so adds to the broader understanding of nutrient fate and transport in urban areas. Many of the studies identified for this review use one or more source tracking methods described above.

N and P cycles in urban environments

To identify the different N and P inputs into an urban watershed, it is necessary to review the contributing sources of those nutrients. Afterward, it is important to assess how these nutrients are transformed and mobilized toward waterbodies in urban settings (Figures 2 and 3). Typical inputs of N and/or P in urban environments include fertilizers, atmospheric deposition, septic systems, wastewater treatment plant outflows (including reclaimed water), plant residues (e.g., grass clippings, tree leaves, and other yard waste), compost, and animal waste. These components containing N and P can be transformed into more simple molecules, and then lost from the soil by plant uptake, denitrification and volatilization (in the case of N), erosion (in the case of P), or they could be transported towards water bodies through runoff or leaching.

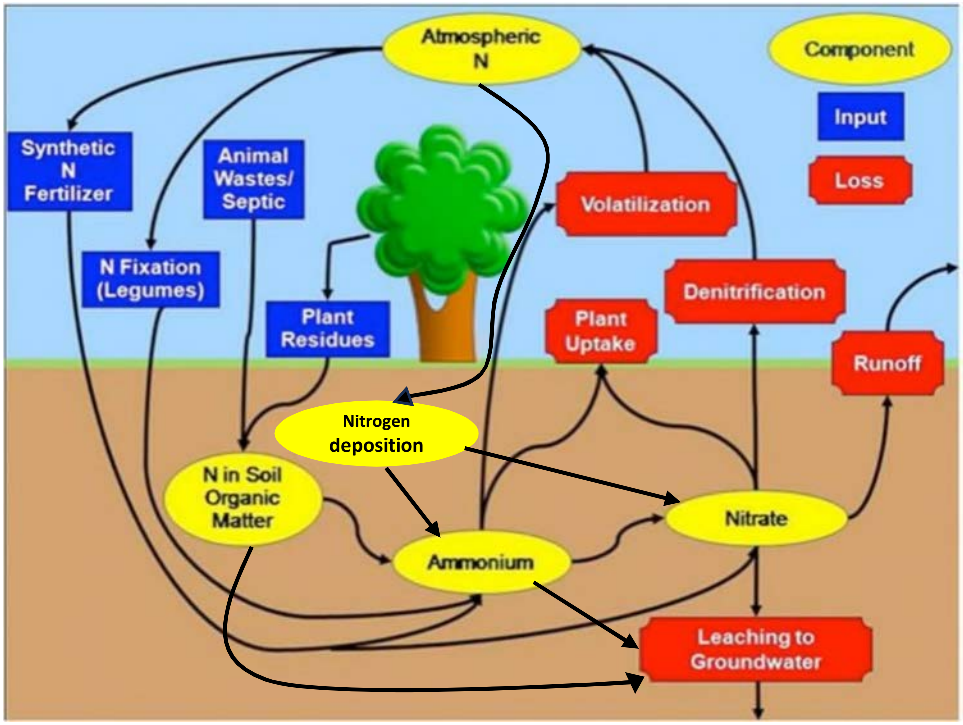

Figure 2. The nitrogen cycle in urban environments. Credit: Modified from UF/IFAS. Source: Shober and Reisinger (2022).

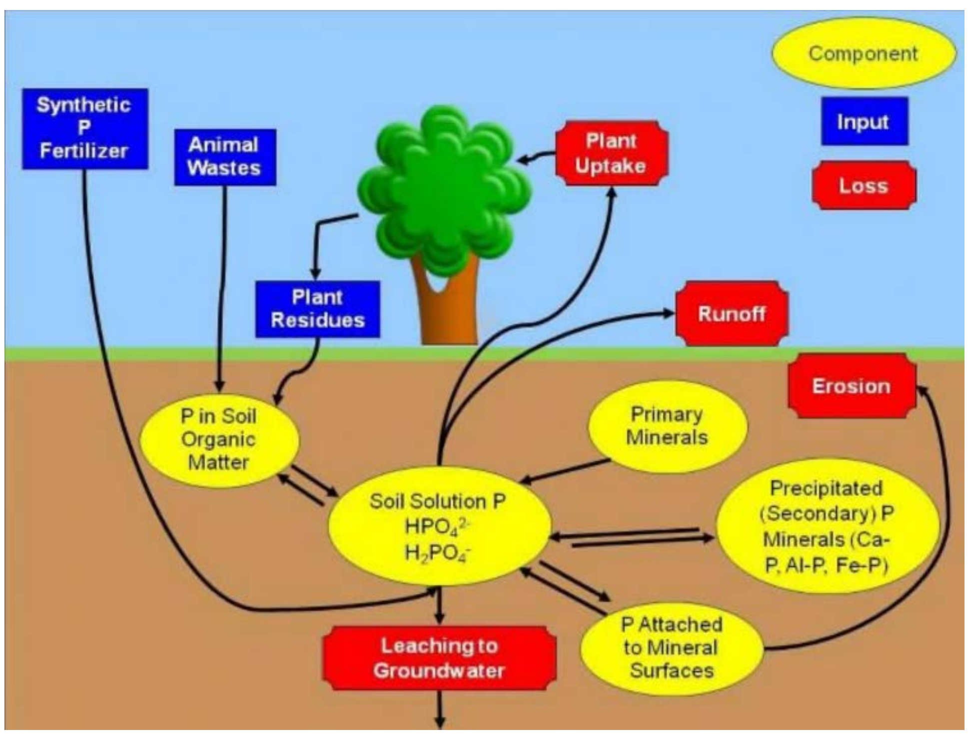

Figure 3. The phosphorous cycle in urban environments. Credit: UF/IFAS. Source: Shober, 2018).

For example, researchers in Minneapolis, MN, conducted a nutrient budget study where they quantified various pathways by which N moves into and out of urban watersheds. They found that residential fertilizer was the largest input of N into the watershed, whereas household pet waste was the largest source of P. Atmospheric deposition was also an important source of N and P across multiple watersheds that were studied (Hobbie et al., 2017).

At the regional or watershed scale, urban watersheds can be highly effective at retaining N under certain circumstances. Natural processes within waterbodies can temporarily or permanently remove nutrients, reducing their influence on other water quality indicators and possibly masking changes in watershed nutrient management. There is a history of research investigating the influence of urbanization and human actions on nutrient export in Baltimore, MD, through a long-funded National Science Foundation research project. For example, Bettez et al. (2015) found that urban watersheds in Baltimore, MD, can typically retain 70% to more than 90% of the total nitrate inputs into the watershed on an annual basis. However, the degree of urbanization and increasing annual precipitation can reduce this level of N retention (Bettez et al., 2015). Among numerous other studies on lawn, forest, stream, and wetland nutrient cycling studies from Baltimore, MD, Suchy et al. (2021) found that N export from residential lawns may not be driven by N fertilization rates. Additional work in Baltimore has revealed that the implementation of green infrastructure to capture and treat stormwater runoff, coupled with a reduction of unintentional discharges of untreated wastewater can reduce annual N and P export to coastal water bodies (Reisinger et al., 2019). In addition, residential lawns can accumulate both C and N at degrees comparable to or even higher than nearby forests (Raciti et al., 2011).

We note that although these studies provide important information about how nutrient cycles interact with various urban factors, the environmental contexts of Minneapolis, MN, and Baltimore, MD, are much different than in Florida due to differences in geology, soils, climate, and social factors. To date, a comprehensive study to understand N and P sources and pathways within Florida's urban watersheds has not been conducted. Having such information would better inform landscape maintenance recommendations, including fertilizer management.

Septic systems

Around 80% of Florida's residents live within 10 miles of the coast. Approximately 30% of Florida's residents rely on septic systems for their wastewater disposal needs, with around 2.6 million systems in operation. Of those 2.6 million septic systems, around 40% are in coastal areas with high water tables (FDEP, 2023). Under these circumstances, septic systems (Figure 4) have been reported as the main contributor of the N load in coastal locations of Florida (Lapointe et al., 2015; Lapointe et al., 2017; Barile, 2018; Herren et al., 2021; Brewton et al., 2022; Tyre et al., 2023; Lapointe et al., 2023), as well as in urbanized estuaries of the northeastern US (Sham et al., 1995; McClelland et al., 1997; Valiela et al., 1997; Valiela et al., 2000; Kinney and Valiela, 2011; Lloyd, 2014).

For example, a recent study by Brewton et al. (2022) reported that more than 80% of the sampled conventional septic systems in the Caloosahatchee River and Estuary, in Lee County, Florida, were too shallow for an adequate filtration function (Figure 5). While they did not analyze nutrients from fertilizers, they found that both groundwater and surface water were contaminated by septic system effluent, impairing the water quality and intensifying harmful algal blooms.

A large-scale study was conducted by Tyre et al. (2023) in various drainage basins throughout Lee County, Florida (Figure 6), where they collected surface water samples as well as particulate organic matter and macrophyte (aquatic plant) tissue samples at 25 sites, between January 2020 and January 2021. They reported that fecal bacteria were elevated in 66% of the samples, while stable isotope analyses revealed sources of N derived from human waste at 44%, 68%, or 100% of locations based on isotopic analyses of nitrate-nitrogen (NO_3–N), particulate organic matter, and plant tissue, respectively. These results provide evidence of widespread human waste contamination in the basins of Lee County, Florida (Figure 7). The authors recommend infrastructure improvements to improve water quality and minimize harmful algal blooms.

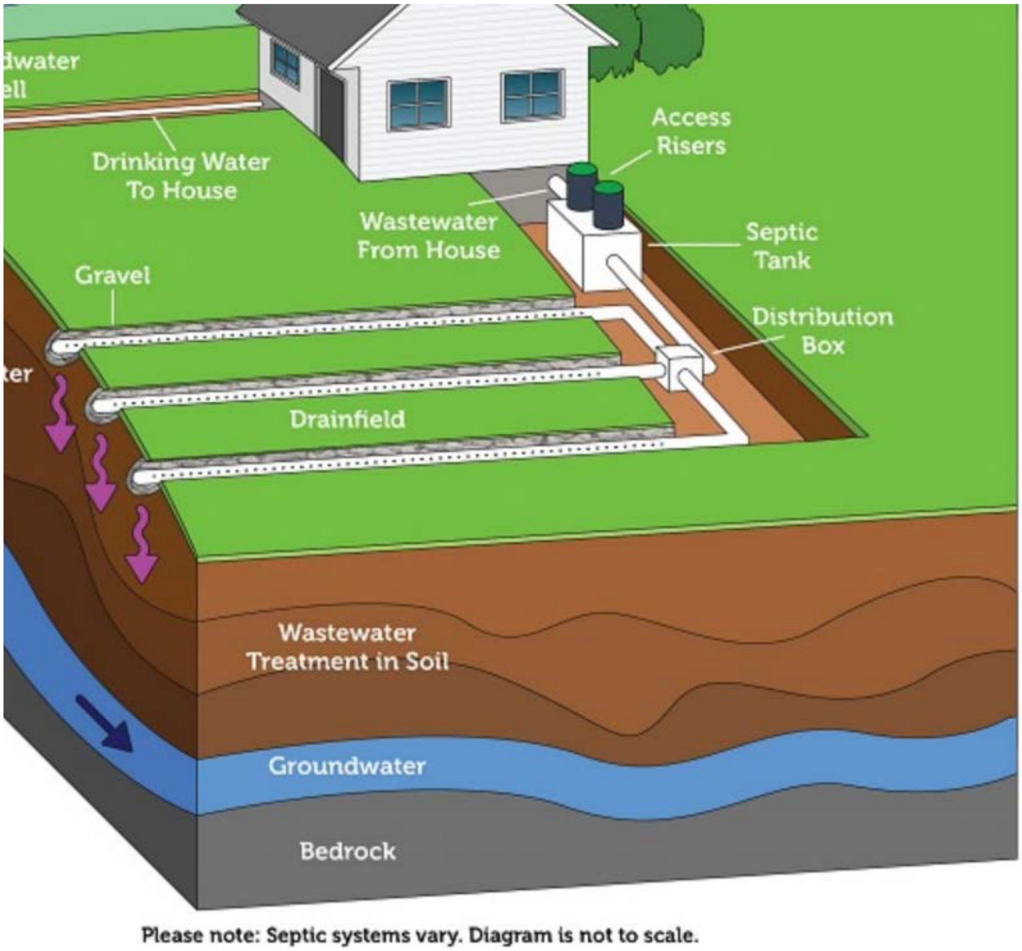

Figure 4. A conventional septic system consisting of a septic tank and drainfield. The septic tank is buried in the soil and collects household waste. As waste collects in the septic tank, solids settle to the bottom of it and the liquid (called effluent) flows out through perforated pipes to the drainfield, or soil area through which effluent percolates downward. (Source: EPA, 2023a)

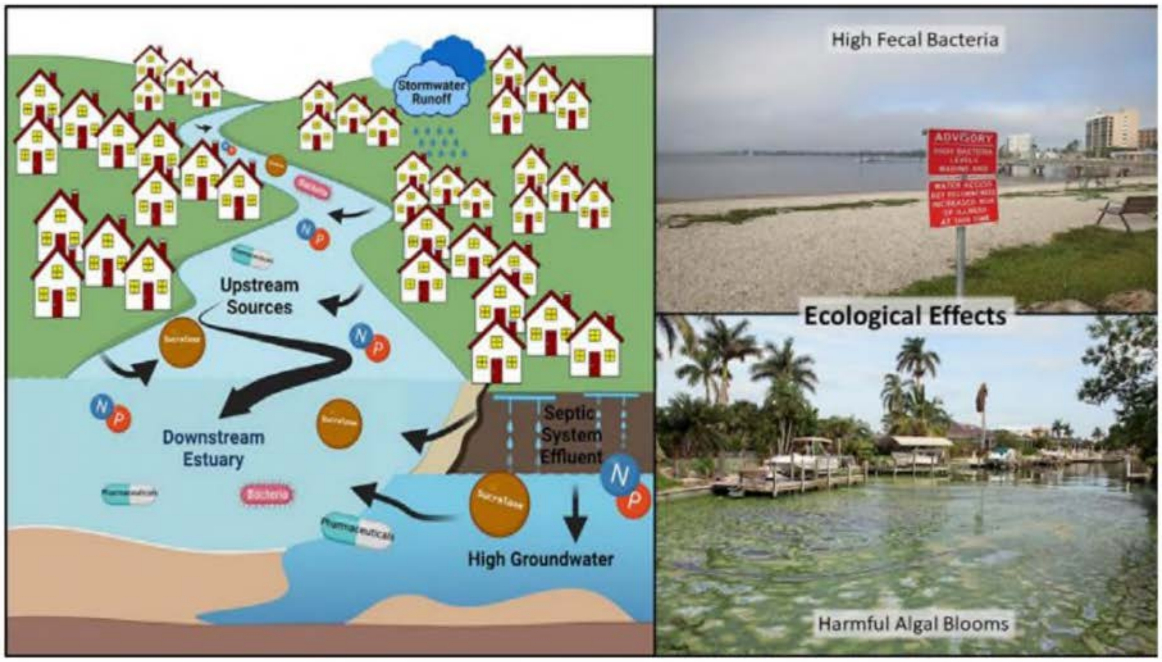

Figure 5. Septic system–groundwater–surface water couplings in waterfront communities. (Source: Brewton et al., 2022)

