Microbial Source Tracking in Lee County Waterways

Final Report

Brian E. Lapointe, Kevin N. Tyre, Rachel A. Brewton, and Diana Baladi

March 30, 2022

Harbor Branch Oceanographic Institute

at Florida Atlantic University

5600 US 1 N.

Fort Pierce, FL 34946

Executive Summary

Water quality degradation due to widespread urbanization is a global issue. Coastal areas in Florida have experienced an unprecedented amount of population growth over the last several decades concurrent with declines in water quality and increases in harmful algal blooms (HABs). Since 2010, the population of Lee County has increased by 29% and continues to grow at an annual rate of 2%. Coincident with this growth, signs of degrading water quality have been increasingly observed. High concentrations of fecal indicator bacteria have raised concerns over the safety of engaging in water-based activities, leading to the closure of affected waterbodies for recreational use. Further, increasingly high nutrient concentrations (nitrogen and phosphorus) and low dissolved oxygen levels have led to the classification of the Caloosahatchee River Estuary as an impaired waterbody by the Florida Department of Environmental Protection (FDEP).

To assess sources contributing to these water quality issues, a microbial source tracking (MST) study was conducted between 2017 and 2020 in North Fort Myers through a partnership between Harbor Branch Oceanographic Institute at Florida Atlantic University, Lee County Department of Natural Resources, and FDEP. This study concluded that human waste contamination from septic systems was adversely impacting water quality in North Fort Myers, which prompted the Lee Board of County Commissioners to request more information regarding the extent of similar impacts throughout the county. Therefore, an MST study with a larger geographic scale was designed for the purpose of assessing sources contributing to the recurring nutrient and bacterial pollution observed in surface waters throughout Lee County. This study involved the identification of 24 key locations with impaired water quality where contamination by septic system effluent was feasible and one reference site in a sewered area. Surface water samples were then collected during eight sampling events from January 2020 through January 2021 and analyzed for fecal indicator bacteria, nutrients, the molecular human waste marker HF183, chemical tracers of human waste, and stable nitrogen and oxygen isotopes. These data were compared between seasons (wet vs. dry) and against the reference site to better understand the prevalence of human waste contamination within Lee County surface waters. A detailed analysis by site is provided in the Appendix of this report.

Evidence of human waste contamination was observed at every site, although to varying degrees of severity. High median concentrations of fecal indicator bacteria, exceeding FDEP water quality standard threshold values, were present at 68% of all sites. Additionally, the human specific HF183 molecular marker was detected at least once at 88% of all sites and positively correlated with fecal indicator bacteria, indicating a potential human waste source. High median concentrations of HF183 were detected at San Carlos Park (1600 copies/100mL) and Heritage Farms (694 copies/100mL), demonstrating persistent human waste contamination at these locations. High HF183 concentrations were observed sporadically at other sites as well. For example, the highest single sample HF183 concentration was observed at Tidewater Island 02 (23,700 copies/100mL), but 75% of all samples collected from this site contained HF183 at concentrations that were below the detection limits (<3 copies/100mL). These data suggests that, while human waste may not be a persistent problem at some sites, periodic loadings may still present a threat to surface water quality and warrant continued monitoring.

The presence of human waste throughout Lee County was further demonstrated by the detection of sucralose in 99% and pharmaceuticals in 44% of all samples. High median concentrations of sucralose, exceeding 380 ng/L, were found at 60% of sites, including the Reference site. The highest median concentrations of sucralose were observed at Heritage Farms (3,750 ng/L) and Overlook Drive (3,400 ng/L), demonstrating persistent human waste contamination at these locations. High median concentrations of ammonium were also observed at 52% of sites and were positively correlated with HF183 (Spearman r = 0.24, p = 0.001), suggesting that a statistically significant fraction of these nutrients originate from a human waste source. Interestingly, few seasonal differences were observed suggesting that sources of contamination are present year-round. Potential human waste sources may include septic systems, aging sewer infrastructure, reclaimed water application, or upstream inputs from wastewater treatment plant effluent. A detailed site analysis is provided as an appendix to this report which examines the evidence to identify potential human waste sources at each site.

Prior research in North Fort Myers demonstrated that ammonium concentrations in groundwater exceeded nitrate by a factor of 6.5 in some residential areas serviced by septic systems, indicating significant nutrient pollution from the leachate. Five sites in the present study were found to have ammonium enriched surface water with median ammonium to nitrate ratios exceeding 2, indicating incomplete ammonium oxidation that may result from rapid transport of ammonium rich septic system effluent to surface water. The sites with the highest median ammonium to nitrate ratios were Summerwood Drive (74.3) and San Carlos Park (15.0). Median nitrogen isotope values >7‰ in dissolved nitrate ($\delta^{15}N\text{-}NO_3^-$) implicate human waste influence at Fort Myers Shores, Mobile Manor, and Page Park. Median nitrogen isotope ($\delta^{15}N$) values >4.9‰ in particulate organic matter (POM) and macrophytes implicate human waste influence at Deep Lagoon, Fort Myers Shores, Heritage Farms, Lake McGregor, Laurelin Court, Ligon Court, Mobile Manor, North Town & River, Orange River, Overlook Drive, Page Park, San Carlos Park, Summerwood Drive, Waterway Estates 02, Yacht Club, and the Reference site.

Though the study provides evidence showing contamination of Lee County surface waters with human waste, there were some important differences between locations. Some sites demonstrated human waste influence that could readily be attributed to septic systems due to a lack of other sources (e.g., Fort Myers Shores and Mobile Manor), whereas in other locations primary contaminant sources were not as easily identified (e.g., Heritage Farms, Lake McGregor, Overlook Drive) and further investigation could yield better understanding. In some of these locations the nearby application of reclaimed water makes it challenging to identify the source of the human waste indicators. A fine scale source tracking effort could provide more information. Additionally, sites along the Caloosahatchee River Estuary (e.g., Reference site, Deep Lagoon, North Town & River, Yacht Club) tended to exhibit lower concentrations of nutrients and bacteria, yet human waste indicators were regularly observed, and sucralose was found in moderate to high concentrations. These results may indicate an upstream source in the Caloosahatchee River Estuary. Additional sampling may be useful to determine if the contaminant sources are localized or exogenous.

This study provides compelling evidence of human waste pollution in Lee County surface waters. Wastewater infrastructure upgrades and septic to sewer conversion programs in highly impacted areas could help reduce pollutant loading to nearby surface waters. These changes should yield improvements in water quality and help minimize the occurrence and intensity of HABs in downstream waterbodies. Lee County has been proactive in the development of a Countywide Wastewater Management Plan and this report may aid resource managers in making decisions for improved wastewater infrastructure. As local populations continue to grow, effective use of these data and continued water quality research will be essential for the health of the community and a sustainable economic future.

Summary Table. Median and overall average values for key water quality parameters for each site in the Lee County Microbial Source Tracking Study. The relative concentrations of each analyte are indicated by red shading, with low concentrations in white and high concentrations in red. High and low threshold values were derived from FDEP water quality standards and statewide surface water quality values for rivers and streams as described in the Methods. BDL = Below Detection Limits. No Data = samples unable to be analyzed.

Site Name	Enterococci	*E. coli*	BOD	HF183	Sucralose	Carbama-zepine	Acetami-nophen	Ibuprofen	Naproxen	NH_4^+	NO_x	SRP	TN	TP	$\delta^{15}N\text{-}NO_3^-$
Reference	20	42	0.9	BDL	470	BDL	BDL	BDL	BDL	0.029	0.066	0.076	1.20	0.11	4.5
Billy Creek	2750	2330	1.1	106	140	BDL	BDL	BDL	BDL	0.193	0.163	0.126	1.25	0.21	2.9
Briarcliff	26	15	1.1	BDL	460	BDL	BDL	BDL	BDL	0.056	0.088	BDL	0.89	0.03	4.4
Daughtry Creek	244	317	0.8	BDL	280	BDL	BDL	BDL	BDL	0.053	0.081	0.119	0.90	0.19	6.1
Deep Lagoon	12	24	1.2	BDL	515	BDL	BDL	BDL	BDL	0.042	0.031	0.055	0.97	0.08	4.0
Fort Myers Shores	659	588	1.0	60	215	BDL	BDL	BDL	BDL	0.031	0.173	0.050	1.02	0.08	11.3
Hendry Creek	238	346	1.2	84	505	0.8	BDL	BDL	BDL	0.092	0.068	0.022	0.89	0.06	4.2
Heritage Farms	707	110	1.2	694	3750	3.4	BDL	BDL	BDL	0.336	0.192	0.020	1.55	0.06	2.1
Lake McGregor	2077	398	0.8	128	130	BDL	BDL	BDL	BDL	0.123	0.032	0.040	0.80	0.08	4.7
Laurelin Court	263	154	0.8	BDL	305	BDL	BDL	BDL	BDL	0.055	0.160	0.065	1.20	0.15	3.9
Ligon Court	3630	391	1.1	BDL	2300	4.5	BDL	BDL	BDL	0.427	0.074	0.027	2.80	0.15	No Data
Mobile Manor	1173	894	0.7	64	1200	4.0	BDL	BDL	BDL	0.041	0.149	0.099	0.82	0.19	8.9
North Town & River	27	11	1.0	BDL	420	BDL	BDL	BDL	BDL	0.025	0.043	0.063	1.02	0.10	5.3
Orange River	898	389	0.8	122	245	BDL	BDL	BDL	BDL	0.041	0.212	0.103	1.20	0.14	4.0
Overlook Drive	2203	255	2.1	68	3400	4.0	BDL	BDL	BDL	0.356	0.097	0.035	2.20	0.17	0.6
Page Park	528	841	0.7	BDL	610	12.0	BDL	BDL	BDL	0.076	0.070	0.008	0.62	0.03	9.7
Pine Island	15	70	0.9	34	130	BDL	BDL	BDL	BDL	0.039	0.016	0.017	0.91	0.05	1.0
Pine Island 02	BDL	BDL	1.3	BDL	160	BDL	BDL	BDL	BDL	0.079	0.012	0.030	0.93	0.07	0.2
San Carlos Park	614	256	0.7	1600	525	0.9	BDL	BDL	BDL	0.239	0.069	0.010	0.71	0.04	2.2
Summerwood Drive	733	193	1.1	45	890	BDL	BDL	BDL	BDL	0.392	BDL	0.085	1.70	0.17	No Data
Tidewater Island 01	77	97	0.8	BDL	640	1.2	BDL	BDL	BDL	0.068	0.050	0.008	0.77	0.03	4.2
Tidewater Island 02	50	450	0.7	BDL	685	1.1	BDL	BDL	BDL	0.058	0.057	0.007	0.77	0.03	4.6
Waterway Estates 01	366	137	1.2	41	405	0.8	BDL	BDL	BDL	0.109	0.114	0.122	1.30	0.15	4.8
Waterway Estates 02	582	270	1.0	15	375	BDL	BDL	BDL	BDL	0.085	0.068	0.100	1.20	0.14	4.4
Yacht Club	32	44	1.2	BDL	200	BDL	BDL	BDL	BDL	0.036	0.102	0.066	1.10	0.12	4.4
Overall Average	**789**	**487**	**1.1**	**363**	**726**	**1.4**	**BDL**	**BDL**	**BDL**	**0.130**	**0.110**	**0.061**	**1.11**	**0.11**	**4.6**
Low	< 22	< 97	< 1	< 94	< 23	< 0.4	< 8	< 20	< 8	< 0.012	< 0.021	< 0.014	< 0.69	< 0.031	< 2
High	> 130	> 410	>2	>525	> 380	> 1.0	> 8	> 20	> 8	> 0.061	> 0.21	> 0.081	> 1.54	> 0.120	> 7
Units	MPN/100mL	MPN/100mL	mg/L	copies/100mL	ng/L	ng/L	ng/L	ng/L	ng/L	mg/L	mg/L	mg/L	mg/L	mg/L	‰