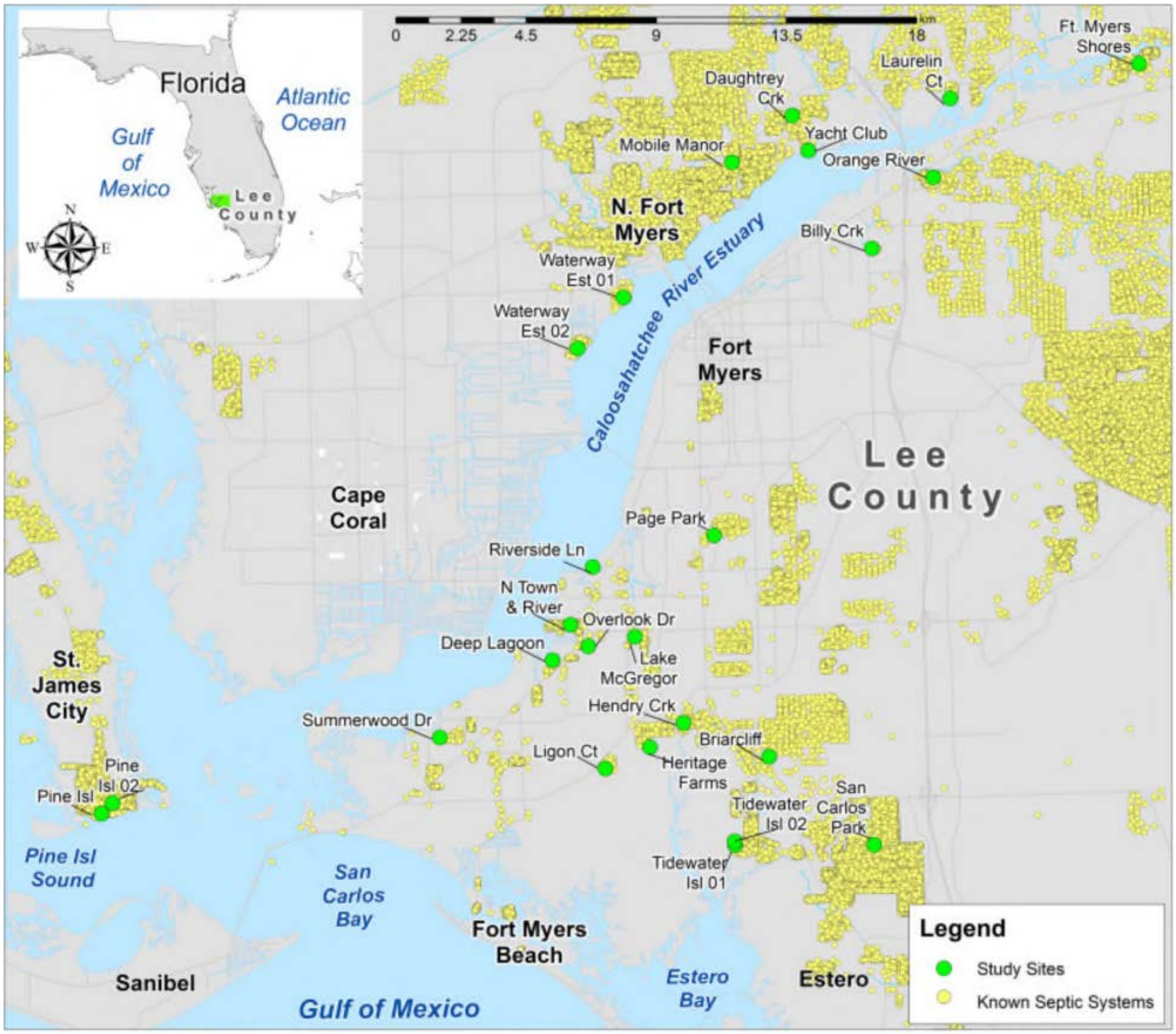

Figure 6. Surface water study sites (green circles) and known septic systems (yellow dots) in Lee County, Florida, from a study conducted by Tyre et al. (2023).

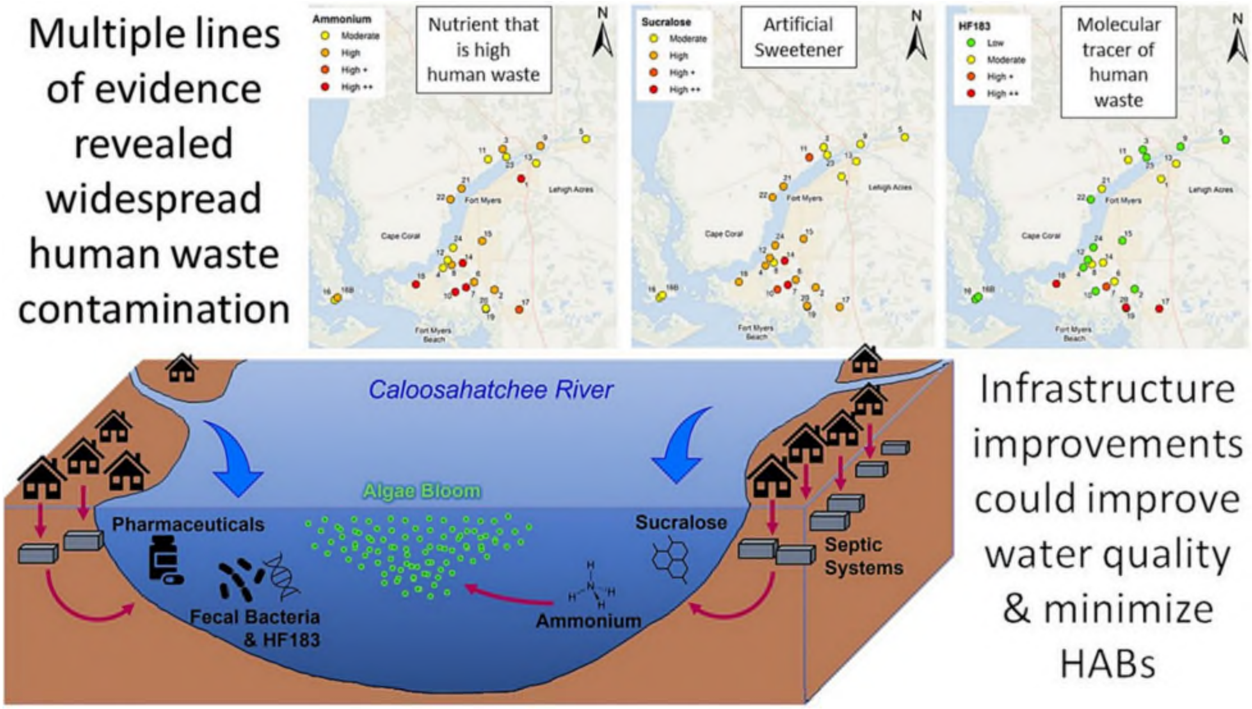

Figure 7. Graphical abstract of the study by Tyre et al. (2023).

Moreover, a recent study by Lapointe et al. (2023) concluded that in Florida’s Indian River Lagoon (IRL) 79% of the N loading was from septic systems, while residential fertilizers contribution was 21%. The authors conclude that current fertilizer restrictions are insufficient to mitigate the ongoing eutrophication at the IRL and that it would be prudent to prioritize reducing human waste nutrient inputs.

Other studies in urbanized estuaries of the northeastern US have reported that the largest N source driving eutrophication and harmful algal blooms come from septic systems (Sham et al., 1995; McClelland et al., 1997; Valiela et al., 1997; Valiela et al., 2000; Kinney and Valiela, 2011; Lloyd, 2014). For example, in Waquoit Bay, MA, 48% of the N load to estuaries came from septic systems, 29% from atmospheric deposition and 16% from fertilizer (Sham et al., 1995; Valiela et al., 1997). Likewise, on different bays of Long Island, NY, around 50% of the N loads came from septic system effluent (Kinney and Valiela, 2011; Lloyd, 2014).

Therefore, septic systems close to waterbodies (or shallow water tables) might result in widespread human waste contamination. Herren et al. (2021), who studied nutrients loadings to the IRL, concluded that low-lying septic systems should be removed. Accordingly, Cox et al. (2019) warned that the functionality of an increasing number of septic systems in coastal areas will be compromised by sea level rise in the future, highlighting the need for alternative wastewater management approaches to protect coastal water quality. In addition, Tyre et al. (2023) suggested the replacement of septic systems in coastal areas of Lee County, Florida, with centralized sewer (or other alternatives) and include adequate wastewater infrastructure with advanced nutrient removal capabilities.

Reclaimed water

Reclaimed water used for urban irrigation has varying levels of both N and P, in both inorganic and organic forms. The concentrations of nutrients in reclaimed water depend on the nutrient removal technology used at the wastewater treatment plant, as well as storage of the water that takes place before it is used as irrigation. Only a few studies have investigated the role of reclaimed water in nutrient transport to waterbodies in Florida. In ideal circumstances, the reclaimed water is applied only to lawns and other urban green spaces, where nutrients may be assimilated by plant roots and soil organic matter. However, urban irrigation can be highly inefficient. A recent study showed that approximately 34% of the total irrigation volume being wastefully applied to adjacent impervious surfaces such as streets and sidewalks (Barr, 2023). Application of irrigation in this way may be problematic especially for waterfront communities where neighborhood stormwater systems quickly route runoff of reclaimed water with elevated N and P to canals or bays.

In a study in Martin County, FL, Barr (2023) collected reclaimed water at sprinkler heads in two residential communities multiple times over 12 months and estimated (based on the assumption that 34% of all irrigation volume was wastefully applied to streets and sidewalks) that yearly N loads from irrigation-driven runoff ranged from about 0.3 to 3.0 lbs N/lawn. For a watershed with several thousand homes using reclaimed water for irrigation, this can translate to thousands or tens of thousands of pounds of N per year. Continued studies are needed to better understand the extent to which irrigation-driven runoff of reclaimed water is impacting downstream waterbodies, and how that varies by the level of wastewater treatment in a watershed and by homeowner behaviors (such as how efficiently they operate their sprinkler systems).

Nutrients from reclaimed water may also be a source of N and P leaching in urban landscapes, if not all the irrigation water's nutrient load is assimilated by plant roots or soil organic matter. It is reasonable to conclude that some fraction of the nutrient load in reclaimed water used for irrigation is taken up by turfgrass or other landscape plants, but studies are lacking on the extent to which all of the nutrient load is taken up, how much of it may leach on short time frames, and how much of it may be incorporated into soil organic matter and leach more slowly over long time periods. A study performed by Cardenas and Dukes (2016) in the locality of Palm Harbor, FL, found that homes irrigating with reclaimed water, and no additional technology other than their irrigation timer, over-irrigated 4.4 times more than the calculated gross irrigation requirement (water needed by the turfgrass). Thus, leaching of nutrients is possible if not likely.

Atmospheric deposition

Rainfall and atmospheric dust naturally contain both N and P. Atmospheric deposition of N is especially recognized as a source of N to waterbodies in Florida, with it accounting for 0.39 lb N/acre in one single storm event in the Tampa Bay area, according to a study by Lusk et al. (2023). Work by the Tampa Bay Estuary Program (TBEP, 2023) estimated that 17% of the total N load (566 tons per year) to Tampa Bay during the 2010s was deposited directly to Bay waters from atmospheric deposition.

Grass clippings and landscape wastes

In established lawns, recycling grass clippings improves the sequestration of N in the soil (Hull and Liu, 2005; Qian et al., 2003; Starr and DeRoo, 1981), since clippings can store between 25% and 60% of the applied N (Petrovic and Easton, 2005). In addition, when clippings are returned to the lawn, turf quality may not be adversely impacted when decreasing N fertilization by 50% to 75% (Heckman et al., 2000; Kopp and Guillard, 2002). Regarding P, returning clippings did not affect P runoff (Bierman et al., 2010).

Grass clippings and other vegetation that is allowed to stay on impervious surfaces can be a source of nutrients for stormwater runoff. Grass clippings and seasonal leaf litter (live oak leaves) that accumulated on urban impervious surfaces were identified as a major source of total N in stormwater runoff from a residential community in Hillsborough County (Lusk et al., 2020). In this case, the N fraction from grass clippings and leaves was in the form of organic N, and while the inorganic forms of N (nitrate and ammonium) are known to be bioavailable and drivers of algal blooms in receiving water bodies, there is a growing body of evidence that organic N in stormwater can also be highly bioavailable (Lusk and Toor, 2016; Muni-Morgan et al., 2023). The Lusk et al. (2020) study also observed that particulate organic N (PON) displayed a seasonal first flush, in which the majority of PON was carried by stormwater early in the summer rainy season, presumably as early summer rains mobilized and transported organic materials that had accumulated on urban impervious surfaces during the preceding dry season. Thus, an important best management practice for nutrients in urban landscapes is to keep grass clippings and landscape wastes from accumulating on impervious surfaces, through practices such as street sweeping, composting, or removal.

Compost amendments

Addition of organic compost increases the potential for N and P leaching, with the majority of nutrient leaching occurring within the first 30 days of incorporation (Radovanovic and Bean, 2020). When applying compost to lawns, Easton and Petrovic (2004) found a higher P loss, on a percent applied P basis, compared to synthetic fertilizer. Time course or chronosequence studies focused on the long-term effects of compost incorporation are needed.

Fertilizers

The amount of fertilizers purchased in the state of Florida containing N declined 64% between 2003 and 2017, from 204,000 tons to 73,000 tons, respectively (Figure 8). Fertilizers containing phosphorus (as P_20_5) have declined 70% over the same period, from 70,000 tons to 21,000 tons, respectively (EPA, 2023b). [Note: P_20_5 (or phosphate) is 44% phosphorus. By convention, the amount of phosphorus in fertilizers is expressed in this form.]

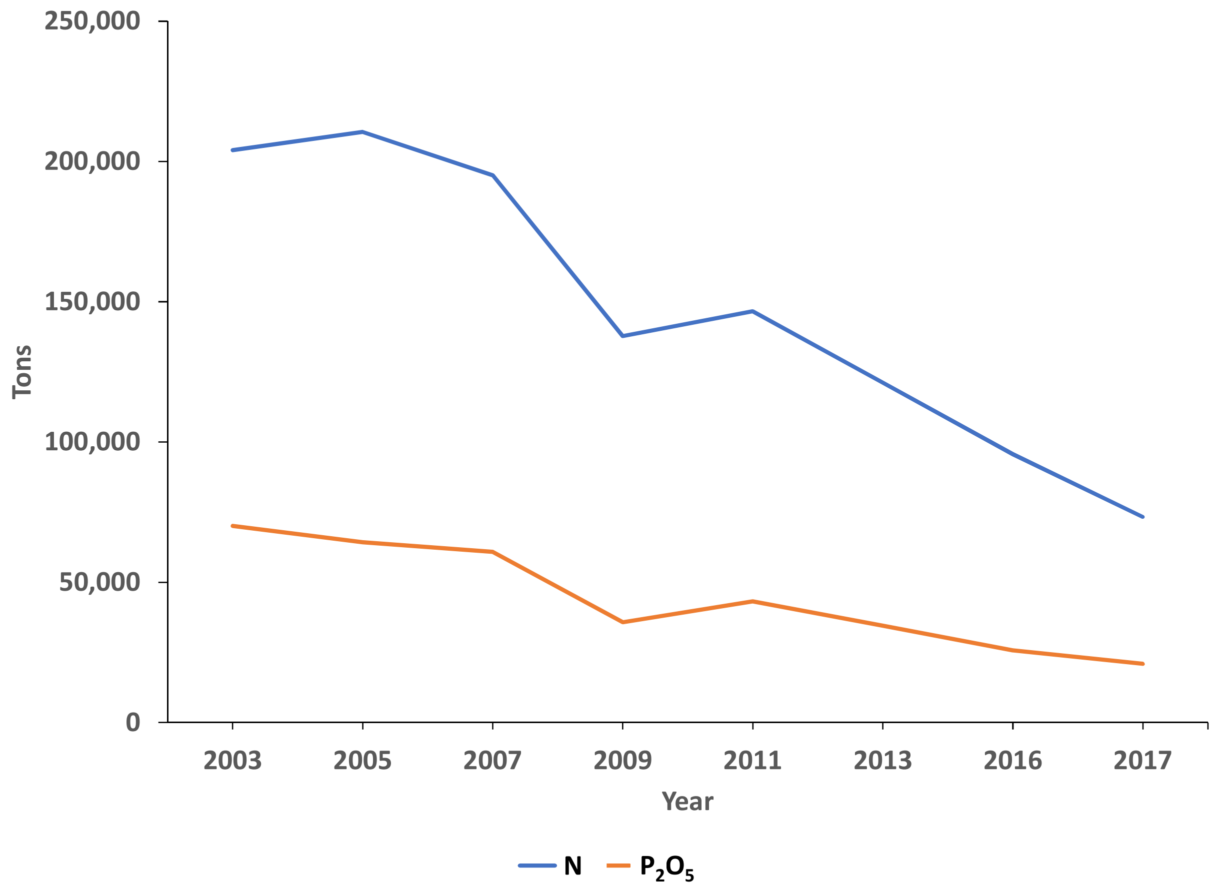

Figure 8. Amounts of fertilizers containing nitrogen (as N) or phosphorus (as P_20_5) purchased in the state of Florida between 2003 and 2017, in terms of sales or shipments, as submitted by state fertilizer control offices. (From data extracted from EPA, 2023b.)

These reported amounts of fertilizers purchased involve all market sectors. Between July 2011 to June 2012, the major consumer of fertilizers was the farm sector, with 83% of the N and 87% of the phosphate fertilizers (Figure 9). Conversely, the lawn sector represented only 6% for the N and 3% for the phosphate market at that time (FDACS, 2017).

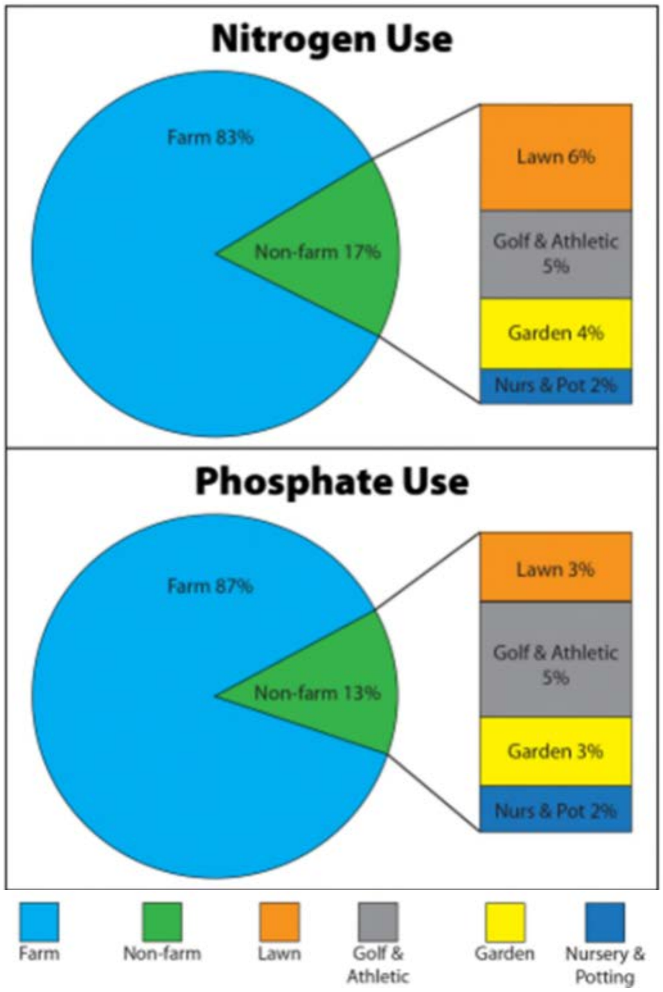

Figure 9. Percentage of total nitrogen and phosphate use by Florida market sector from July 2011 to June 2012. Credit: Shaddox and Unruh, 2017. Source: FDACS (2017).

Despite these trends in fertilizer use, around half of homeowners apply fertilizer to their yards in any given year, according to a telephone survey across the United States to residents with yards (n = 9,480; Polsky et al. 2014). This survey included cities throughout the USA, including Miami, FL. In Miami, FL, over 50% of urban and more than 75% of suburban households self-reported the use of fertilizer in the year before the survey conducted in 2011 (Polsky et al., 2014). Fertilizer use trends by Floridians are currently unknown. Garnering this information would be useful for better understanding consumer behavior and then targeting educational messaging appropriately.

Nutrient Runoff

Runoff is the lateral movement of nutrients, beyond the target location, above or near the soil surface. When nutrients move below the root zone, the process is defined as leaching. The loss of nutrients through runoff can be influenced by factors such as irrigation rate, precipitation rate, topography, soil type, soil compaction, soil water content, and the type of fertilizer used.

Most Florida soils are sandy with a rapid water infiltration capacity, making surface water movement (i.e., runoff) less common than water percolation (i.e., leaching) into the soil. For example, Shaddox and Sartain (2001) reported that when N was applied on a 10% slope subject to intense irrigation rates, the N found in runoff was less than 0.1% of that applied. Krimsky et al. (2021) found that soil and organic N nutrient pools contributed more than one-third of the NO_3−N runoff, whereas fertilizers contributed ~10% to 30% of N runoff during both dry (no fertilizer ban) and wet seasons (fertilizer ban). Studies in the Tampa Bay region revealed that atmospheric deposition is the dominant source of N in runoff, but that fertilizer, soil, and organic sources also contribute (Yang and Toor 2017). In Minnesota, Bierman et al. (2010) reported that P runoff can be reduced by not applying a P fertilizer to soils with high P test results, while returning clippings to the lawn did not increase P runoff.

In Ohio, Cheng et al. (2014) found that disturbed soil lawns due to the urban development process had a significantly shorter runoff initiation time compared to topsoil lawns. Moreover, disturbed soil lawns showed substantially higher total runoff volume (540%) and sediment loss (410%). Gregory et al. (2006) states that construction activity increases the potential for urban runoff. In urban soils, compaction contributes to issues related to soil drainage, aeration, nutrient cycling, and plant growth. Interconnected spaces facilitate water movement through the soil. Smaller pores in compacted soil hold less water which can diminish infiltration rates and lead to increased runoff and erosion. Erosion can increase the delivery of nutrients and other pollutants to nearby water bodies. Additional investigation into the runoff of pollutants resulting from soil compaction in urban developments is needed. Likewise, further research focused on urban landscape runoff under Florida conditions (sandy, low nutrient holding capacity soils, and intense rainfall events) is lacking and should be further investigated.

Nutrient Leaching

Nutrients are considered leached from the plant system if they move vertically beyond the rootzone. There are many factors that can influence leaching from turfgrass areas which include turfgrass species, fertilizer source and application rate, irrigation management, maturity and health of the grass, and root architecture. The influence of turfgrass species on N leaching losses is largely a factor of the turfgrass root system. Deeper-rooted turfgrasses tend to reduce N leaching losses compared to shallow-rooted turfgrasses (Bowman et al., 1998). Management practices that encourage deep rooting (such as deep and infrequent irrigation) are factors that shape UF/IFAS landscape fertilizer recommendations.

Numerous turfgrass leaching studies, under different conditions and in three locations statewide, were performed by UF/IFAS with funds from the FDEP. The main findings of these studies are summarized below and were used to adjust fertilizer recommendations (Table 1).

Nutrient leaching from newly planted grasses and ornamental beds

Generally, the potential for nutrient leaching losses increases during plant establishment periods. This is likely due to the lack of an extensive root system that is capable of assimilating the applied and soil nutrients, and the increase in irrigation typically applied to establishing plants. Trenholm et al. (2013) found that NO_3–N leaching was greater during the establishment period compared to established turf regardless of N application rate or timing. Additionally, waiting to apply fertilizers containing P until 30 days after sod installation reduced orthophosphate-P leaching losses compared to fertilizing at installation (Erickson et al., 2010). Loper et al. (2012) also reported that nutrient leaching was higher for ornamental beds compared to turf areas during establishment and that the application of compost in addition to fertilizer increased nutrient loads in leachate. Results of this research indicate that N and P fertilization should be withheld for a minimum of 30 to 60 days after laying sod to reduce potential nutrient leaching.

Nutrient leaching from established grasses

Proper irrigation and fertility are essential components of producing and maintaining quality turfgrass. However, inappropriate fertilizer application timings and excessive fertilizer and irrigation rates can potentially increase nutrient leaching. In Florida, fertilizer applied at UF/IFAS's recommendations (Table 1) to a healthy stand of turfgrass resulted in negligible nutrient leaching from all the various turfgrasses used in Florida, which are bahiagrass (*Paspalum notatum* Flügge), bermudagrass (*Cynodon* spp.), centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.], St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze.], and zoysiagrass (*Zoysia* spp.) (Gonzalez et al., 2013; Maia et al., 2021; McGroary et al., 2017; Shaddox et al., 2016a and 2016b; Telenko et al., 2015; Trenholm et al., 2012). These UF/IFAS recommendations for N (Table 1) are often 50% to 75% less than the amount of N required to increase N leaching above levels as associated with unfertilized turfgrass (Trenholm et al. 2012; Shaddox et al. 2016a; McGroary et al. 2017). Consequently, these rates are considered conservative, and exceeding them is unnecessary, since any additional increase in turfgrass growth or quality is marginal and may have adverse environmental consequences (Shaddox and Unruh, 2018).

It is possible that 0% to 55% of applied N could be leached, with the higher percentages occurring when UF/IFAS recommendations are not followed (Shaddox and Unruh, 2018). Likewise, reviewing studies outside of Florida, Barton and Colmer (2006) concluded that when the irrigation and fertilizer applied matched the plant requirements, less than 5% of the N applied is lost through leaching.

Table 1. Table 1. UF/IFAS nitrogen (N) recommendations for established turfgrass in Florida, by species and region (lb N/1,000 ft²/year). (Sources: Dukes et al., 2020 and Klein et al., 2023.)

	2004 – 2015			2016 – present		
	North	Central	South	North	Central	South
Species	(lb N/1,000 ft²/year)					
Bahiagrass	2–3	2–4	2–4	1–2	1–2	1–2
Bermudagrass				3–5	4–6	5–7
Centipedegrass	1–2	2–3	2–3	0.4–2	0.4–3	0.4–3
St. Augustinegrass	2–4	2–5	4–6	2–4	2–5	4–6
Zoysiagrass	3–5	3–6	4–6	2–3	2–4	2.5–4.5

Notes:

- *North Florida in this example is anything north of Ocala. Central Florida is defined as anything south of Ocala to a line extending from Vero Beach to Tampa. South Florida includes the remaining southern portion of the state.*
- *Preferences for lawn quality and maintenance level vary; therefore, a range of fertility rates is recommended. Additionally, effects within a localized region (i.e., microenvironmental influences such as shade, drought, soil conditions, and irrigation) necessitate a range of fertility rates.*
- *These recommendations assume that grass clippings are left on the lawn.*

In previous studies, minimal differences in NO_3–N leaching were observed due to fertilizer source or type. However, in general, slow-release N sources further reduce N leaching losses compared with soluble N sources (Shaddox and Unruh, 2018). Slow-release N fertilizers differ from soluble N sources because over time small portions of N are released, which increases the likelihood of plant uptake of applied N and decreases potential for N leaching losses (Guillard and Kopp, 2004). For slow-release fertilizers, nutrient release rate is influenced by environmental conditions. Initial and long-term release of N is significantly different based on the slow-release source (Shaddox, 2023).