Low Moderate High

Table of Contents

List of Tables

List of Figures

List of Acronyms

BOD	Biochemical oxygen demand
DIN	Dissolved inorganic nitrogen
DO	Dissolved oxygen
FAU	Florida Atlantic University
FIB	Fecal indicator bacteria
FDEP	Florida Department of Environmental Protection
HAB	Harmful algal bloom
HBOI	Harbor Branch Oceanographic Institute
LCEL	Lee County Environmental Laboratory
MDL	Method detection limit
MST	Microbial Source Tracking
N	Nitrogen
NH_4^+	Ammonium
NO_x	Nitrate + nitrite
P	Phosphorus
POM	Particulate organic matter
SRP	Soluble reactive phosphorus
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TP	Total phosphorus

1. Introduction

Sustainable coastal population growth and development continues to be a global challenge as nearly 40% of the world's population lives within 100 km of a coast (Stauber et al., 2016). Humans have significantly contributed to increased concentration of pollutants flowing into surface and groundwater, resulting in the worldwide degradation of water quality in rivers, estuaries, and coastal oceans (Meybeck, 2004). These pollutants, including nutrients, pathogens, and chemical contaminants, present a global environmental and human health concern (Wade et al., 2003; Kaown et al., 2012; Buxton et al., 2016; EPA, 2017). Excess loading of nitrogen (N) and phosphorus (P) accelerates eutrophication, promotes harmful algal blooms (HABs), and depletes dissolved oxygen (DO) in urbanized watersheds, resulting in fish kills, habitat loss, and overall water quality degradation (Howarth et al., 2000; NRC, 2000; Heisler et al., 2008).

Human enteric pathogens may be found in untreated domestic wastewater and can cause serious illness (Gerardi & Zimmerman, 2004; Gerardi, 2006; Wen et al., 2009; Al-Gheethi et al., 2018). The abundance of fecal indicator bacteria (FIB), such as *Escherichia coli* and enterococci, is commonly used to gauge the potential risk to human health from fecal pathogens, as these bacteria are present in high concentrations in the feces of mammals (Sadowsky & Whitman, 2011; Byappanahalli et al., 2012). Gastrointestinal diseases and infections in swimmers are linked with elevated FIB concentrations in surface waters (Haile et al., 1999; Wade et al., 2003; Zmirou et al., 2003). Fecal pathogens from wastewater are responsible for a variety of waterborne diseases including cholera, hepatitis, shigellosis, encephalitis, and others (Yates, 1985; Ferguson et al., 2003). Over 60% of the United States population engages in water sports like swimming and boating (DeFlorio-Barker et al., 2018), so waterborne illnesses caused by pathogenic microorganisms represent a substantial human health and economic risk.

Onsite sewage treatment and disposal systems, commonly called "septic systems," are a significant source of nutrient and pathogenic pollution in surface waters via subsurface transport through contaminated groundwater (Griffin et al., 1999; Lipp et al., 2001; Drake & Bauder, 2005; Hossain et al., 2010). In the United States, 20% of homes (26.1 million) currently rely on septic systems to treat and dispose of human waste (EPA, 2008). The U.S. Bureau of the Census reports that at least 10% of septic systems are dysfunctional and state agencies report that these systems are the third most common source of groundwater contamination (EPA, 2000b). Florida accounts for 12% of the United States' septic systems (2.6 million; FDOH, 2020) and statewide, septic systems discharge more than 426 million gallons of effluent per day into the subsurface soil environment (Meeroff et al., 2008).

Florida's coastal areas are especially vulnerable to pollution from septic tanks because of shallow water tables and porous soils that allow for rapid transport of contaminants to groundwater and adjacent surface waters (Lapointe et al., 1990; Corbett et al., 2002; Meeroff et al., 2008). Improperly located septic systems may contaminate groundwaters and surface waters even when functioning properly, as they are not designed to remove nutrients and pathogens from the effluent (Mallin, 2013). A septic system that is installed in an area with a high water table decreases the efficiency of effluent treatment by reducing the unsaturated soil depth through which the effluent moves before mixing with groundwater (Bicki and Brown, 1990). Florida State code requires a minimum of 6" of cover on top of a septic system drainfield, a ~1' drainfield depth (may be less in some soil types), and 2' of separation from the bottom of the drainfield to the seasonal high water table (FAC Rule 64E-6). Therefore, at least 3.5' of separation is needed from the ground surface to the water table to meet the minimum requirements for adequate treatment of septic system effluent. Similarly, the US EPA recommends between 2 to 5' of separation between the drainfield and groundwater for adequate treatment of septic system effluent, depending on soil conditions and other factors (EPA, 2002). In some coastal systems, up to 80% of septic systems do not meet the minimum separation requirements with variation throughout the year and 30% of those systems are never in compliance (Cox et al., 2020).

In Florida nearly 40% of the septic systems are found in susceptible coastal areas with high water tables (Toor et al., 2011). Accordingly, prior studies have found evidence of improperly sited septic systems leading to groundwater and surface water contamination in various watersheds throughout the state (Lapointe et al., 1990; Anderson, 1998; Arnade, 1999; Lipp et al., 2001; Lapointe & Bedford, 2007; Lapointe et al., 2015; Lapointe et al., 2016; Lapointe et al., 2017; Lapointe et al., 2021). For example, septic systems in Jupiter (Lapointe & Krupa, 1995) and Port St. Lucie (Lapointe et al., 2020) were found to often not meet the minimum separation needed for septic system effluent treatment due to high water tables during the wet season. In North Fort Myers this issue persisted throughout the year with ~87% of depth to groundwater measurements made from October 2017 to April 2020 not meeting the minimum FAC requirements (Lapointe et al., 2021). Similarly, surface water contamination issues relating to high water tables have been documented in other locations, such as coastal North Carolina (Mallin, 2013), Cape Cod, MA (Swartz et al., 2006), Long Island, NY (Gobler & Sañudo-Wilhelmy, 2001), upstate New York (Chen, 1988), Rhode Island (Cox et al., 2020), and other locations, demonstrating that this issue is widespread in urbanized waterfront areas. Furthermore, rising groundwater tables and increased precipitation due to climate change are expected to increase the threat of pathogen and nutrient pollution from coastal septic systems (Cox et al., 2019).

With a population of 770,577 (as of 2019; US Census), Lee County is the 8th largest county in Florida and the estimated number of septic systems within the county exceeds 100,000 units (FDOH, 2018). A four-year study in North Fort Myers demonstrated that improperly located and aging septic systems in waterfront developments negatively impacted water quality (Lapointe et al., 2021). High water tables and porous sandy soils in this area facilitate rapid percolation of septic system effluent to the groundwater (Wedderburn et al., 1982; EPA, 2000a). The Caloosahatchee River and Estuary, which flows east to west from Lake Okeechobee to the Gulf of Mexico and bisects the county, is a major discharge area for contaminated groundwater and surface water, especially during the wet season (Wedderburn et al., 1982). High dissolved nutrient concentrations are characteristic of the Caloosahatchee River Estuary, contributing to increased HABs (Lapointe & Bedford, 2007) and decreased DO levels (Liu et al., 2009). Additionally, elevated concentrations of FIB that do not meet the FDOH safety criteria are often observed in the Caloosahatchee River Estuary, indicating a potential risk to human health from fecal pathogens (Braun, 2015, 2016).

While water quality in Lee County has degraded over the last several decades (W. Dexter Bender and Associates, 1995; Bailey et al., 2009; Liu et al., 2009), the occurrence of HABs has increased. Blooms of the dinoflagellate *Karenia brevis* are often reported annually off the Lee County coast. Commonly referred to as "red tides," *K. brevis* blooms can contain high concentrations of brevetoxins, a type of neurotoxin that causes fish kills, shellfish contamination, and human respiratory ailments when aerosolized (Lee et al., 1989). In the last 30 years, these blooms have become increasingly common, especially in nearshore environments (Brand & Compton, 2007; Yentsch et al., 2008) where they are fueled by terrestrial nutrient sources (Medina et al., 2020). Additionally, red drift macroalgae blooms have occurred along beaches and coastal waters of Lee County (Lapointe & Bedford, 2007) and blooms of the blue-green algae *Microcystis aeruginosa* have occurred in the Caloosahatchee River, Estuary, and residential canals (Lapointe et al., 2006; Urakawa et al., 2020; Lapointe et al., 2021). These HABs can negatively impact local economies through the mortality or closure of commercial seafood fisheries and by inhibiting ecotourism activities (Anderson, 1998). Therefore, it is important to minimize excess nutrients flowing into these coastal ecosystems to mitigate HABs.