Fertilizer applications to turf stands that do not have adequate ground cover (e.g., bare patches), are stressed, or are damaged, could result in greater nutrient losses due to leaching. For example, St. Augustinegrass or zoysiagrass damaged from herbicide stress, winter kill or disease that resulted in the lack of turf cover showed a greater potential for N leaching (Shaddox et al., 2016a; Telenko et al., 2015; Trenholm et al., 2012). Normally, stresses manifest themselves as reductions in turfgrass density and growth, which correspond to a reduction in N uptake. These stresses are largely environmental, caused by pests, late-season frosts, and changes in season. However, stress can also be anthropogenic caused by misapplications of nutrients or pest control products. When stresses occur, further applications of N may not cure the problem and may, in fact, exacerbate it and increase N leaching (Shaddox and Unruh, 2018). Further research regarding how to manage nutrient applications to stressed or damaged turf is needed.

Turfgrasses can uptake a large amount of nutrients when fertilizers are applied during times when plants are actively growing (Figure 10). In South Florida, Snyder et al. (1984) found the highest N leaching during periods when turfgrass was less active. In general, from a plant physiology-perspective, once warm-season turfgrass is established, the potential for nutrient losses is lower during the summer growing period compared to spring, fall, and winter. In the spring, root growth is typically still developing, which may account for greater potential NO_3–N losses. In the fall, growth (both shoot and root) begins to taper off, which may also account for greater leaching potential. The reduction in N leaching from winter to summer is largely a factor of increased plant growth (Figure 10) and increased evapotranspiration, which reduce the amount of N in the soil solution and the amount of moisture in the rootzone, respectively (Barton and Colmer, 2006; Shaddox and Unruh, 2018). However, these seasonal patterns of nutrient losses due to changing nutrient demand by plants assumes all other factors are equal and fertilizers are applied at the right rate (Table 1), the right place, and the right times. Nonetheless, the turfgrass growth potential throughout the year varies at different latitudes in Florida (Figure 10). For example, locations with warmer winters, like Key West, have higher growth potential during the winter months than locations with cooler winters, such as Tallahassee or Pensacola.

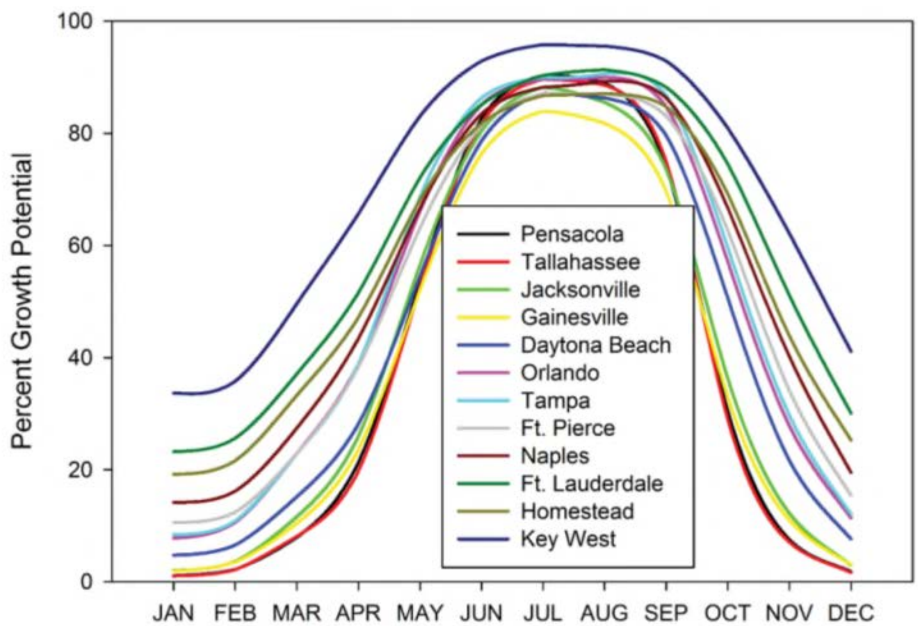

Figure 10. Potential turfgrass growth as a function of temperature throughout an entire year at different latitudes in Florida. Credit: J. Bryan Unruh, UF/IFAS, unpublished data (2023).

Fertilizer ordinances effects

The few studies performed in Florida regarding the efficacy of the fertilizer restrictions have shown mixed results. Some studies have revealed certain positive impacts on water quality (Lasso de la Vega and Ryan, 2016; Smidt et al., 2022; Lapointe et al., 2023), whereas other studies have found inconclusive results (Motsch, 2018; Souto et al., 2019) or no significant ordinance effects (Krimsky et al., 2021) in both inland and coastal environments. These varied conclusions can be attributed to the fact that not all ordinances exert the same influence on the water quality parameters analyzed. Additionally, many of these studies were focused on individual ordinances at local levels, where external factors other than fertilizer ordinances may interact with ordinance impacts, making it difficult to disentangle the causality of water quality improvements (or lack thereof).

Motsch (2018) studied two estuarine canal systems in Florida, one with a fertilizer ordinance – Cape Coral—and one without a fertilizer ordinance—Fort Lauderdale. In the three years post ordinance, Cape Coral estuarine canals exhibited a 24% reduction in total N, which initially may appear to suggest the efficacy of the fertilizer ordinance. However, in Fort Lauderdale, total N declined by 33% over the same period without a fertilizer ordinance. These results suggest that there may be other factors driving the reduction in total N within both canals, although it would be difficult to attribute an effect to the ordinance in either direction given the lack of replication and the anecdotal, case-study nature of this study. Therefore, to mitigate the impacts of nutrients at the local level, further studies are necessary to understand and manage the large-scale nutrient loading sources. The author suggests that in addition to fertilizer ordinances, other management strategies and policies might be necessary.

Research conducted by Souto et al. (2019) in three adjacent counties in Florida (Pinellas, Manatee, Hillsborough) of the Tampa Bay area focused on N reductions resulting from community education and fertilizer restrictions. They were not able to draw a conclusion on the effect of the fertilizer restrictions due to the short time frame of the study (18 months). They recommended 10 years of storm water monitoring to confidently measure a 20% reduction in N concentrations.

Smidt et al. (2022) analyzed the changes in water quality of 160 lakes throughout Florida (Figure 11) from samples collected between 1987 through 2018. This large data set was then used to analyze trends in water quality parameters not just before and after implementation of county-wide fertilizer ordinances, but relative to the type of ordinance (winter fertilizer ban, summer ban, nonseasonal ban, no ban).

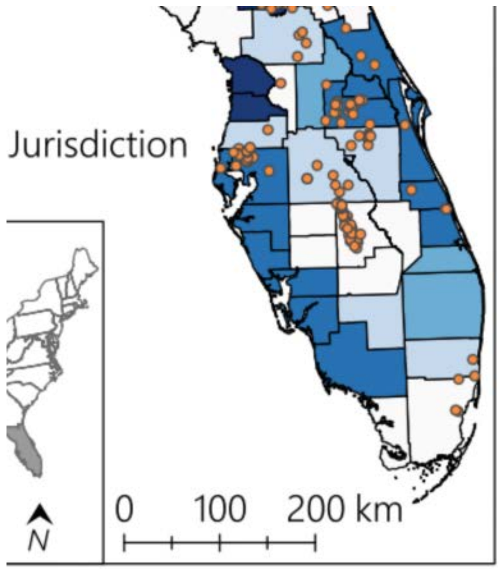

Figure 11. Florida site map (A) and county fertilizer ordinance by type (B), with lake locations of filtered LakeWatch samples, as reported in the study by Smidt et al. (2022).

A summary of the ordinance type and its effect on the different water quality parameters is shown in Table 2. Results from this study showed that winter fertilizer bans exhibited the most comprehensive and consistently positive effect (i.e., large improvement) on all water quality parameters. Summer fertilizer bans had a medium effect (e.g., medium improvement) on total P and Secchi depth (a measure of clarity of the water) but no effect on total N and chlorophyll *a* (a measure of the amount of algae growing in a waterbody). Non-seasonal fertilizer bans had a slight effect (e.g., small improvement) on total P and total N but no effect on chlorophyll *a* and Secchi depth. These results agree with other studies where most leaching of nutrients occurred during the dormant season (Shaddox et al., 2016a; Telenko et al., 2015; Trenholm et al., 2012).

Table 2. Ordinance impacts on water quality trends for different ordinance types and water quality responses. Box colors denote the trajectory (orange: degrading, blue: improving) and magnitude (darker colors denote larger effects) of ordinance impacts on water quality trends. (Source: Smidt et al., 2022).

	Ordinance impact on water quality trend			
Ban type	Total phosphorus	Total nitrogen	Chlorophyll *a*	Secchi depth
No ban	Small degradation	No change	No change	Small improvement
Non-seasonal	Small improvement	Small improvement	No change	No change
Summer	Medium improvement	No change	No change	Medium improvement
Winter	Large improvement	Large improvement	Large improvement	Large improvement

In addition, summer (wet season) fertilizer restrictions had no impact on total N and a medium effect on total P (Table 2). This result agrees with studies concluding that most nutrient uptake occurs during the active growth period, which coincides with the wet season (Carey et al., 2012; Hochmuth et al., 2012), and less nutrients are leached towards the groundwater (Gonzalez et al., 2013; Maia et al., 2021; McGroary et al., 2017; Shaddox et al., 2016a and 2016b; Telenko et al., 2015; Trenholm et al., 2012). Moreover, locations with no ban or a non-seasonal ban unexpectedly resulted in no significant impact on the water quality trend, including total P and total N. Based on the results of their study and supported by other studies, the authors recommended that counties without a winter ban should consider whether a winter ban would be useful to achieve the counties objectives with respect to water quality, particularly northern counties with significant winter dormancy (see Figure 10). However, this study was limited to the lakes monitored by the Florida LakeWatch program, and only one county with winter ordinances was included in the study (Alachua County). The winter ordinance effect could be an artifact of other behaviors/changes/programs being implemented within Alachua County. Finally, the authors stated that fertilizer restrictions are not likely to be a standalone solution.

As in the study by Smidt et al. (2022), Krimsky et al. (2021) did not find the expected influence of a summer (wet season) fertilizer ban, when measuring surface runoff from 10 homeowner lots close to the IRL. However, this research was a short-term (one year) study, and it was estimated that 5-10 years of data are necessary to confidently measure changes due to the fertilizer ban and overcome the influence of legacy nutrients from previous land uses and fertilizer applications.

Conversely, data from the volunteer monitoring Pond Watch Program was used to assess the impacts of the summer season fertilizer ban on water quality in some stormwater ponds in Lee County (Lasso de la Vega and Ryan, 2016). Levels of N, P, and chlorophyll *a* were compared across nine similar urban stormwater ponds during the wet months of 2004 through 2008 (prior to the fertilizer ordinance enforcement) and from 2009 through 2013 (post-fertilizer ordinance). The results showed a statistically significant reduction of total P and chlorophyll *a*, which decreased by 25% and 34% respectively, between pre- and post-ordinance. However, total N did not significantly differ before and after the ordinance was implemented.

Lapointe et al. (2023) collected samples at 20 sites in the IRL, before a wet season fertilizer ban and five years after. Water samples were analyzed to determine chlorophyll *a* and multiple dissolved nutrient concentrations, including NO_3^-, total dissolved nitrogen (TDN), soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), ammonium (NH_4^+), and nitrite (NO_2^-). At the same 20 sites, composite samples of the most abundant macroalgae were collected to characterize, among other analyses, stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) isotope values. Results indicate that the water quality and harmful algal blooms have worsened in parts of the IRL, mainly due to a change in the N source supporting algae blooms post-fertilizer bans. Sources of N, such as atmospheric deposition, fertilizers, and/or biosolids switched to more enriched N sources, such as human or animal waste. They found a 21% contribution of N from residential fertilizers compared to a 79% from septic systems. These loading estimates are similar to those reported in other septic systems impacting urbanized estuaries (Sham et al., 1995; McClelland et al., 1997; Valiela et al., 1997; Valiela et al., 2000; Kinney and Valiela, 2011; Lloyd, 2014). According to Lapointe et al. (2023), the fertilizer restrictions had been insufficient to diminish eutrophication at the IRL and, ultimately, they had diverted “attention, efforts, and funds that potentially could have been more effective if allocated to reducing human waste impacts”.

The effects of fertilizer restrictions have also been studied in other states. For example, a statewide law prohibiting the use of phosphorus lawn fertilizer, except in prescribed instances, was implemented in Minnesota in 2005. A 2007 report to the Minnesota Legislature by the Minnesota Department of Agriculture stated that the improvement of surface water quality was inconclusive (MDA, 2007). In the same state, Vlach et al. (2008) compared P runoff from sites using only P-free fertilizer to other sites using P-containing fertilizer finding no difference of total P between them, but found a 17% lower soluble reactive P at the P-free sites.

In southeast Michigan, Lehman et al. (2011) studied the effect of a 2006 fertilizer ordinance restricting the application of P fertilizer (May – September), unless a soil test demonstrated the need. After three years of data collection, they reported reductions of 11 to 23% in total P and 23 to 35% for dissolved P, but no reductions in NO_3^- or dissolved organic matter which is unsurprising given that the ordinance was only related to P, not N.

In central New Jersey, Qiu et al. (2014); studied the long-term water quality impacts of two P fertilizer application rates: Scenario A) decrease application rates by 25%, and Scenario B) completely eliminate P fertilizer as required by law. Scenario A resulted in 15% reduction of total P, while Scenario B achieved an even higher reduction in total P but had the unintended consequence of increasing N runoff in the watershed. In addition, the reduction in P application under both scenarios did not achieve the Total Maximum Daily Load (TMDL) standard for total P, suggesting the need for additional BMP's.

Human behavior

Even if the most effective biogeochemical approach for fertilizer ordinances is understood, efficacy ultimately hinges on compliance. The social indicators that influence whether an individual will engage in behavioral action are complex and include stakeholder awareness, social norms, perceived control, and behavioral intent. A 2015 study of Florida residents indicated that only 32% of Florida residents were accurately able to identify whether they resided in a city or county with a fertilizer blackout period, and more than half of respondents were unsure (Ryan et al., 2019). Regionally specific surveys support these results. A highly educated, older population who responded to a survey in Sarasota County, FL, similarly resulted in findings that only 35% of respondents were familiar with the county fertilizer ordinance (Kirkpatrick et al., 2014). Similarly, only 16% and 32% of residents in a master-planned community in Manatee County, FL, were aware of the year-round P ban and seasonal N ban, respectively (Persaud et al., 2019). Finally, a tri-county (Pinellas, Manatee, Hillsborough) study within the watershed of the Tampa Bay, FL, found that 24% to 44% of respondents were aware of local fertilizer regulations. Pinellas County, FL, residents, which had the most restrictive ordinance of the three counties and a substantial awareness campaign, had significantly greater ordinance awareness and knowledge (Souto et al., 2019).

Knowledge about the existence of a fertilizer ordinance does not automatically ensure that individuals will engage in those behaviors associated with the ordinance. As described above, most fertilizer ordinances are comprised of numerous individual behaviors, the application of which may not be universal. Social psychological theories can be used to determine which social factors are the greatest predictors of residents' fertilizer practices. For example, a 2018 household survey (n = 3,836) collected data on lawn fertilizer practices in the metropolitan area of Baltimore, MD (Groffman et al., 2023). They divided the sampled households in "Class 1" (households who care strongly about their lawns, ~55% of respondents) and in Class 2 (households that favor policies to reduce fertilizer use, ~45% of respondents). Class 1 households demonstrated a decreased likelihood of endorsing policies involving fertilizer surcharges and strict limitations on fertilizer application frequency (no more than one application per year). In contrast, households in Class 2 exhibited favorable inclinations toward moderate regulations, limited to no more than 3 applications of fertilizer per year and surcharges on lawn fertilizer.

A study of Florida residents measuring the likelihood of an individual's willingness to adopt fertilizer BMPs on a five-point scale from *never* to *always*, suggests that some BMPs will always have adoption by ~30% of the population whereas some BMPs are unlikely to ever be adopted. For example, more than 30% of the survey respondents indicated that they never test their soil or apply fertilizer based on soil test results, and less than 10% of the respondents were always willing to comply with these behaviors. As soil testing is a conventional requirement for the application of P in Florida's fertilizer ordinances, the water quality benefits associated with this behavior are less likely to be realized.

Similar behaviors that had low frequency of adoption were selecting slow-release N fertilizers and ensuring landscape professionals have the necessary certification to apply fertilizers (Warner et al., 2019). These results are consistent with those of a 2020 survey of more than 1,000 Florida homeowners that suggests only 23% of the survey respondents were classified as fertilizer conscious, preferring landscapes with low fertilizer requirements. While fertilizer conscious residents also indicated a moderate preference for low irrigation inputs, a greater proportion of the population (27%) prioritized low irrigation over fertilizer inputs (Knuth et al., 2023). While attitudes about fertilization are important predictors of engagement in fertilizer BMPs, this and other studies indicate that Floridians prioritize water conservation over water quality (Warner et al., 2018; Knuth et al., 2023). This prioritization of water quantity over quality may be associated with the belief that most Floridians (80%) do not believe that their landscapes have a negative impact on water quality (Ryan et al., 2019).

Social norms are the shared set of behaviors perceived to be acceptable by groups of people. There are several studies reported in the literature that indicate the social norms associated with residing in a Homeowners Association (HOA) influence the adoption of residential landscape practices, including fertilization (Fraser et al., 2013; Warner et al., 2021). This is especially important for Florida which boasts the second highest proportion of residents living within an HOA as compared with any other state (Community Associations Institute, 2021). Warner et al. (2021) also found that in addition to social norms, individuals' belief about whether they have control over their ability to engage in a behavior, referred to as perceived behavioral control, was a strong predictor of adopting fertilizer BMPs. They also found that compatibility of fertilizer BMPs with residents' existing values, yard care routines and expectations, and budget were important for adoption. This outcome is consistent with the findings that suggest that the more complicated a behavior is, or is perceived to be, the less likely it is to be adopted (Rogers, 2003).

A study on homeowners' awareness and perception of sustainable landscaping practices revealed that Florida homeowners who possess more knowledge about sustainable landscape programs, like Florida-Friendly Landscaping™, are more inclined to participate in sustainable landscaping practices (Zhang et al., 2021). In line with this, Brevard County is currently implementing an outreach program that covers topics such as fertilizer application, management of grass clippings, excess irrigation, maintenance of stormwater ponds, septic systems, and sewer laterals. This initiative is expected to contribute to raising awareness and understanding of these issues (Lapointe, 2023). Moreover, initiatives rooted in community participation, such as fertilizer restrictions, play a crucial role in engaging local populations in environmental protection endeavors (Krimsky et al., 2021).

SUMMARY AND RECOMMENDATIONS

A key takeaway message from the scientific literature (including recent studies from Florida and other parts of the USA) is that there are multiple sources of nutrients N and P in urban watersheds with the potential to contribute to nutrient pollution of Florida's waterbodies (i.e., septic systems, reclaimed water, atmospheric deposition, grass clippings and landscape wastes, compost amendments, fertilizers, nutrient runoff, and nutrient leaching). Therefore, it is important to place the various sources in context with each other to learn which sources might be the most important in a given location and time. In this document, we summarized the research conducted on these sources of N and P and analyzed their effect on water quality.

Only six studies (five in the peer-reviewed scientific literature) analyzing the efficacy (pre/post) of fertilizer ordinances in Florida were found. The first study was published in 2016, with data from five years pre- and five years post-fertilizer ordinance, from a volunteer program monitoring nine urban stormwater ponds. The results showed a post-ordinance reduction in total P and chlorophyll *a*, but not for total N.

In 2018, a second study included two estuarine canal systems in Florida, one with a fertilizer ordinance –Cape Coral—and one without a fertilizer ordinance—Fort Lauderdale. In the three years post ordinance, Cape Coral estuarine canals resulted in a reduction of 24% in total N, suggesting the efficacy of the fertilizer ordinance. However, in Fort Lauderdale, total N declined

33% over the same period, without a fertilizer ordinance. Based on the results of this study, the fertilizer ordinance did not appear to influence total N in the canals. However, the lack of true replication makes any inference drawn from this study questionable. A research study published in 2019 and conducted in three adjacent counties within the watershed of the Tampa Bay, Florida, was not able to draw a conclusion on the effect of the fertilizer restrictions due to the short sampling time frame of the study (18 months). They recommended 10 years of storm water monitoring to confidently measure a 20% reduction in N concentrations.

A study published in 2021, measured the surface runoff from 10 homeowner lots close to the IRL and found that NO_3^- and NH_4^+ -based fertilizers contributed a combined 31% to 44% of NO_3^- in lawn surface water runoff, however, it did not find the expected influence of a summer (wet season) fertilizer ban. The largest spatial area covered by a study and for the longest time frame (21 years) was published in 2022, which analyzed the changes in water quality of 160 lakes throughout Florida. This study utilized water quality data from lakes in locations with no ban, no seasonal ban, summer ban, and winter ban. Results from this study generally showed some improvement in water quality across the type of fertilizer ordinance in 8 of 12 ordinance water quality parameter trend categories. In locations with no ban or non-seasonal ban, a small or no change was found. In addition, summer bans had no impact on total N and only a medium effect in total P. The authors concluded that there was a tendency for positive impacts of fertilizer ordinances on water quality, with winter fertilizer bans being the most comprehensive and effective compared to other ordinance types. These results agree with other studies that suggest that most nutrient leaching occurs during the winter dormant season. However, the authors remarked that to prevent nutrient pollution in water bodies, fertilizer restrictions are not likely to be a standalone solution.

Conversely, a new study published in 2023 that sampled 20 sites in the Indian River Lagoon found that current N loading estimates represent a 21% contribution from residential fertilizers compared to 79% from septic systems. These results are in line with a multitude of studies highlighting the importance of multiple different N sources exporting N from urban watersheds. Furthermore, these results agree with other studies showing that human waste is often the largest contributing N source driving eutrophication and harmful algal blooms in coastal environments. The authors concluded that the fertilizer bans had "ultimately diverted attention, efforts, and funds that potentially could have been more effective if allocated to reducing human waste impacts".

These varied conclusions can be attributed to the fact that not all ordinances exert the same influence on the water quality parameters analyzed. In addition, the divergence in the effectiveness of fertilizer restrictions depends on various factors, including the specific regulations in place, the (lack of) enforcement, education, or awareness of those regulations, the willingness of stakeholders to comply, and the ecological and environmental conditions of the area. These factors may limit the efficacy of ordinances or the ability of scientific studies to separate the effects of ordinances from other natural or human-caused factors affecting water quality. Additionally, waterbodies can respond in a variety of different ways to increasing nutrient inputs. Natural processes within these waterbodies can temporarily or permanently remove nutrients, reducing their influence on other water quality indicators and possibly masking changes in watershed nutrient management.

Despite these knowledge gaps and disparate impacts, fertilizer ordinances continue to be adopted and promoted as an environmental management strategy throughout Florida. The assumption that residential fertilizer restrictions will reduce pollution to waterbodies and improve water quality remains largely unclear. The few studies that have attempted to establish the effectiveness of ordinances have been generally limited to small or specific areas and analyzed the fertilizer restriction impacts in relatively short time scales (less than 5 years). The single study that incorporated a larger area and a longer time frame was not able to directly document mechanistic effects given the opportunistic study design (i.e., the study repurposed data collected for other reasons, allowing a large dataset but an unbalanced experimental design).

To fully understand the effect of fertilizer ordinances and other strategies to mitigate nutrient pollution and improve water quality, thorough study of the topic is required. Such a study would be characterized by a comprehensive monitoring program spanning multiple watersheds that seeks to quantify sources, transport, and fate of nutrients, and ecological responses of downstream receiving waters. This study would need to be geographically distributed across a range of urban watersheds spanning social, environmental, and economic gradients. There would need to be a combination of water quality monitoring through both manual field sampling and continuously deployed electronic monitoring equipment, nutrient source tracking (e.g., with isotopic analysis and direct measurement), and experimental manipulations in the lab and in the field to assess causal mechanisms. This research would need to be carried out for multiple years to account for known annual and decadal climate patterns, shifting regulatory environments, and demographic trends throughout our state. Results from this study would facilitate better decisions regarding future regulations and public funding to remediate this ongoing problem statewide.

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Effects of nutrient enrichment on seagrass population dynamics: evidence and synthesis from the biomass–density relationships

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Summary

1. The available data from experimental and descriptive studies on seagrass biomass and density responses to nutrient enrichment were analysed to assess the intraspecific mechanisms operating within seagrass populations and whether biomass–density relationships can provide relevant metrics for monitoring seagrasses.

2. The response of shoot biomass and density to nutrient enrichment was dependent on the type of study; the short-term positive response of biomass and density in experimental studies reveals context-specific nutrient limitation of seagrasses. The long-term negative response of descriptive studies probably results from ecosystem-scale events related to nutrient enrichment such as increased turbidity, algal blooms, epiphyte loads and anoxia.

3. Most seagrass species analysed lie in the nonthinning part of the theoretical biomass–density curves. A simultaneous increase in biomass and decrease in density, evidence of self-thinning, were only observed in 4 of 28 studies. The analysis of both the static and the dynamic biomass–density relationships revealed that the slopes increase under nutrient enrichment. Surprisingly, the species-specific slopes (log B-log D) were higher than one, revealing that the B/D ratio, that is, the average shoot biomass, increases with density in all seagrass species analysed. Nutrient enrichment further enhanced this effect as biomass–density slopes increased to even higher values. The main drivers behind the increasing biomass–density slopes under nutrient enrichment were the increase in shoot biomass at densities above a species-specific threshold and/or its decrease below that threshold.

4. *Synthesis*. Contrasting short- and long-term responses of both biomass and density of seagrasses to nutrient enrichment suggest that the former, positive ones result from nutrient limitation, whereas the later, negative ones are mediated by whole ecosystem responses. In general, shoot biomass of seagrasses increases with density, and nutrient enrichment enhances this effect. Experimental testing of facilitation processes related to clonal integration in seagrasses needs to be done to reveal whether they determine the low incidence of self-thinning and the intriguing biomass–density relationships of seagrass species. The increasing slopes and decreasing

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intercepts of the species-specific dynamic biomass–density relationships of seagrasses and the decreasing coefficients of variation of both biomass and density constitute relevant, easy-to-collect metrics that may be used in environmental monitoring.

Key-words: biomass–density relationship, facilitation, intraspecific competition, monitoring, nutrient enrichment, plant population and community dynamics, seagrass

Introduction

The relationships between plant biomass and density ultimately reflect the competitive mechanisms operating within populations and how they respond to the environment (Weller 1987). Changing environmental conditions (e.g. nutrient availability) may modify such competitive mechanisms, affecting the biomass–density relationships of plant populations (Morris & Myerscough 1985, 1991; Morris 1995, 1999, 2002, 2003; Steen & Scrosati 2004; Cabaço, Machás & Santos 2007; Chu *et al.* 2010). These relationships may be structured along the same or along different biomass–density lines as the environment varies (Morris 2003). Matching biomass–density lines indicates that the biomass packing does not change with environmental conditions, even though the rate of propagation along the line may vary. Different biomass–density lines resulting from higher availability of resources reflect different competition processes occurring within populations as a consequence of biomass accumulation (Morris 2003). Ultimately, increased intraspecific competition due to increased biomass of individuals in crowded plant populations may result in a density decrease, a process known as self-thinning (Yoda *et al.* 1963; White 1981; Westoby 1984; Weller 1987). In addition to competition, facilitation, that is, the positive effect of plants on the establishment or growth of neighbouring plants (Brooker *et al.* 2008), may also be involved. Chu *et al.* (2008) demonstrated that facilitation could also affect the biomass–density relationships, playing an important role in plant–plant interactions and in the population dynamics outcome. Intraspecific facilitation is common in clonal plants such as seagrasses, where clonal integration results in particularly active spatial and temporal dynamics involving the continuous recruitment and mortality of shoots within the same individual (Duarte *et al.* 2006).