To address these human and environmental health concerns, Lee County Department of Natural Resources partnered with Harbor Branch Oceanographic Institute at Florida Atlantic University (HBOI-FAU) and Florida Department of Environmental Protection (FDEP) to conduct a microbial source tracking (MST) study to determine the source(s) of nutrient and fecal bacterial pollution in Lee County surface waters. Source identification of nutrient and fecal pollution is necessary to determine human health and environmental risks for the development of mitigation strategies. Because FIB are found in a variety of animals, they provide no reliable indication regarding the source of fecal pollution where many potential non-point sources of fecal contaminants exist (Hagedorn et al., 2011; Tran et al., 2015). Therefore, more sophisticated tools must be employed to determine the source. MST methods utilize source-specific gene fragments, synthetic chemical compounds, and stable isotope values to identify sources of pollution (Bernhard & Field, 2000; McQuaig et al., 2006; Albertin et al., 2012).

Molecular markers are valuable tools for determining fecal pollution sources. The HF183 gene fragment is found within the 16s rRNA gene of the *Bacteroides* sp. that reside exclusively in the human colon as part of the normal gut microbiota. These bacteria are expelled in excrement and because they are obligate anaerobes, they are unable to survive outside of their human hosts. Therefore, detection of the HF183 molecular marker in oxygenated groundwater and surface waters serves as a reliable indicator of human fecal pollution (Ahmed et al., 2008; Sauer et al., 2011; Ahmed et al., 2019). Some of the limitations of this method include relatively short half-life of HF183 in the environment (from 0.4 up to 8 days; Seurinck et al., 2005; Walters & Field, 2009). Thus, due to long holding times, the abundance of HF183 in septic system and wastewater treatment plant effluent may be less than in fresh, raw wastewater (Boehm et al., 2013). Additionally, humic acid (which can be prevalent in the Caloosahatchee River and its tributaries) is detrimental to HF183 qPCR analysis (Green & Field, 2012). Therefore, it is useful to supplement HF183 analyses with other MST methods.

Several chemical compounds are suitable tracers of human waste pollution in surface water. Widespread human consumption of the artificial sweetener sucralose in conjunction with its resistance to degradation and frequent detection in wastewater makes it a reliable indicator of human waste pollution (Scheurer et al., 2009; Wolf et al., 2012; White et al., 2019; Yuan et al., 2020). In Florida, multiple studies have successfully used sucralose to identify areas where septic systems are leaching into surface water (Stoner et al., 2016; Lapointe et al., 2016; Lapointe et al., 2017; Silvanima et al., 2018; Lapointe, et al., 2020; Herren et al., 2021). In Singapore, raw wastewater and surface water samples were analyzed to determine presence of pharmaceutical contaminants in a highly urbanized area and rural forested area. Acetaminophen and carbamazepine were not detected in any of the rural samples but were detected in 90% of the sewered area samples and all the raw wastewater samples (Tran et al., 2014). This suggests that these chemical compounds can also be used as indicators of human waste pollution. Ibuprofen is also useful for source tracking human waste contamination because it is mostly removed during wastewater treatment (>85% removal; Mhuka et al., 2020) but only slightly removed by microbes in saturated soils (18% removal; Carr et al., 2011). Therefore, the detection of ibuprofen in the environment is indicative of untreated human waste. By contrast, naproxen is resistant to degradation and removal by traditional wastewater treatment, and may be found in both raw human waste or wastewater treatment plant effluent (Kanakaraju et al., 2015; Xu et al., 2019; Mhuka et al., 2020; Wojcieszyńska & Guzik, 2020).

Stable isotope values are an effective tool for source identification of reactive N species in urbanized watersheds (Luu et al., 2020). Dual stable isotope analysis of nitrate ($\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-) in water samples can discriminate between different pollution sources, such as fertilizers and human waste (Heaton, 1986; Aravena et al., 1993; Olsen et al., 2010; Li et al., 2016). Further, analysis of stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$) in macrophyte tissue can be used to discriminate between natural and anthropogenic nutrient sources (Risk et al., 2009; Lapointe et al., 2015; Lapointe et al., 2017). Enriched $\delta^{15}N$ values of +3‰ to +19‰ are indicative of a human waste N source (Heaton, 1986; Costanzo et al., 2001), whereas N from synthetic fertilizers ranges from -2‰ to +2‰ (Bateman & Kelly, 2007), atmospheric N ranges from -3‰ to +1‰ (Paerl & Fogel, 1994), and natural N source values are close to 0‰ (Heaton, 1986; France et al., 1998).

This MST study combines the use of molecular marker, chemical indicators, and stable isotopes to identify sources of nutrient and bacterial pollution in Lee County surface waters. The goal of this research was to better understand the drivers contributing to degraded surface water quality throughout Lee County. The results of this study will be useful for Lee County resource managers and elected officials as they strive to maintain and improve surface water quality and mitigate worsening HABs.

2. Methods

2.1 Sampling sites

Twenty-four sites within Lee County were selected for water quality sampling. One site that was in an area serviced by centralized sewer served as a Reference site (site 24) and the remaining 23 sites were in residential areas serviced by septic systems (Figure 1). Site selection involved extensive preliminary assessment of the study area, including surveying areas with documented water quality issues and identifying available sampling locations with surface water access. Sixteen sites were accessible by land, while eight sites in the Caloosahatchee River Estuary were sampled by boat. Detailed site descriptions are provided as an Appendix.

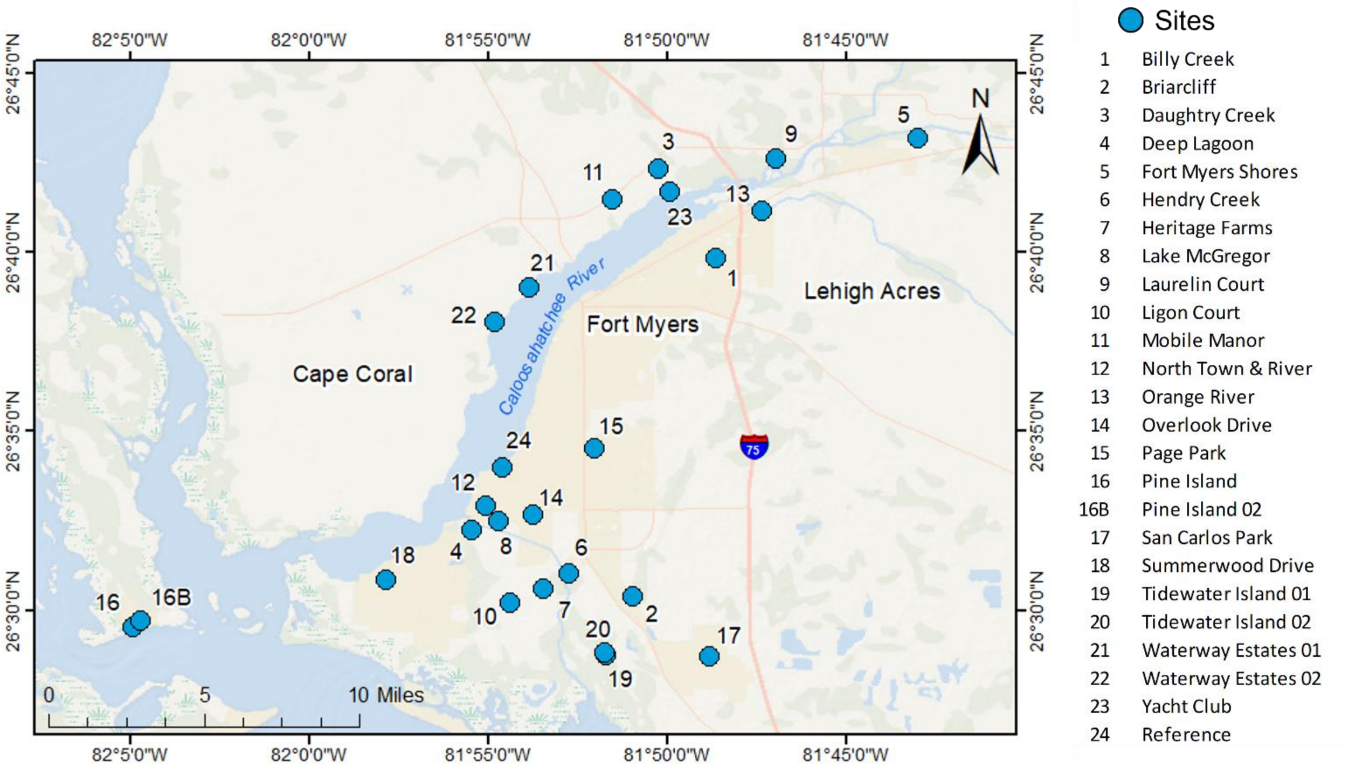

Figure 1. Surface water sites within Lee County, FL, sampled during the 2020-2021 Microbial Source Tracking study.

2.2 Sample collection and handling

Surface water samples were collected at 24 sites during eight sampling events from January 2020 to January 2021 and were evenly divided between wet and dry seasons. The sampling schedule was interrupted due to the coronavirus pandemic, resulting in no sampling events occurring from March through June 2020. All sampling events occurred over three days with approximately eight sites sampled per day. One sampling event was split between October and November 2020 due to staff illness. For this event, 8 of 24 sites were sampled in October and the remaining 16 sites were sampled in November.

Sampling was conducted following FDEP standard operating procedure (SOP) FS 2100 for surface water. At each site, temperature (°C), salinity (ppt), conductivity (µS/cm), pH, and DO (% saturation, mg/L) were measured with a calibrated YSI ProPlus or YSI Pro1030. Water samples were collected by hand or using a pole sampler into clean high-density polyethylene (HDPE) bottles for analysis of biochemical oxygen demand (BOD), HF183, enterococci, *E. coli*, ammonium (NH_4^+), nitrate + nitrite (NO_x), total Kjeldahl nitrogen (TKN), total nitrogen (TN), total phosphorus (TP), color, and soluble reactive phosphorus (SRP). Upon collection, enterococci and *E. coli* samples were preserved with sodium thiosulfate; NH_4^+, NO_x, TKN, TN, and TP samples were acidified to a pH of <2 with sulfuric acid; and color and SRP samples were field-filtered with 0.45µm filter. Water samples were also collected into amber glass bottles for analysis of sucralose, acetaminophen, carbamazepine, ibuprofen, and naproxen. For analysis of %C, %N, %P, $\delta^{13}C$, and $\delta^{15}N$, particulate organic matter (POM) samples were coarse filtered through a 200 µm sock filter to remove macrodetritus per Savoye et al. (2003) and collected into HDPE bottles. When present, macroalgae and/or macrophytes were collected and stored in plastic bags for analysis of %C, %N, %P, $\delta^{13}C$, and $\delta^{15}N$. For determination of dissolved $\delta^{15}N$ and $\delta^{18}O$, additional water samples were field-filtered (0.45µm) into amber glass vials containing 18% (v/v) aqueous hydrochloric acid solution to lower the pH to < 3. Samples for dissolved $\delta^{15}N$ and $\delta^{18}O$ were not collected in January or February 2020. All water samples were immediately submerged in ice in a dark cooler, while macrophytes and algae were placed on a towel or in a bin above the ice to prevent cell rupture due to freezing. Samples were transported to Lee County Environmental Laboratory (LCEL) in Fort Myers, Florida or processed at the HBOI-FAU temporary field laboratory within required holding times for analysis.

2.3 Sample analysis

Water samples for BOD, enterococci, *E. coli*, NH_4^+, NO_x, SRP, TKN, TN, TP, and color were hand-delivered within five hours to LCEL. In brief, the following National Environmental Laboratory Accreditation Conference (NELAC) approved standard methods were used for analyses: 5-Day BOD Test (Standard Method 5210 B), 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, EPA Method 351.2 for TKN, and Standard Method 2120 C for color. Method detection limits (MDLs) were 0.3 mg/L for BOD, 2 MPN/100mL for enterococci and *E. coli*, 0.014 mg/L for NH_4^+, 0.01 mg/L for NO_x, 0.004 mg/L for SRP, 0.05 mg/L for TKN, and 0.006 mg/L for TP. TN was calculated by LCEL as TKN + NO_x.

HF183 samples were shipped overnight to Source Molecular Corporation (SMC), Miami Lakes, FL. At SMC, samples were prepared by filtering through a 0.45µm membrane filter(s) and placing each filter in separate, sterile 2 ml disposable tubes containing a mix of beads and lysis buffer. Each sample was homogenized for 1 min before extracting DNA using the Generite DNA-EZ ST1 extraction kit (GeneRite, NJ), as per manufacturer's protocol. Extracted DNA was then amplified and analyzed with a Bio-Rad QX200 Droplet Digital PCR System (Cao et al., 2015). Absolute quantification of HF183 was achieved by software Poisson Distribution Analysis (Sivaganesan et al., 2011). The MDL for HF183 was 3 copies/100 mL.

At HBOI-FAU, water samples for dissolved $\delta^{15}N$ and $\delta^{18}O$ were stored at 4°C until NO_x data for the samples was received from LCEL. After which, samples with nitrate concentrations > 0.04 mg/L were shipped to Beta Analytic Testing Laboratory (BATL), Miami, FL. At BATL, samples were analyzed by the chemical reduction of nitrate to nitrous oxide followed by continuous flow (CF) Isotope Ratio Mass Spectrometry (IRMS) (Altabet et al., 2019; Casciotti et al., 2002; Foreman et al., 2016).

Sucralose, acetaminophen, carbamazepine, ibuprofen, and naproxen samples were shipped overnight to the FDEP laboratory in Tallahassee, FL. At the FDEP lab, samples were analyzed by high performance liquid chromatography (HPLC), coupled with both thermospray-mass spectrometry (TS-MS) and an ultraviolet (UV) detector (EPA method 8321B). MDLs were 100 ng/L for sucralose, 8 ng/L for acetaminophen, 0.8 ng/L for carbamazepine, 20 ng/L for ibuprofen, and 8 ng/L for naproxen.

POM samples were vacuum filtered onto a pre-combusted 0.45 µm glass fiber filter within 12 h of collection and dried in an oven at ~65°C. The dried filters were folded and cut in half. One half was shipped to the University of Georgia's Stable Isotope Ecology Laboratory (UGA-SIEL) where they were analyzed for %C, %N, $\delta^{13}C$, and $\delta^{15}N$ 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 shipped to the University of Missouri's Soil and Plant Testing Laboratory (UM-SPTL) for analysis of %P by Inductively Coupled Plasma Atomic Emission Spectroscopy (Viso & Zachariadis, 2018). The resulting stable isotope and C:N:P data were used to identify nutrient sources and characterize temporal and spatial variation in phytoplankton nutrient status, which allow for inferences regarding nutrient availability in relation to various natural and anthropogenic nutrient sources and climate-related phenomena. Results were compared to the Redfield ratio of 106:16:1 (Redfield, 1958) where C:N ratios >6.6 indicate increasing N-limitation.

Macroalgal and macrophyte tissue were held in a cooler until return to HBOI-FAU where they were rinsed briefly in DI water, cleaned of extraneous materials, and dried at 65°C. The dried tissue was finely ground with mortar and pestle and shipped to the UGA-SIEL and UM-SPTL, where they were analyzed similarly to the POM samples as described above.

2.4 Precipitation

Precipitation data for Lee County from December 1, 2019 to January 31, 2021 was obtained from the National Oceanic and Atmospheric Administration National Centers for Environmental Information (https://www.ncdc.noaa.gov/data-access). Fourteen weather stations within Lee County were selected and mean daily precipitation across all stations was calculated. These stations included US1FLLE0006, US1FLLE0010, US1FLLE0022, US1FLLE0029, US1FLLE0037, US1FLLE0055, US1FLLE0057, US1FLLE0058, US1FLLE0059, US1FLLE0062, US1FLLE0065, US1FLLE0068, USW00012835, and USW00012894.

2.5 Data analysis

For nutrient and bacterial parameters, half of the MDL was substituted for any results flagged as below detection limits. For chemical tracers, zeros were substituted for any results flagged as below detection limits. This is because the chemical tracers measured in this study are not naturally occurring (unlike nutrients and bacteria) and therefore a concentration of zero is feasible. Molar ratios (DIN:SRP, TDN:TDP) of dissolved nutrients were calculated using molar concentrations (μM). NH_4^+:NO_X was calculated and sites with low values (< 0.5) were considered to have high or near-complete ammonium oxidation, whereas sites with high values (> 2) were considered to have low ammonium oxidation.

Water quality parameter classifications of *low*, *moderate*, and *high* were established to provide context for observed values. These classifications were defined based on numerical FDEP water quality standards, statewide data, and the Redfield ratio (Redfield, 1958). For enterococci, *E. coli*, TN, and TP, the *high* concentration thresholds were defined to align with the requirements for Class III waters and peninsular streams as found in FDEP 62-302 Surface Water Quality Standards. For HF183, no surface water quality standard threshold value is established by FDEP, therefore a *high* concentration threshold value of 525 copies/100mL is used to align with recent work by Boehm and Soller (2020). Elemental ratios of carbon to nitrogen to phosphorus (C:N:P) in POM and macrophytes were compared to a modified Redfield ratio as described in Geider and La Roche (2002), where C:N values < 6 and > 8 were interpreted as being indicative of C and N-limitation, and N:P values < 15 and > 30 indicative of N and P-limitation, respectively. For dissolved $\delta^{15}N$ values, the *high* and *low* concentration thresholds were defined as 7‰ and 2‰ based on previous work by Heaton (1986), Kendall (1998), Kendall et al. (2007), Aravena and Mayer (2009), and Zhang et al. (2018). Where $\delta^{15}N$-NO_3^- values greater than 7‰ are interpreted as having a human waste N source, $\delta^{15}N$ values less than 2‰ are interpreted as having a fertilizer N source, and $\delta^{15}N$ values between 2 and 7‰ are interpreted as having a mixed or soil N source. Stable nitrogen isotope values ($\delta^{15}N$) found in POM and macrophyte tissue were interpreted as having an untreated domestic waste N source if greater than 4.9‰ based on previous work by Hinkle et al. (2008).

For all other parameter values: *high, moderate,* and *low* classification thresholds were derived from statewide surface water quality values for rivers and streams in Florida as described below.

Statewide surface water quality data for Florida's rivers and streams were accessed from the FDEP WIN WAVES database with a date range from 2010 to present. Where possible, data were filtered to include only water quality data which were analyzed by the same standard methods used in this study. Regional differences were not considered. Descriptive statistics were calculated on the filtered data to determine the first quartile (Q_1) and third quartile (Q_3) values for each parameter. Parameters in this study were compared to statewide data and considered *low* if below the statewide Q_1 value, *high* if above the statewide Q_3 value, and *moderate* if between the statewide Q_1 and Q_3 values.

Descriptive statistics, including mean (average), median, standard deviation, standard error, minimum, and maximum, were calculated for all parameters. Prior to statistical analyses, normality, skewness, kurtosis, and other parametric assumptions were assessed. Paired sample t-test (normal data) or Mann-Whitney U tests (non-normal data) were conducted to determine if water quality parameters varied significantly between seasons (wet/dry) and sites (septic/reference). A Spearman correlation was conducted to determine the pairwise correlation coefficients between water quality parameters. Differences were considered significant at $p < 0.05$. Analyses were conducted in MiniTab v. 19, figures were created in GraphPad Prism 9, and maps were created in ArcMap v. 10.8.1.

3. Results

3.1 Precipitation

Precipitation during the study period was seasonally variable (Figure 2). A cumulative total of 61.93 in. of precipitation 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 precipitation events occurring outside of this window. Dry seasons persisted from January 1 to May 14, 2020, and from November 16, 2020, to January 31, 2021. A cumulative total of 52.87 in. of precipitation was recorded during the wet season with a daily mean of 0.29 in., whereas a cumulative total of 9.06 in. of precipitation was recorded during the dry season with a daily mean of 0.04 in. The cumulative rainfall 10 days prior to the wet season sampling events were 2.98 in., 1.64 in., 2.35 in., 3.1 in., and 0.8 in. for July, August, September, October, and November, respectively. The cumulative rainfall 10 days prior to the dry season sampling events were 0.02 in., 0.03 in., 0.20 in., and 0.17 in. for January (2020), February, December, and January (2021), respectively.