Nutrients affect both the structure and dynamics of the populations of seagrasses mainly through changes in plant architecture, morphology and mortality (Short 1983; Romero *et al.* 2006; Fertig, Kennish & Sakowicz 2013) and therefore may have an effect on the biomass–density relationships. The meta-analysis of Hughes *et al.* (2004) revealed that experimental additions of inorganic nutrients to sediments generally stimulate seagrass growth, suggesting nutrient limitation of plant production. However, the excessive growth of epiphytes, macroalgae and phytoplankton under high nutrient loads decreases seagrass growth and survival (Lee, Park & Kim 2007; Schmidt *et al.* 2012). Excessively high nutrient regimes also result in built-up of organic matter, which may result in conditions unfavourable to seagrasses, such as sediment anoxia or sulphide toxicity (Koch 2001; Koch *et al.* 2006). Direct nutrient toxicity effects on seagrass growth and survival have also been reported (van Katwijk *et al.* 1997; Brun *et al.* 2002; Burkholder, Tomasko & Touchette 2007). These have been considered the major factors contributing to seagrass decline world-wide (Short & Wyllie-Echeverria 1996; Ralph *et al.* 2006; Waycott *et al.* 2009).

To date, self-thinning processes have not been explicitly reported for seagrasses in established, natural populations.

Here, we analyse how the biomass–density relationship of seagrass meadows responds to nutrient enrichment. As changes to the biomass–density relationship are determined by the growth and survival responses of individual plants, this relationship may reveal the competitive and resource allocation mechanisms operating within seagrass meadows under increasing nutrient loads. The only report on this subject is Cabaço, Machás & Santos (2007), who observed that the slope of the biomass–density relationship of *Zostera noltii* increased along a gradient of anthropogenic nutrient enrichment and that this was mainly driven by biomass changes. Our analysis is based on a world-wide data set of biomass and density responses of seagrass species both under small-scale, controlled nutrient additions (experimental studies) and under large-scale contrasting nutrient levels in natural settings (descriptive studies), obtained from published and unpublished data sources. We analyse the effects of nutrient enrichment on biomass and density separately, as well as on the biomass–density relationships. We also investigate whether the life strategy of different species influences the population's response to nutrients. Size and growth of seagrasses are linked to their life-history strategy as small-size species tend to have high growth rates and large-size species tend to have low growth rates (Duarte *et al.* 2006). In order to test this, the species responses were scaled to their specific shoot weight, rhizome diameter, leaf length, and both horizontal and vertical rhizome elongation rates.

The biomass and density of seagrass populations are easily measurable and, in fact, have been widely used both in regional-scale monitoring programs (e.g. Mediterranean basin; Lopez y Royo *et al.* 2010) and in global-scale monitoring programs (e.g. SeagrassNet, www.seagrassnet.org). If the biomass–density relationships vary with nutrient loadings, reflecting the outcome of the competitive mechanisms operating within populations, this relationship can constitute a sound metric for coastal monitoring based on seagrass stands. The biomass–density relationships could then be used as early warning indicators of the negative effects of excessive nutrient loadings on coastal ecosystems dominated by seagrasses.

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Materials and methods

RESPONSE OF SEAGRASS BIOMASS AND DENSITY TO NUTRIENT ENRICHMENT

Data on the effects of nutrient enrichment on both the above-ground biomass and shoot density (per square metre) of monospecific seagrass meadows were compiled both from the literature and from unpublished data sources (Table 1). Both descriptive studies and field experiments were included in the analysis. The sources of nutrient enrichment in descriptive studies were mainly urban wastewater, aquaculture and agriculture (Table 1). Most of the experimental studies were performed by enriching the sediment with slow-release fertilizers (Table 1). Only the studies reporting both the above-ground biomass and density responses for the same sites and time period were included in the analysis. Mesocosms and laboratory experiments were not considered in this study as they generally involve plant manipulations and we consider that they do not represent natural conditions. As well, these data are not expressed per unit of area as required for the analysis.

Nutrient levels were classified as low and high because the absolute nutrient concentrations varied widely, with the differences between undisturbed (low) and enriched (high) conditions ranging from 2- to 97-fold (Table 1). When more than one experiment per study was conducted on the same species in different locations (sites), the mean values were used in the analysis.

The biomass and density responses to nutrient enrichment were quantified for each study case as the percentage change $((H-L)/L) \times 100$, where H is the biomass or density at high nutrient levels and L is the biomass or density at low nutrient levels. The response is negative when biomass or density of seagrass meadows declines with nutrient enrichment and positive when they increase. Linear regression analyses were used to examine the effects of seagrass growth or size on the biomass or density responses to nutrient enrichment (Sokal & Rohlf 2012). The species-specific average values of individual shoot weight (g DW), rhizome diameter (mm) and leaf length (cm) were used as size parameters, and the horizontal and vertical rhizome elongation rates (cm yr^{-1}) as growth parameters. All these parameters were derived from literature data (see Table 2 for details).

BIOMASS–DENSITY RELATIONSHIPS

The 'static interspecific biomass–density relationship' (Weller 1989; Scrosati 2005) was obtained using data collected a single time, the season of maximum above-ground biomass. The static relationships under low and high nutrient levels were compared. When the temporal variation of biomass and density of a species at low and high nutrient levels was available, the 'dynamic biomass–density relationship' was analysed. This was possible for seven seagrass species, *Cymodocea nodosa* (Spain), *Halophila ovalis* (Thailand), *Thalassia hemprichii* (Thailand), *Thalassia testudinum* (Belize, Colombia and Mexico), *Zostera capricorni* (Australia), *Zostera marina* (USA) and *Zostera noltii* (Portugal), in a total of nine study cases. These were all descriptive studies (Table 1). The linear log–log relationships between biomass and density were determined by principal component analysis (PCA), because both variables are random (Weller 1987; Scrosati 2005). The PCA yields an orthogonal regression, which minimizes deviations perpendicularly to the fitted line and therefore does not rank variables as independent or dependent. PCA was performed on the covariance matrix with the linear fit corresponding to the first eigenvalue (Manly 1986; Jackson 1991). The slope was estimated by dividing the biomass loading by the density loading. The linear dependence of the biomass–density relationships was measured by Pearson's correlation coefficient, and its statistical significance was determined by testing the null hypothesis that log biomass and log shoot density were uncorrelated (Sokal & Rohlf 2012).

To estimate the variance of the slopes and intercepts of PCA regressions of the static interspecific biomass–density relationship, so that the differences between low and high nutrient levels could be tested, a bootstrap resampling technique (random sampling with replacement) was done 50 times to the original set of biomass–density variables and a PCA was performed to each bootstrap sample. Fifty bootstrap samples are considered adequate to estimate standard errors (Timmerman, Kiers & Smilde 2007). The variables of interest, that is, the slope and intercept, were estimated for each PCA, and their average values (±SE) were calculated. A Student's *t*-test was used to assess the significant differences between the mean slope and intercept of the static interspecific biomass–density relationship obtained under low and high nutrient levels.

For the dynamic intraspecific biomass–density lines, a chi-square test was used to investigate the response trends to nutrient enrichment of the slopes and intercepts, testing the null hypothesis that the number of cases showing increasing or decreasing responses was equal. Statistical analyses were performed using SYSTAT. Levels of significance were established at $\alpha = 0.05$.

Results

Responses of above-ground biomass and shoot density to increasing nutrient loads were obtained from 28 studies (17 descriptive and 11 experimental) of 14 species (Table 1), including a wide range of plant sizes and geographical distribution. In 22 of the studies, biomass and density covaried simultaneously, either increasing together (six studies, all experimental, Fig. 1 upper right quarter) or decreasing together (three experimental plus 13 descriptive studies, Fig. 1 lower left quarter). Only in four studies were there increases in biomass accompanied by density decrease as expected under self-thinning (two descriptive and two experimental). Interestingly, descriptive and experimental studies showed opposite trends in the biomass and density responses to nutrients. A simultaneous decrease in biomass and density was the most common response in descriptive studies (13 of 16 cases, 76%, Fig. 1) as opposed to experimental studies (2 of 11 cases, 18%). No descriptive studies reported simultaneous increases in biomass and density. In experimental studies, biomass increased in 8 of the 11 cases (72%).

The magnitude of the biomass–density responses to nutrient enrichment (Fig. 1) was much higher in the species showing a positive response (up to 269% for biomass and 125% for density) than in the species showing a negative response (down to −84% and −79%, respectively). The biomass–density regression line is above the 1:1 line (Fig. 1) showing that, overall, the above-ground biomass of seagrasses responds more than density to nutrient increase.

The analysis of the static interspecific relationship between biomass and density shows that the slope of the line is significantly higher under high nutrient conditions in experimental studies (Fig. 2a), but not in descriptive studies (Fig. 2b) due to higher variability of biomass and density. The intercept of

Table 1. List of seagrass reports of monospecific meadows for which the biomass–density relationships at low and high nutrient levels were available. Nutrient sources, enrichment levels (x fold) and responses of above-ground biomass and density ('+' for increase and '−' for decrease) of descriptive (D) and experimental (E) studies are presented

Species (abbreviation)	Location	Nutrient source		Enrich. (× fold)	Response: Biomass	Response: Density	Reference
Cymodocea nodosa (Cn)	Alfacs Bay, Spain	Agriculture run-off (rice paddy fields)	D	30	−	−	O. Mascaró, M. Pérez & J. Romero unpubl. data
Cymodocea rotundata (Cr)	Cape Bolinao, NW Philippines	Slow-release fertilizer in sediment	E	18	+	+	Agawin, Duarte & Fortes (1996)
Cymodocea serrulata (Cs)	Moreton Bay, Australia	Slow-release fertilizer in sediment	E	97	−	−	Udy & Dennison (1997a)
Halophila ovalis (Ho)	Trang and Satun, Thailand	Mangrove run-off	D	3	−	−	A. Prathep unpubl. data
Heterozostera tasmanica (Ht[a])	Port Phillip Bay, Australia	Slow-release fertilizer in sediment	E	6	+	+	Bulthuis, Axelrad & Mickelson (1992)
Heterozostera tasmanica (Ht[b])	Westernport, Australia	Agriculture and urbanization	D	2	−	−	Miller, Campbell & Scudds (2005)
Halodule uninervis (Hu[a])	Moreton Bay, Australia	Slow-release fertilizer in sediment	E	97	+	+	Udy & Dennison (1997a)
Halodule uninervis (Hu[b])	Green Island (GBR), Australia	Slow-release fertilizer in sediment	E	41	+	+	Udy *et al.* (1999)
Posidonia australis (Pa)	Rottnest Island, Australia	Slow-release fertilizer in sediment	E	6	−	−	Udy & Dennison (1999)
Posidonia oceanica (Po[a])	Aegean Sea, Greece	Fish farm	D	4	−	−	Apostolaki *et al.* (2009), E.T. Apostolaki unpubl. data
Posidonia oceanica (Po[b])	NE Spain	Urbanization, sewage effluents, agriculture	D	4	−	−	Martínez-Crego *et al.* (2008), Romero *et al.* (2007)
Syringodium isoetifolium (Si)	Green Island (GBR), Australia	Slow-release fertilizer in sediment	E	41	−	−	Udy *et al.* (1999)
Thalassia hemprichii (Th[a])	Cape Bolinao, NW Philippines	Slow-release fertilizer in sediment	E	18	+	+	Agawin, Duarte & Fortes (1996)
Thalassia hemprichii (Th[b])	Trang, Thailand	Mangrove run-off	D	3	−	−	A. Prathep unpubl. data
Thalassia testudinum (Tt[a])	Sarasota Bay, Florida, USA	Run-off, baseflow, point sources, septic tanks, rainfall	D	12	−	−	Tomasko, Dawes & Hall (1996)
Thalassia testudinum (Tt[b])	St. Joseph Bay, Florida, USA	Slow-release fertilizer in water	E	13	+	−	Heck *et al.* (2000)
Thalassia testudinum (Tt[c])	CCB and LLM, Texas, USA	Ammonium enrichment in sediment	E	9	+	−	Lee & Dunton (2000)
Thalassia testudinum (Tt[d])	Puerto Morelos, Mexico	Mangrove run-off	D	2	+	−	B.I. van Tussenbroek unpubl. data
Thalassia testudinum (Tt[e])	Lighthouse Atoll, Punta Gorda and Placencia, Belize	Run-off, water pollution	D	5	−	+	SeagrassNet unpubl. data
Thalassia testudinum (Tt[f])	Tayrona and Rosario Is., Colombia	Human waste in nearby developments	D	2	−	−	SeagrassNet unpubl. data
Zostera capricorni (Zc[a])	Moreton Bay, Australia	Slow-release fertilizer in sediment	E	97	+	+	Udy & Dennison (1997a)
Zostera capricorni (Zc[b])	Moreton Bay, Australia	Sewage, septic effluent, prawn-farm effluent, river discharge	D	28	−	+	Udy & Dennison (1997b)
Zostera capricorni (Zc[c])	Lake Macquarie and Tuggerah Lakes, NSW, Australia	Agriculture run-off, urban stormwater	D	4	+	−	R. Gruber unpubl. data
Zostera marina (Zm[a])	Gulf of St. Lawrence, Canada	Bivalve aquaculture, fish processing plants, peat mining, agriculture	D	20	−	−	Schmidt *et al.* (2012)
Zostera marina (Zm[b])	British Columbia, Canada	Urbanization	D	7	−	−	Robinson, Yakimishyn & Dearden (2011)
Zostera marina (Zm[c])	Humboldt Bay, California, USA	Aquaculture, agriculture run-off	D	3	−	−	SeagrassNet unpubl. data
Zostera noltii (Zn[a])	Ria Formosa, Portugal	Urban wastewater effluent	D	49	−	−	Cabaço, Machás & Santos (2007), Cabaço, Santos & Sprung (2012), Peralta *et al.* (2005)
Zostera noltii (Zn[b])	Cadiz Bay, Spain	Fish aquaculture effluent	D	4	−	−	García-Marín *et al.* (2013)

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Table 2. Growth and size characteristics of the seagrass species examined. HE, horizontal rhizome elongation rate (cm yr^{-1}); VE, vertical rhizome elongate rate (cm yr^{-1}); RD, rhizome diameter (mm); SW, shoot weight (g DW); LL, leaf length (min.-max., cm); n/a, data not available

Species	HE	VE	RD	SW	LL
Cymodocea nodosa	40.2	1.43	2.73	0.09	10–30
Cymodocea rotundata	209.9	1.53	2.44	0.065	7–15
Cymodocea serrulata	153.0	13.10	2.78	0.121	6–15
Halophila ovalis	357.5		1.30	0.016	1–4
Heterozostera tasmanica	102.8	8.91	1.74	0.080	7–35
Halodule uninervis	101.2	4.10	1.37	0.027	6–15
Posidonia australis	9.3	1.42	7.21	0.875	30–60
Posidonia oceanica	2.8	2.70	10.75	0.657	40–50
Syringodium isoetifolium	109.1	8.55	1.74	0.038	7–30
Thalassia hemprichii	54.1	3.25	3.63	0.156	10–40
Thalassia testudinum	69.3	3.89	5.96	0.238	10–60
Zostera capricorni	78.0		1.40	0.091	7–50
Zostera marina	26.1		3.50	0.323	n/a
Zostera noltii	68.4		1.54	0.011	6–22

Data compiled by Duarte (1991), Marbà & Duarte (1998), Duarte *et al.* (1998), Hemminga & Duarte (2000), and Marbà & Duarte (2003). Data on seagrass leaf length (minimum and maximum leaf length) were obtained from Phillips & Meñez (1988) and Kuo & den Hartog (2001). Data on the horizontal rhizome elongation rate for *Zostera capricorni* were obtained from Turner *et al.* (1996) and Turner (2007). Updated data on *Zostera noltii* by Cabaço, Santos & Duarte (2008) were used.

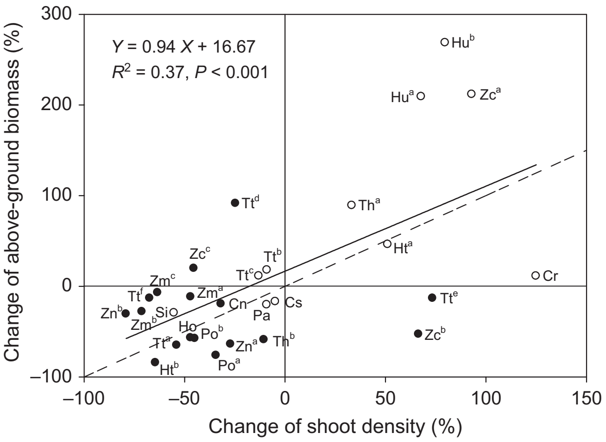

Fig. 1. Relationship between the percentage of change of seagrass above-ground biomass and shoot density in response to nutrient enrichment. The coefficient of determination (R^2) and significance (P) of linear regression is shown. Dashed line represents the 1:1 biomass–density line. Open and closed circles represent experimental and descriptive studies, respectively. See Table 1 for species name abbreviations.

the biomass–density relationship shows the opposite response, decreasing with nutrient enrichment in both experimental and descriptive studies.

The slopes of the intraspecific, dynamic biomass–density relationships (in log scale) for the nine species of seagrasses from which data were available were all positive and higher than one (Table 3). This revealed, respectively, that data are in the nonthinning part of the theoretical biomass–density curves and that the B/D ratio increases with density in all seagrass species tested, that is, that the average biomass of each individual increases with density. Nutrient enrichment further increased the slopes and thus the positive effect of density on the average biomass of each seagrass shoot. This was observed in all reported cases, except one of *Thalassia testudinum* where the slope decreased from 1.38 to 1.09 with nutrients (Tt^d, Table 3). The intercept of the dynamic biomass–density line showed the opposite trend of the slopes. The coefficients of variation of density and biomass decreased with nutrient enrichment in seven of nine cases (Table 3).

Figure 3 shows how the biomass–density data fit around the intraspecific, dynamic biomass–density relationships to help understanding the main drivers of the slope responses to nutrient enrichment (Table 4). In species where the lines intercept within the range of data dispersal (Fig. 3), the biomass–density ratio (B/D), that is, the biomass of each shoot, decreased below a certain density threshold, whereas above that threshold it increased. This was observed in five of the nine seagrass species. However, the density thresholds are near the range limits of the observed density values in four species. Consequently, the main driver of the slope response to nutrient enrichment was a progressive increase in B/D with density, observed in *Thalassia hemprichii* (Th^b), *Thalassia testudinum* (Tt^f), *Zostera capricornii* (Zc^c) and *Zostera marina* (Zm^c). In *Cymodocea nodosa*, *Halophila ovalis and Thalassia testudinum* (Tt^e), there was a progressive reduction in the B/D ratio at lower densities, whereas in *Thalassia testudinum* (Tt^d), B/D increased progressively at lower densities. In *Zostera noltii*, shoot biomass decreases with density at densities lower than 8511, whereas it increases above that threshold (Table 4). This is the only species where the density threshold is in the middle of the density distribution range (Fig. 3).

The analysis of the interspecific responses of biomass and density to different levels of nutrient enrichment shows that biomass is significantly related to nutrients (Fig. 4A), only when biomass increased with nutrients (most experimental studies). In descriptive studies, where biomass decreased with nutrients (negative values of change, Fig. 4A), there was no significant relationship between biomass and nutrient levels. In contrast, density was not related to nutrient enrichment both in the positive and negative sets of responses (Fig. 4B).

Neither the seagrass biomass nor the shoot density responses to nutrients were related to seagrass size or growth characteristics analysed (Table 5). Therefore, the hypothesis that the species-specific life strategy of seagrasses (expressed as size and growth) influences the populations' response to nutrients was not supported.

Discussion

The analysis of the available data on the effects of nutrient enrichment on seagrass biomass and density revealed that the most common reaction was a synchronized response of both variables to increasing nutrients, which was reflected in the significant relationship between biomass and density

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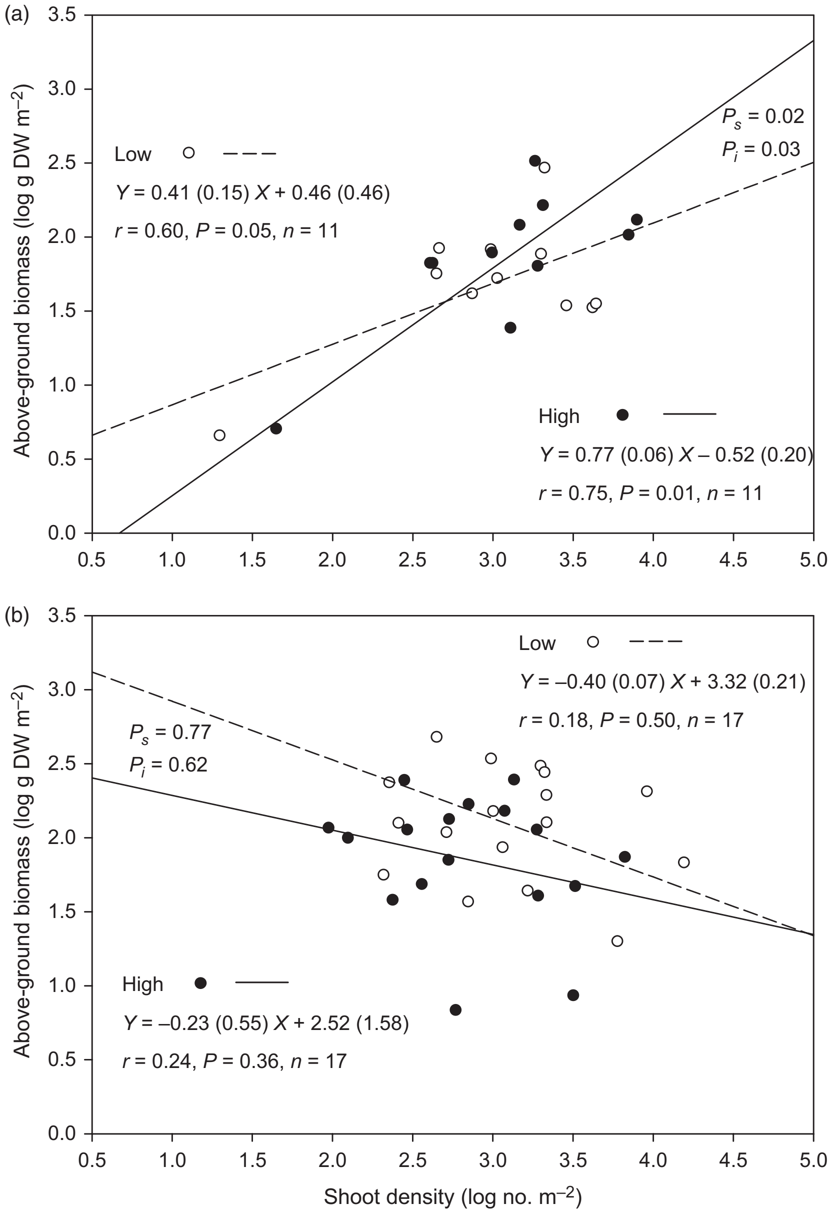

Fig. 2. Static interspecific relationship between above-ground biomass and shoot density (both log-transformed) of seagrasses at low and high nutrient levels for experimental (a) and descriptive (b) studies. Mean slopes and intercepts (±SE) were obtained using PCA bootstrapping (n = 50); P in italics shows the significance levels of the t-tests between the low and high nutrient levels for the slope (s) and intercept (i). Pearson's correlation: r – correlation coefficient, P – significance level of linear relationship and n – number of studies included in the analysis.

responses (Fig. 1). The analysis also revealed two opposite responses that corresponded to the type of study performed. Biomass and density tended to increase simultaneously under high nutrient levels in short-term experimental studies, whereas they tended to decrease simultaneously in descriptive studies where the seagrass populations were exposed to the long-term effects of nutrient increase. Experimental and descriptive studies may reveal different time frames of the nutrient enrichment response curve, indicating that experiments are context-dependent and that the limited temporal scales of experimental approaches may result in conclusions that cannot be extrapolated to a long-term ecosystem scale.

The short-term responses observed in experimental studies suggest a general nutrient limitation of seagrasses in the systems where these experiments were carried out. These responses were driven primarily by changes in biomass, rather than density as shown both by the statistically significant response of seagrass biomass to different nutrient levels, which was not observed for density (Fig. 4), and by the observation that the linear regression of biomass and density responses is above the 1:1 line (Fig. 1). Nutrient enrichment in nutrient limited conditions will result in higher biomass per shoot, increasing potential competitive interactions (Morris 2003), for example, for light. A similar trend was reported for terrestrial herbaceous clonal plants that responded to increasing nutrients

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Table 3. Dynamic relationship between above-ground biomass and shoot density (both log-transformed) for seagrass species (descriptive studies) at low and high nutrient levels obtained using PCA. *r*, correlation coefficient, *P*, significance level of linear relationship and *n,* number of samples included in the analysis. Coefficient of variation (CV,%) of density (D) and biomass (B) is shown. See Table 1 for species name abbreviations

Species	Nutrients	Slope		Intercept		*r*	*P*	*n*	CV_D		CV_B	
Cn	Low	2.55		−6.09		0.81	< 0.001	36	6.46		24.67	
	High	2.71	↑	−6.63	↓	0.64	< 0.001	36	5.20	↓	17.02	↓
Ho	Low	1.27		−3.63		0.87	< 0.001	242	9.30		46.79	
	High	1.36	↑	−4.00	↓	0.64	< 0.001	916	7.28	↓	37.92	↓
Th[b]	Low	1.18		−1.59		0.69	< 0.001	65	7.19		11.67	
	High	1.23	↑	−1.61	↓	0.76	< 0.001	40	9.19	↑	15.42	↑
Tt[d]	Low	1.38		−2.38		0.57	< 0.001	72	4.87		10.99	
	High	1.09	↓	−1.15	↑	0.77	< 0.001	78	4.10	↓	6.57	↓
Tt[e]	Low	1.98		−2.89		0.68	< 0.001	126	11.70		28.59	
	High	3.23	↑	−6.41	↓	0.44	< 0.001	99	9.82	↓	28.23	↓
Tt[f]	Low	2.37		−5.13		0.47	< 0.001	144	11.29		25.95	
	High	2.68	↑	−5.15	↓	0.41	0.002	58	10.93	↓	23.57	↓
Zc[c]	Low	1.02		−1.50		0.10	0.033	127	10.18		18.37	
	High	1.72	↑	−3.10	↓	0.56	< 0.001	116	9.01	↓	18.08	↓
Zm[c]	Low	1.91		−2.61		0.69	< 0.001	356	17.30		38.43	
	High	2.02	↑	−1.98	↑	0.55	< 0.001	333	15.52	↓	25.10	↓
Zn[a]	Low	1.02		−1.89		0.65	< 0.001	283	8.18		15.70	
	High	2.23	↑	−6.64	↓	0.38	< 0.001	252	9.53	↑	28.39	↑

Table 4. Drivers of the slope responses to nutrient enrichment based on the biomass–density data point distribution of seagrass species presented in Fig. 3. See Table 1 for species name abbreviations

Species	Log D intercept	D intercept (shoots m^{-2})	Driver of slope response
Cn	3.41	2570	B/D ratio decrease at D < 2570 and increase at D > 2570
Ho	4.35	22387	B/D ratio decrease at D < 22387 and increase at D > 22387
Th[b]	out of range	out of range	B/D ratio increase with density
Tt[d]	out of range	out of range	B/D ratio increase with lowering density
Tt[e]	2.80	631	B/D ratio decrease at D < 631 and increase at D > 631
Tt[f]	out of range	out of range	B/D ratio increase with density
Zc[c]	2.28	191	B/D ratio decrease at D < 191 and increase at D > 191
Zm[c]	out of range	out of range	B/D ratio increase with density
Zn[a]	3.93	8511	B/D ratio decrease at D < 8511 and increase at D > 8511

by increasing their biomass (Müller, Schmid & Weiner 2000). These findings are also supported by the meta-analysis done by Hughes *et al.* (2004), which revealed that experimental additions of inorganic nutrients to sediments generally stimulated seagrass growth. On the other hand, the long-term negative responses of both biomass and density of seagrasses observed in descriptive studies probably result from ecosystem-scale events related to nutrient enrichment such as increased turbidity, algal blooms, epiphyte loads and anoxia (Ralph *et al.* 2006). The major role of these factors on seagrass decline is well known (Burkholder, Tomasko & Touchette 2007; Ralph *et al.* 2007; Fertig, Kennish & Sakowicz 2013).