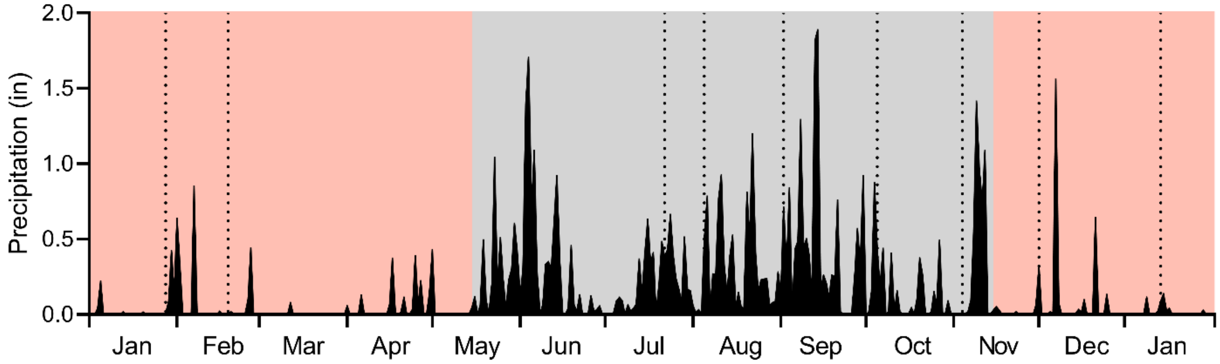

Figure 2. Daily precipitation (mean) across fourteen weather stations within Lee County, FL from January 1, 2020 to January 31, 2021. Dashed bars represent sampling events. Note that sampling efforts in October and November 2020 were combined as one event. Red and grey shaded areas represent dry and wet seasons, respectively.

3.2 Environmental parameters

Surface water measurements for environmental parameters, including pH, temperature, salinity, DO, and color, varied by location and sampling event (Table 1). Mean pH ranged from 7.05 ± 0.12 at Pine Island to 7.80 ± 0.06 at Mobile Manor 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 temperature ranged from 21.4 ± 1.7°C at Summerwood Drive to 27.4 ± 1.7°C at Orange River and was significantly lower in the dry season (20.7 ± 0.3°C) than the wet season (27.90 ± 0.3°C; t-test, $p < 0.001$). Mean salinity ranged from 0.3 ± 0.02 at Billy Creek to 25.3 ± 2.3 at Pine Island with no significant difference between the dry season (4.7 ± 0.7) and wet season (4.0 ± 0.8; t-test, $p = 0.51$). Mean DO concentrations ranged from 1.11 ± 0.15 mg/L at San Carlos Park to 6.58 ± 0.40 mg/L at North Town & River 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 DO saturation ranged from 14.64 ± 2.17% at San Carlos Park to 82.24 ± 3.75% at North Town & River and was significantly higher in the dry season (54.89 ± 2.3%) than the wet season (42.64 ± 2.18%; t-test, $p < 0.001$) Mean color ranged from 19.05 ± 4.06 at Pine Island to 120.7 ± 17.3 at Orange River and was significantly lower in the dry season (59.1 ± 2.8) than the wet season (84.1 ± 4.9; t-test, $p < 0.001$).

Table 1. Environmental parameters (mean ± standard error) measured in surface water at each site within the Lee County study area.

Site Name	pH	Color (CU)	Salinity (ppt)	Conductivity (µS)	Temperature (°C)	DO (mg/L)	DO (%)
Reference	7.66 ± 0.07	79.3 ± 17.7	7.2 ± 2.2	12225 ± 3634	25.2 ± 1.85	6.16 ± 0.53	74.3 ± 4.36
Billy Creek	7.51 ± 0.09	90.9 ± 3.8	0.3 ± 0.0	578 ± 32	24.6 ± 2.22	3.25 ± 0.29	37.7 ± 2.98
Briarcliff	7.53 ± 0.04	61.8 ± 4.0	0.3 ± 0.0	665 ± 24	26.0 ± 1.60	4.10 ± 0.49	50.1 ± 5.30
Daughtry Creek	7.62 ± 0.05	97.7 ± 24.9	0.8 ± 0.3	1555 ± 545	24.0 ± 1.61	3.39 ± 0.48	38.8 ± 4.59
Deep Lagoon	7.64 ± 0.09	74.3 ± 16.8	10.8 ± 2.6	17788 ± 4175	24.9 ± 1.94	5.79 ± 0.58	72.6 ± 5.66
Ft Myers Shores	7.38 ± 0.08	74.3 ± 10.0	0.8 ± 0.3	1526 ± 540	23.4 ± 1.57	4.47 ± 0.40	53.0 ± 4.05
Hendry Creek	7.41 ± 0.03	64.8 ± 4.9	3.8 ± 0.9	6734 ± 1550	23.7 ± 1.55	2.38 ± 0.36	28.7 ± 4.08
Heritage Farms	7.47 ± 0.04	101.1 ± 3.7	0.7 ± 0.0	1382 ± 79	23.5 ± 1.60	2.17 ± 0.32	26.4 ± 3.98
Lake McGregor	7.39 ± 0.12	53.3 ± 1.5	0.4 ± 0.0	868 ± 38	22.4 ± 1.68	1.86 ± 0.40	21.3 ± 4.16
Laurelin Court	7.47 ± 0.05	114.3 ± 20.6	1.1 ± 0.5	1896 ± 829	24.4 ± 1.90	4.61 ± 0.58	54.1 ± 5.47
Ligon Court	7.32 ± 0.07	118.2 ± 39.1	1.3 ± 0.1	2598 ± 202	20.7 ± 3.54	3.15 ± 1.06	39.6 ± 12.00
Mobile Manor	7.80 ± 0.06	60.2 ± 4.9	1.0 ± 0.4	1669 ± 690	24.4 ± 1.42	2.81 ± 0.49	32.6 ± 5.19
North Town & River	7.75 ± 0.06	66.3 ± 18.5	10.8 ± 2.7	16501 ± 3770	24.7 ± 1.90	6.58 ± 0.40	82.2 ± 3.75
Orange River	7.64 ± 0.04	120.7 ± 17.3	1.1 ± 0.4	2130 ± 787	27.4 ± 1.69	3.32 ± 0.43	42.7 ± 4.98
Overlook Drive	7.27 ± 0.18	61.3 ± 3.4	0.5 ± 0.0	1013 ± 43	22.3 ± 2.13	4.25 ± 0.49	50.9 ± 6.42
Page Park	7.54 ± 0.09	27.9 ± 1.9	0.6 ± 0.0	1125 ± 53	24.7 ± 1.53	4.81 ± 0.68	57.1 ± 7.26
Pine Island	7.05 ± 0.12	19.1 ± 4.1	25.3 ± 2.3	39626 ± 3343	24.9 ± 1.82	4.42 ± 0.67	58.4 ± 7.59
Pine Island 02	7.42 ± 0.12	26.5 ± 5.2	23.4 ± 3.3	36891 ± 4931	26.2 ± 2.30	2.48 ± 0.66	34.8 ± 9.02
San Carlos Park	7.41 ± 0.06	45.4 ± 3.7	0.3 ± 0.0	578 ± 78	26.2 ± 1.60	1.11 ± 0.15	14.6 ± 2.17
Summerwood Dr	7.18 ± 0.14	66.1 ± 3.8	0.6 ± 0.0	1249 ± 65	21.4 ± 1.68	2.48 ± 0.58	28.7 ± 6.15
Tidewater Isl 01	7.49 ± 0.05	55.1 ± 5.4	6.6 ± 1.6	11443 ± 2556	24.1 ± 1.69	4.57 ± 0.49	56.6 ± 5.53
Tidewater Isl 02	7.65 ± 0.05	55.8 ± 5.0	6.5 ± 1.5	11149 ± 2512	23.5 ± 1.94	4.63 ± 0.62	55.4 ± 6.78
Waterway Ests 01	7.37 ± 0.06	84.3 ± 14.3	4.7 ± 1.5	8235 ± 2585	24.6 ± 1.77	4.81 ± 0.55	58.4 ± 5.81
Waterway Ests 02	7.65 ± 0.08	84.0 ± 16.4	4.8 ± 1.5	7888 ± 2316	24.5 ± 1.89	6.08 ± 0.66	73.4 ± 6.15
Yacht Club	7.56 ± 0.06	97.1 ± 14.5	1.5 ± 0.7	2572 ± 1104	24.9 ± 1.80	5.37 ± 0.52	64.9 ± 5.54

3.3 Bacteria

The overall median enterococci concentration in surface water across all 25 sites was 276 MPN/100mL, with some individual counts exceeding the upper limits of detection (>4840 MPN/100mL; Figure 3). Enterococci concentrations exceeding the FDEP 62-302 ten percent threshold value for Class III waters (> 130 MPN/100mL) were detected in 63% of all samples and at least once at every site, except Yacht Club. The mean enterococci concentration at the Reference site was 75 ± 41 MPN/100mL (Table 2). There were 18 sites with mean enterococci concentrations that exceeded FDEP standards. Of those sites, 16 had significantly higher mean enterococci concentrations than the Reference site (Mann-Whitney U, $p < 0.05$). No significant differences in enterococci concentrations were observed between the dry season (760 ± 132 MPN/100mL) and wet season (817 ± 108 MPN/100mL; Mann-Whitney U, $p = 0.15$).

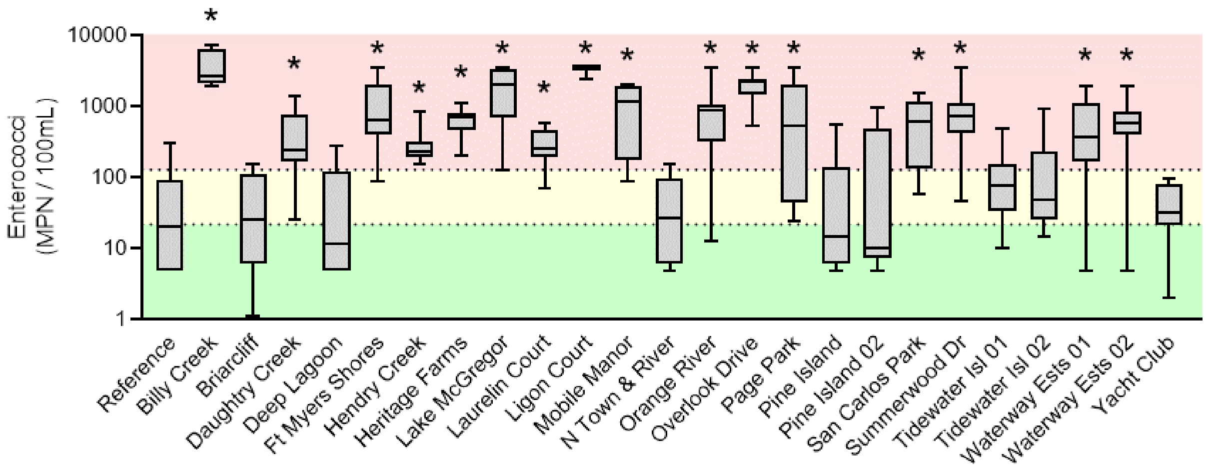

Figure 3. Enterococci concentrations in Lee County, FL surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median *E. coli* concentration in surface water across all 25 sites was 172 MPN/100mL, with some samples exceeding the upper limits of detection (>4840 MPN/100mL; Figure 4). Concentrations of *E. coli* exceeding the FDEP 62-302 ten percent threshold value for Class III waters (> 410 MPN/100mL), were detected in 31% of all samples and at least once at 71% of sites with septic systems. The mean *E. coli* concentration at the Reference site was 54 ± 16 MPN/100mL (Table 2). There were 16 sites with significantly higher mean *E. coli* concentrations than the Reference site (Mann-Whitney U, $p < 0.05$), of which 12 had concentrations that exceeded FDEP standards. Four sites had low mean *E. coli* concentrations (< 97 MPN/100mL), including Briarcliff (43 ± 18 MPN/100mL), Deep Lagoon (49 ± 19 MPN/100mL), North Town & River (32 ± 14 MPN/100mL), and Yacht Club (49 ± 9 MPN/100mL). However, none of these four sites had significantly lower concentrations than the Reference site (Mann-Whitney U, $p > 0.05$). There were no significant differences in *E. coli* concentrations between the dry season (496 ± 92 MPN/100mL) and wet season (478 ± 81 MPN/100mL; Mann-Whitney U, $p = 0.52$).

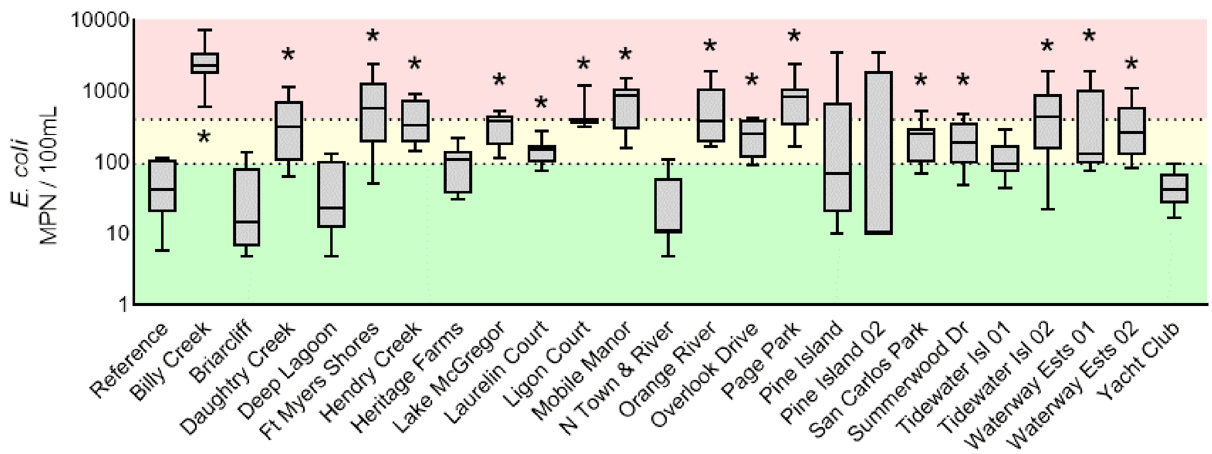

Figure 4. *Escherichia coli* concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median BOD in surface water across all 25 sites was 1.00 mg/L, with individual values ranging from 0.3 to 4 mg/L (Figure 5; Table 2). Mean BOD at Overlook Drive (2.20 ± 0.36 mg/L), Pine Island 02 (1.84 ± 0.48 mg/L), and Waterway Estates 01 (1.25 ± 0.11 mg/L) were all significantly higher than the mean BOD at the Reference site (0.93 ± 0.07 mg/L; Mann-Whitney U, $p < 0.05$). Apart from the Reference site, there were 12 other sites with BOD < 1 mg/L. Mobile Manor (0.60 ± 0.07 mg/L) was the only site with significantly lower mean BOD than the Reference site (Mann-Whitney U, $p = 0.011$). There were no significant differences in BOD between the dry season (0.97 ± 0.06 mg/L) and wet season (1.10 ± 0.06 mg/L; t-test, $p = 0.12$).

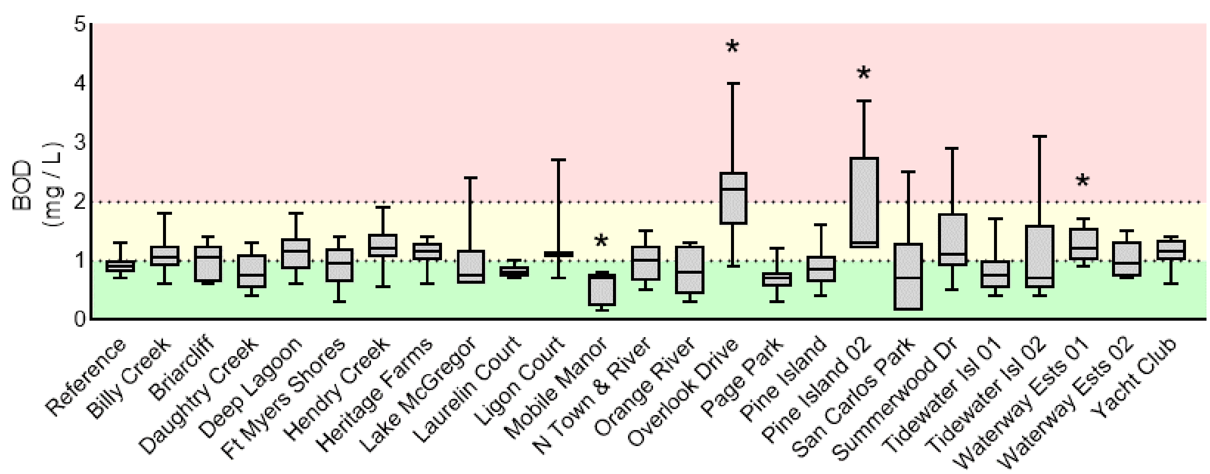

Figure 5. Biochemical oxygen demand (BOD) in Lee County surface water. Whiskers show the maximum and minimum measured value at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

HF183 was detected in 50% of all samples collected in this study. The overall median human *Bacteroides* HF183 concentration in surface water across all 25 sites was 12.6 copies/100mL; however, values ranged up to 23,700 copies/100mL (Figure 6; Table 2). Concentrations of HF183 >525 copies/100mL were detected in 10% of samples, and at least once at 32% of all sites. Heritage Farms (680 ± 214 copies/100mL), San Carlos Park (1,903 ± 412 copies/100mL), Summerwood Drive (1,455 ± 1322 copies/100mL), and Tidewater Island 02 (2,993 ± 2,958 copies/100mL) had high mean HF183 concentrations > 525 copies/100mL. Only Heritage Farms, San Carlos Park, and Hendry Creek had significantly higher mean HF183 concentrations than the Reference site (55.7 ± 43.8 copies/100mL; Mann-Whitney U, $p < 0.05$). Although Tidewater Island 02 had the highest mean HF183 concentration, the difference from the Reference site was not statistically significant (Mann-Whitney U, $p = 0.772$). Apart from the Reference site, there were 14 other sites with a low mean HF183 concentration (< 94 copies/100mL). Of these sites, three had no HF183 detections across all sampling events, including Laurelin Court, Ligon Court, and North Town & River. There were no significant differences in HF183 concentrations between the dry season (314 ± 117 copies/100mL) and wet season (411 ± 252 copies/100mL; Mann-Whitney U, $p = 0.40$).

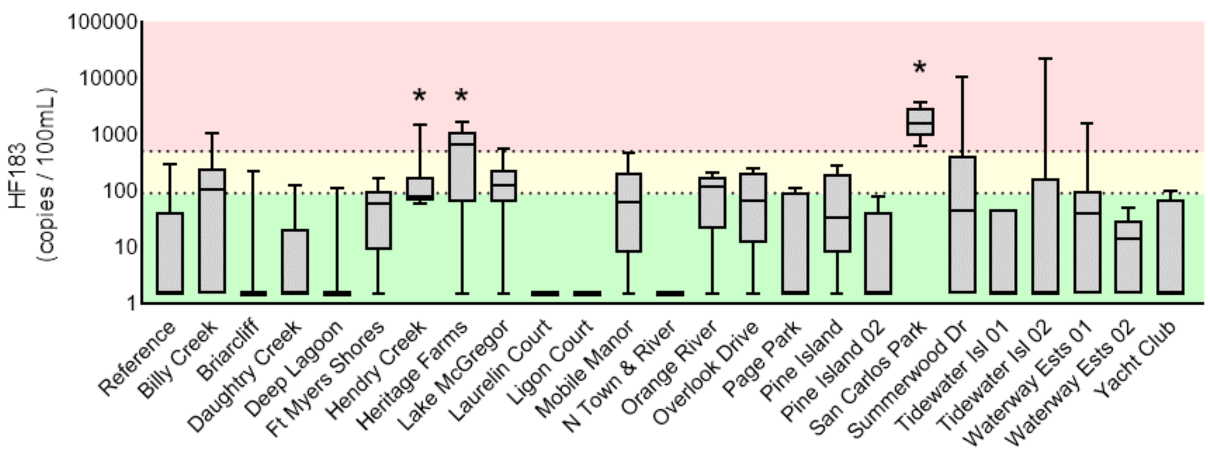

Figure 6. Human *Bacteroides* HF183 gene fragment concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

Table 2. Bacterial parameters (mean ± standard error) measured in surface water at each site within the Lee County study area. Sites with no detections are labeled 'BDL' for 'below detection limits.' Green up arrows indicate a mean that is significantly higher than the Reference site. Red down arrows indicate a mean that is significantly lower than the Reference site (Mann-Whitney U, $p < 0.05$).