Most seagrasses analysed here lie in the nonthinning part of the theoretical biomass–density curves. A simultaneous increase in biomass and decrease in density, evidence of self-thinning (Yoda *et al.* 1963; White 1981; Westoby 1984; Weller 1987), was only observed in 4 of 28 studies (Table 1). As well, the maximum biomass values across seagrass species, 2.67 at low nutrients and 2.51 log g DW m^{-2} at high, are well under the 'ultimate biomass–density line' (4.87 log g DW m^{-2}; Scrosati 2005), which describes the maximum biomass possible at any plant density and constrains all plant populations (Weller 1989; Scrosati 2005). This suggests that density-dependent mortality is not common in seagrasses, probably because of flexible clonal growth patterns or facilitative interactions associated with the species clonal integration (discussed below). Three of these cases were documented on *Thalassia testudinum* (Heck *et al.* 2000; Lee & Dunton 2000; B.I. van Tussenbroek, unpubl. data) and one in *Zostera capricorni* (R. Gruber, unpubl. data), which indicates that self-thinning may occur at least on these species. *T. testudinum* shows a unique regulation of shoot density involving shoots that become dormant at high densities (van Tussenbroek, Galindo & Marquez 2000). Increased dormancy was related to biomass increase (B.I. van Tussenbroek, pers. comm.) as expected in a self-thinning demographic process. Additionally, experimental mesocosm studies have shown a process of self-thinning in *Zostera marina* (Short, Burdick & Kaldy 1995). The species density decreased with reduced light

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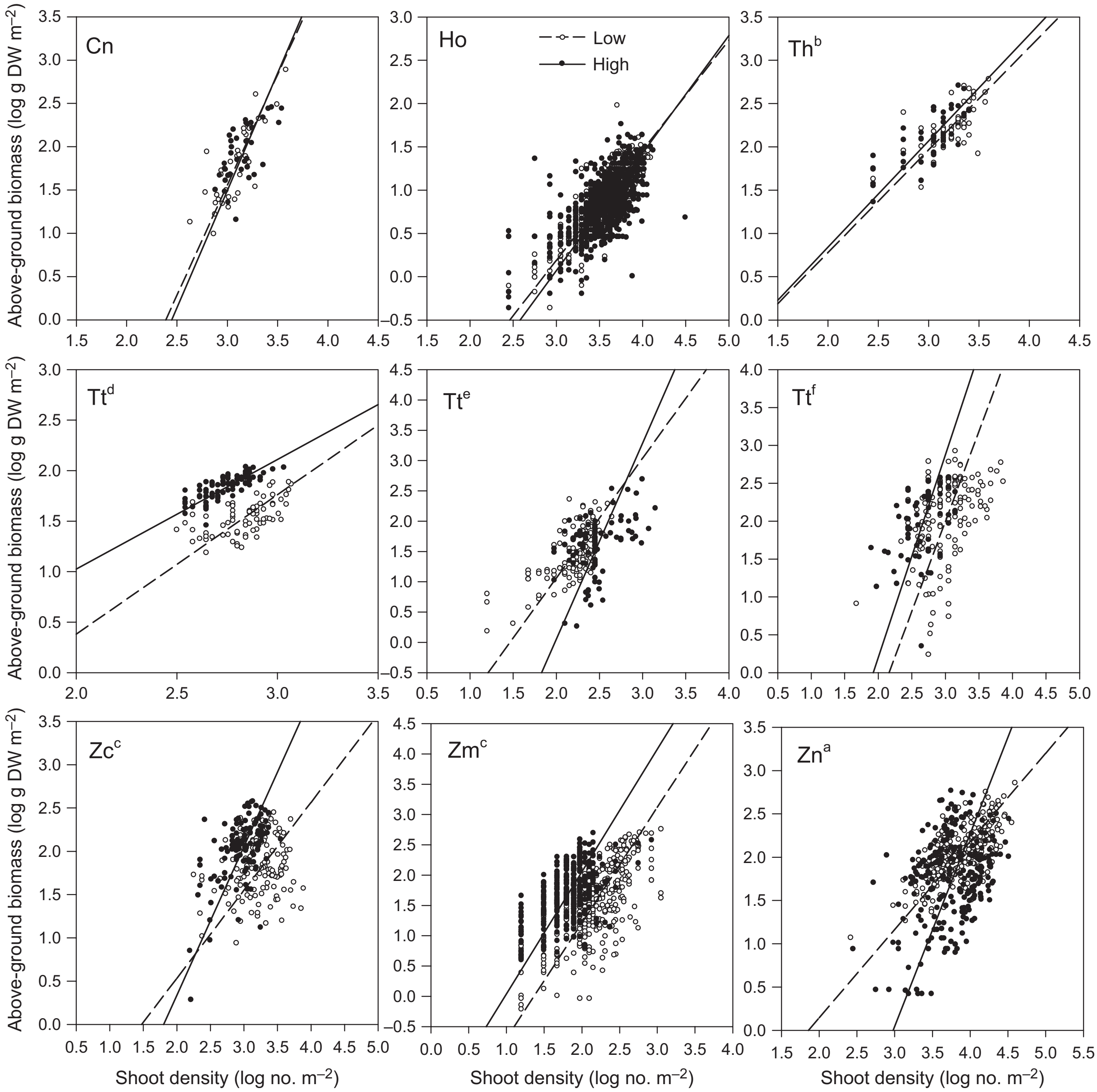

Fig. 3. Intraspecific, dynamic biomass–density relationships in seagrass species. See Table 1 for species name abbreviations.

conditions as expected under self-thinning. This response did not change under nutrient enrichment conditions.

The analysis of both the static and the dynamic biomass–density relationships of seagrasses revealed that the slopes of the biomass–density linear relationships increase under nutrient enrichment. In particular, increasing slopes and decreasing intercepts of the dynamic biomass–density relationship of single seagrass species are good integrative indicators of altered environmental condition of seagrass habitats related to nutrient loading. Higher slopes and lower intercepts under high nutrients were also observed elsewhere in *Z. noltii* (Cabaço, Machás & Santos 2007), macroalgae (Steen & Scrosati 2004) and terrestrial plants (Morris 2003; Chu *et al.* 2010). As biomass and density of seagrass populations are easily measurable and have been widely used in monitoring programs, their linear relationship in monospecific communities, particularly the slope and intercept, can be used as metrics that reveal alterations of the intraspecific competitive mechanisms resulting from nutrient disturbances, adding relevant inputs for the assessment of the ecological quality status of coastal and transitional waters.

The seagrass biomass and density variability can also provide relevant information on the species response to nutrient disturbance. In general, the variability of biomass and density decreased with nutrient enrichment, showing an opposite response to the general trend of ecological responses to disturbance-driven changes (e.g. Sousa 1984; Underwood 1992; Turner 2010). However, a decrease in variability may occur

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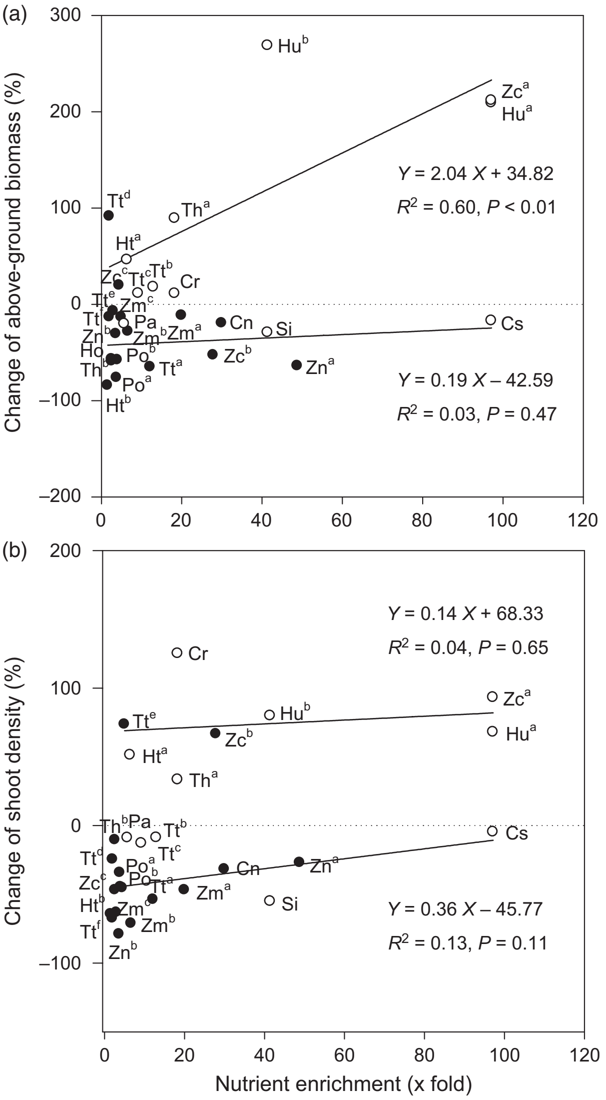

Fig. 4. Relationship between the nutrient enrichment level and the rate of change in seagrass above-ground biomass (a) and in seagrass shoot density (b) in descriptive (black circles) and experimental studies (open circles). R^2 and significance (P) are shown for both the increasing and decreasing biomass–density data sets. See Table 1 for species name abbreviations.

when populations are under chronic rather than discrete disturbances, such as sewage discharge or organic enrichment, due to the absence of response recovery time of continuous disturbance events (Fraterrigo & Rusak 2008).

Even though biomass–density metrics may be useful indicators to monitor seagrass meadows, the intrinsic population mechanisms that drive the biomass–density relationships and their response to nutrients must be understood. The analyses performed here revealed surprising conclusions that need to be tested. The first is that the B/D ratio increases with density in all seagrass species tested (because log B-log D slopes > 1), that is, that the average biomass of each individual increases with density. This is intriguing and probably is a consequence of facilitative processes related to clonal integration in seagrasses. Furthermore, our analysis suggests that nutrient enrichment further increases this effect as biomass–density slopes increased to even higher values. The redistribution of photosynthates through clonal integration to shoots receiving less light due to increasing densities leading to a more efficient production performance of shoots could be an explanation for this, but this hypothesis must be experimentally tested.

Table 5. Results of linear regression analysis between the rate of change of both shoot density and above-ground biomass and the seagrass size and growth characteristics. See Table 2 for size and growth abbreviations. anova F statistics and P-significance level are presented. n, number of studies included in the analysis

		Regression		ANOVA	
Change (%)	Size/growth	R^2	n	F	P
Shoot density	HE	0.047	28	1.277	0.269
	RD	0.056	28	1.536	0.226
	SW	0.058	28	1.604	0.217
	VE	0.022	19	0.386	0.543
	LL_{min}	0.040	25	0.952	0.339
	LL_{max}	0.024	25	0.558	0.463
Above biomass	HE	0.002	28	0.049	0.826
	RD	0.093	28	2.680	0.114
	SW	0.079	28	2.236	0.147
	VE	0.005	19	0.092	0.766
	LL_{min}	0.074	25	1.829	0.189
	LL_{max}	0.026	25	0.603	0.445

Facilitation among plants can affect the course of intraspecific competition to self-thinning under abiotic stress (Chu *et al.* 2010). Abiotic stress results in a steeper biomass–density relationship, as generally observed here for seagrasses, but this effect may be reduced by positive interactions among individuals (Chu *et al.* 2010), delaying the onset of density-dependent shoot mortality. Competitive interactions in clonal plants are not solely determined by the resource itself (Schwinning & Weiner 1998), as their clonal nature and implicit internal resources translocation may alleviate competition within populations (de Kroon 1993). This may explain why seagrasses, in general, do not always show self-thinning. In species with low clonal integration such as *Zostera noltii* (Marbà *et al.* 2002; Cabaço, Alexandre & Santos 2005), where lower facilitative interactions are expected, self-thinning is not observed probably because the high biomass per shoot at high nutrient levels is mediated through high growth and turnover rates (Peralta *et al.* 2005). The role of facilitation as a causal mechanism for the lack of self-thinning in seagrasses is worthy of being tested experimentally in the future.

To test the hypotheses emerging from our analysis of the main drivers behind the increasing biomass–density slopes of seagrasses under nutrient enrichment will be challenging. In four of the nine cases assessed, the biomass of individual shoots increased progressively with increasing densities fitting the facilitation hypothesis, for example, that the redistribution of photosynthates through clonal integration to shoots receiving less light leads to a more efficient production performance of shoots. In three other cases, there was a progressive

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reduction in shoot biomass at lower densities. This suggests that the potential deleterious effects of excessive nutrient conditions are more effective under lower densities, that is, that facilitation may reduce the negative impacts of excessive nutrients. The combination of both of these processes may explain the observations in *Z. noltii*. This was the only species where the density threshold was well in the middle of the density distribution range and consequently where the biomass of each shoot decreased with density at densities lower than 8500 shoots m^{-2}, whereas it increased above that threshold. Interestingly, in *T. testidinum* (Tt^d, from Puerto Morelos, Mexico), the only case where the biomass–density slope decreased and that also showed self-thinning, the biomass of shoots increased more at lower densities.

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