Site Name	BOD (mg/L)	HF183 (copies/100mL)	Enterococci (MPN/100mL)	*Escherichia coli* (MPN/100mL)
Reference	0.93 ± 0.07	56 ± 44	75 ± 41	54 ± 16
Billy Creek	1.09 ± 0.13	220 ± 128	3752 ± 790 ↑	2945 ± 708 ↑
Briarcliff	0.98 ± 0.11	30 ± 28	54 ± 21	43 ± 18
Daughtry Creek	0.81 ± 0.11	21 ± 16	438 ± 168 ↑	430 ± 140 ↑
Deep Lagoon	1.15 ± 0.13	16 ± 14	62 ± 36	49 ± 19
Ft Myers Shores	0.91 ± 0.13	65 ± 20	1162 ± 431 ↑	793 ± 286 ↑
Hendry Creek	1.30 ± 0.10	277 ± 178 ↑	310 ± 82 ↑	443 ± 105 ↑
Heritage Farms	1.13 ± 0.09	680 ± 214 ↑	659 ± 97 ↑	106 ± 24
Lake McGregor	0.98 ± 0.22	187 ± 64	1979 ± 462 ↑	357 ± 56 ↑
Laurelin Court	0.83 ± 0.04	BDL	303 ± 61 ↑	153 ± 22 ↑
Ligon Court	1.50 ± 0.61	BDL	3227 ± 403 ↑	641 ± 282 ↑
Mobile Manor	0.60 ± 0.07 ↓	125 ± 60	1119 ± 301 ↑	796 ± 170 ↑
North Town & River	0.98 ± 0.12	BDL	51 ± 19	32 ± 14
Orange River	0.81 ± 0.14	112 ± 29	1025 ± 397 ↑	699 ± 231 ↑
Overlook Drive	2.20 ± 0.36 ↑	98 ± 37	2049 ± 322 ↑	257 ± 50 ↑
Page Park	0.71 ± 0.09	39 ± 18	1012 ± 467 ↑	903 ± 248 ↑
Pine Island	0.89 ± 0.13	92 ± 41	100 ± 68	625 ± 441
Pine Island 02	1.84 ± 0.48 ↑	18 ± 17	201 ± 190	774 ± 715
San Carlos Park	0.93 ± 0.27	1903 ± 412 ↑	670 ± 196 ↑	244 ± 54 ↑
Summerwood Dr	1.37 ± 0.30	1455 ± 1322	1029 ± 392 ↑	222 ± 55 ↑
Tidewater Isl 01	0.84 ± 0.15	19 ± 9	124 ± 54	126 ± 29
Tidewater Isl 02	1.10 ± 0.33	2993 ± 2958	194 ± 108	625 ± 225 ↑
Waterway Ests 01	1.25 ± 0.11 ↑	236 ± 196	667 ± 242 ↑	528 ± 254 ↑
Waterway Ests 02	1.01 ± 0.11	18 ± 7	709 ± 208 ↑	394 ± 125 ↑
Yacht Club	1.13 ± 0.09	26 ± 16	44 ± 12	49 ± 9

3.4 Chemical tracers of human waste

The overall median sucralose concentration in surface water across all 25 sites was 415 ng/L, with individual values ranging up to 5,900 mg/L (Figure 7; Table 3). High concentrations of sucralose (>380 ng/L) were detected in 54% of all samples and at least once at every site, except Billy Creek, Daughtry Creek, Pine Island, Pine Island 02, and Yacht Club. The mean sucralose concentration at the Reference site was 504 ± 91 ng/L. In addition to the Reference site, 15 other sites had high mean sucralose concentrations

(> 380 ng/L). Of these sites, five had very high mean sucralose concentrations that were significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$), including Heritage Farms (3613 ± 207 ng/L), Ligon Court (1900 ± 400 ng/L), Mobile Manor (1120 ± 184 ng/L), Overlook Drive (3725 ± 361 ng/L), and Summerwood Drive (919 ± 50 ng/L). Although no sites had low mean sucralose concentrations (< 23 ng/L), seven sites in the moderate range (23 – 380 ng/L) had significantly lower mean sucralose concentrations than the Reference site (Mann-Whitney U, $p < 0.05$), including Billy Creek (158 ± 27 ng /L), Daughtry Creek (262 ± 30 ng/L), Fort Myers Shores (182 ± 48 ng/L), Lake McGregor (172 ± 45 ng/L), Pine Island (121 ± 17 ng/L), Pine Island 02 (160 ± 15 ng/L), and Yacht Club (221 ± 28 ng/L). There were no significant differences in sucralose concentrations between the dry season (719 ± 93 ng/L) and wet season (733 ± 108 ng/L; Mann-Whitney U, $p = 0.73$).

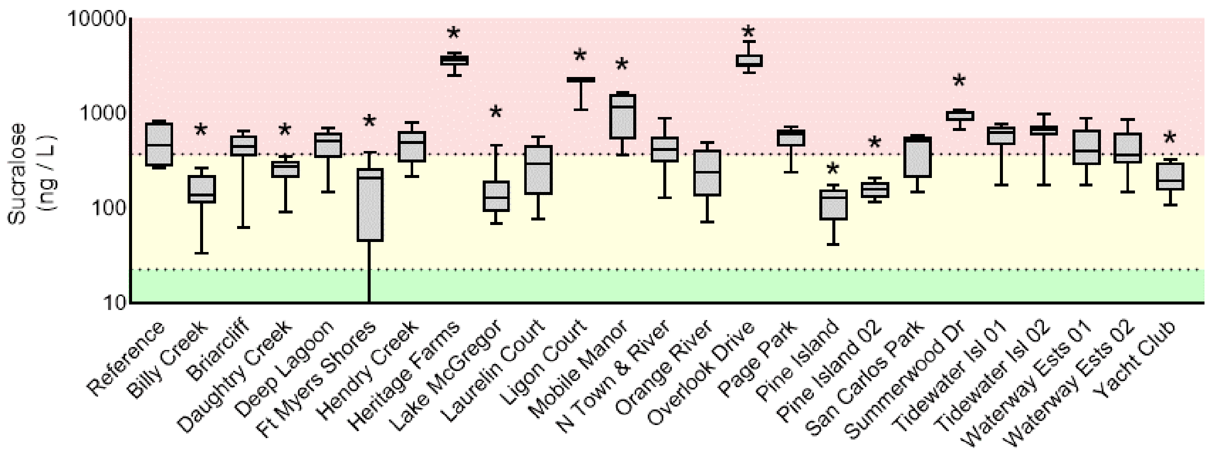

Figure 7. Sucralose concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median carbamazepine concentration in surface water across all 25 sites was below the limits of detection (< 0.8 ng/L); however, some high individual values were detected ranging up to 18 ng/L (Figure 8; Table 3). Carbamazepine was detected in 41% of all samples, including one detection at the Reference site. The mean carbamazepine concentration at the Reference site was 0.136 ± 0.136 ng/L. There were eight sites with high mean carbamazepine concentrations (> 1.0 ng/L), including Heritage Farms (3.19 ± 0.35 ng/L), Ligon Court (4.43 ± 0.75 ng/L), Mobile Manor (3.96 ± 0.73 ng/L), Overlook Drive (4.63 ± 0.60 ng/L), Page Park (12.2 ± 1.35 ng/L), San Carlos Park (1.05 ± 0.37 ng/L), Tidewater Island 01 (1.35 ± 0.34 ng/L), and Tidewater Island 02 (1.14 ± 0.33 ng/L). All these sites had significantly higher mean carbamazepine concentrations than the Reference site (Mann-Whitney U, $p < 0.05$). There were 14 sites with a low mean carbamazepine concentration (< 0.8 ng/L), including seven sites where carbamazepine was not detected. These were Billy Creek, Briarcliff, Fort Myers Shores, Lake McGregor, Pine Island, Pine Island 02, and Summerwood Drive. There were no significant differences in carbamazepine concentrations between the dry season (0.66 ± 0.42 ng/L) and wet season (0.09 ± 0.09 ng/L; Mann-Whitney U, p = 0.37).

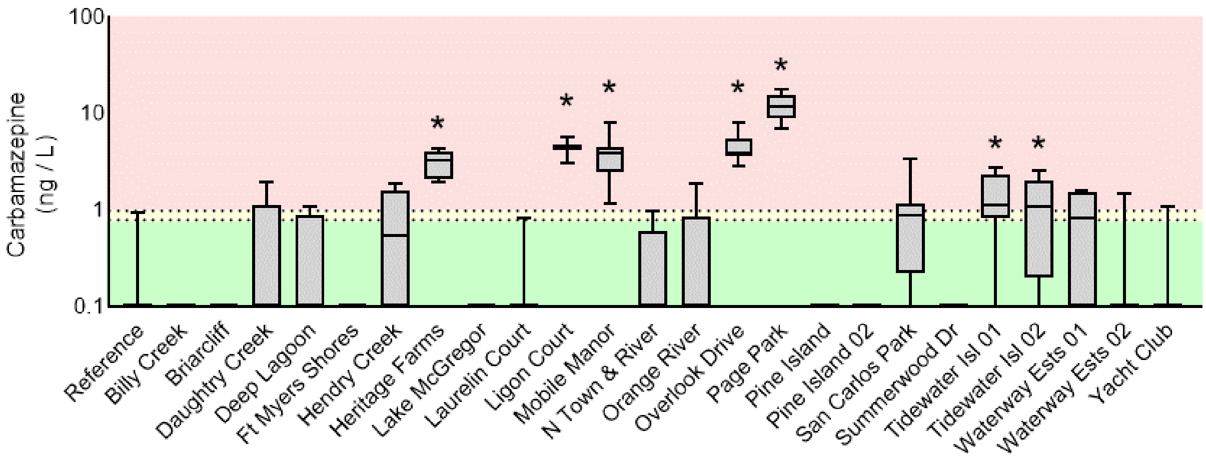

Figure 8. Carbamazepine concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, p < 0.05).

Acetaminophen, ibuprofen, and naproxen were rarely detected in surface water samples (Table 3). Acetaminophen was only detected in four samples, including Briarcliff (21 ng/L, Jan 2021), Daughtry Creek (8.9 ng/L, Oct 2020), Hendry Creek (9.1 ng/L, Dec 2020), and Lake McGregor (33 ng/L, Dec 2020). Ibuprofen was only detected in three samples, including Summerwood Drive (100 ng/L, Jan 2021; 87 ng/L, Dec 2020) and Waterway Estates 02 (58 ng/L, Sep 2020). Naproxen was only detected in one sample at Heritage Farms (42 ng/L, Feb 2020).

Table 3. Chemical tracers of human waste (mean ± standard error) measured in surface water at each site within the Lee County study area. Sites with no detections are labeled 'BDL' for 'below detection limits.' Green up arrows indicate a mean that is significantly higher than the Reference site. Red down arrows indicate a mean that is significantly lower than the Reference site (Mann-Whitney U, $p < 0.05$).

Site Name	Sucralose (ng/L)	Acetaminophen (ng/L)	Carbamazepine (ng/L)	Ibuprofen (ng/L)	Naproxen (ng/L)
Reference	504 ± 91	BDL	0.1 ± 0.1	BDL	BDL
Billy Creek	158 ± 27 ↓	BDL	BDL	BDL	BDL
Briarcliff	438 ± 68	2.63 ± 2.63	BDL	BDL	BDL
Daughtry Creek	262 ± 30 ↓	1.11 ± 1.11	0.4 ± 0.3	BDL	BDL
Deep Lagoon	489 ± 68	BDL	0.4 ± 0.2	BDL	BDL
Ft Myers Shores	182 ± 48 ↓	BDL	BDL	BDL	BDL
Hendry Creek	504 ± 72	1.14 ± 1.14	0.8 ± 0.3	BDL	BDL
Heritage Farms	3613 ± 207 ↑	BDL	3.2 ± 0.3 ↑	BDL	5.25 ± 5.25
Lake McGregor	172 ± 45 ↓	4.13 ± 4.13	BDL	BDL	BDL
Laurelin Court	301 ± 64	BDL	0.1 ± 0.1	BDL	BDL
Ligon Court	1900 ± 400 ↑	BDL	4.4 ± 0.8 ↑	BDL	BDL
Mobile Manor	1120 ± 184 ↑	BDL	4.0 ± 0.7 ↑	BDL	BDL
North Town & River	458 ± 80	BDL	0.2 ± 0.1	BDL	BDL
Orange River	277 ± 54	BDL	0.5 ± 0.2	BDL	BDL
Overlook Drive	3725 ± 361 ↑	BDL	4.6 ± 0.6 ↑	BDL	BDL
Page Park	564 ± 57	BDL	12.2 ± 1.4 ↑	BDL	BDL
Pine Island	121 ± 17 ↓	BDL	BDL	BDL	BDL
Pine Island 02	160 ± 15 ↓	BDL	BDL	BDL	BDL
San Carlos Park	424 ± 66	BDL	1.0 ± 0.4 ↑	BDL	BDL
Summerwood Dr	919 ± 50 ↑	BDL	BDL	23.4 ± 15.4	BDL
Tidewater Isl 01	589 ± 70	BDL	1.4 ± 0.3 ↑	BDL	BDL
Tidewater Isl 02	656 ± 82	BDL	1.1 ± 0.3 ↑	BDL	BDL
Waterway Ests 01	466 ± 90	BDL	0.8 ± 0.3	BDL	BDL
Waterway Ests 02	443 ± 84	BDL	0.2 ± 0.2	7.3 ± 7.3	BDL
Yacht Club	221 ± 28 ↓	BDL	0.1 ± 0.1	BDL	BDL

3.5 Nutrients and stable isotopes

The overall median NH_4^+ concentration in surface water across all 25 sites was 0.067 mg/L, with some individual values ranging up to 1.34 mg/L (Figure 9; Table 4). The mean NH_4^+concentration at the Reference site was 0.05 ± 0.02 mg/L. There were 15 sites with high mean NH_4^+ concentrations (> 0.06 mg/L), seven of which were significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$), including Billy Creek (0.24 ± 0.06 mg/L), Hendry Creek (0.10 ± 0.01 mg/L), Heritage Farms (0.36 ± 0.05 mg/L), Ligon Court (0.61 ± 0.30 mg/L), Overlook Drive (0.36 ± 0.08 mg/L), Page Park (0.09 ± 0.01 mg/L), and Summerwood Drive (0.60 ± 0.17 mg/L). Additionally, we identified eight NH_4^+ rich sites with mean NH_4^+:NO_x > 2, including Heritage Farms (2.2), Lake McGregor (6.82), Ligon Court (18.46), Overlook

Drive (3.76), Pine Island (2.15), Pine Island 02 (2.84), San Carlos Park (22.63), and Summerwood Drive (95.16). NH_4^+ concentrations in the dry season (0.12 ± 0.02 mg/L) were significantly lower than concentrations in the wet season (0.15 ± 0.02 mg/L; Mann-Whitney U, $p < 0.001$).

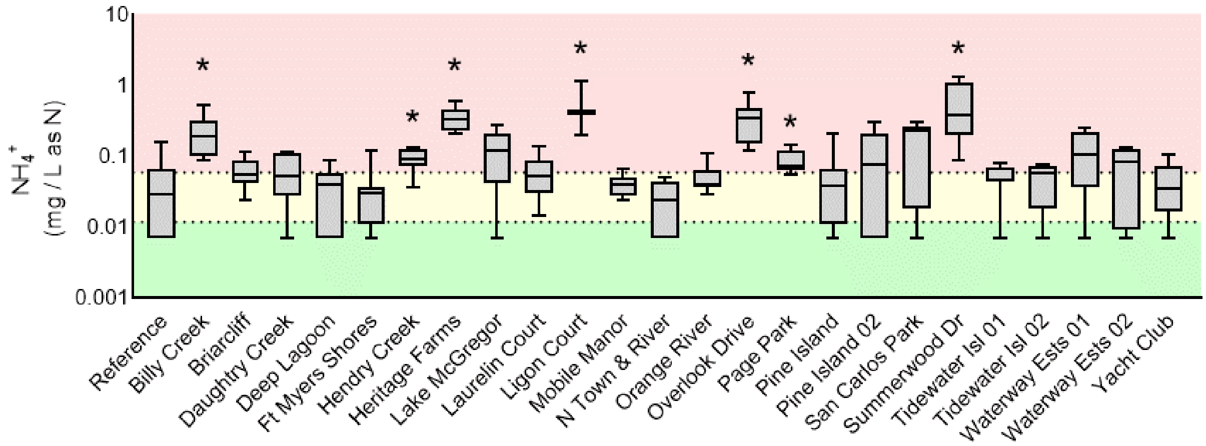

Figure 9. Ammonium (NH_4^+) concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median NO_x concentration in surface water across all 25 sites was 0.085 mg/L, with some individual values ranging up to 0.487 mg/L (Figure 10; Table 4). The mean NO_x concentration at the Reference site was 0.14 ± 0.05 mg/L. Billy Creek (0.24 ± 0.05 mg/L) was the only site with a high mean NO_x concentration (> 0.21 mg/L), although the difference from the Reference site was not statistically significant (Mann-Whitney U, $p = 0.272$). Summerwood Drive (0.009 ± 0.003 mg/L) was the only site with a low mean NO_x concentration (< 0.02 mg/L) and the only site with a significantly lower mean NO_x concentration than the Reference site (Mann-Whitney U, $p = 0.007$). The NO_x concentration across all sites was 0.11 mg/L in both wet and dry seasons and there was no significant difference between seasons (Mann-Whitney U, $p = 0.75$).

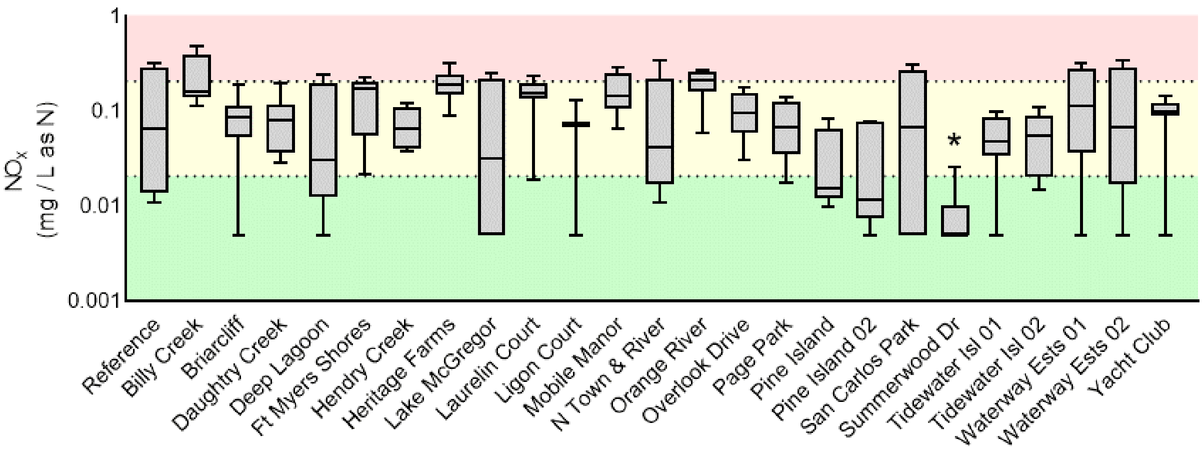

Figure 10. Nitrate + nitrite (NO_x) concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median DIN concentration in surface water across all 25 sites was 0.186 mg/L, with some individual values ranging up to 1.35 mg/L (Figure 11; Table 4). The mean DIN concentration at the Reference site was 0.189 ± 0.069 mg/L. There were six sites with high mean DIN concentrations (> 0.271 mg/L), including Billy Creek (0.474 ± 0.090 mg/L), Heritage Farms (0.555 ± 0.047 mg/L), Ligon Court (0.682 ± 0.335 mg/L), Overlook Drive (0.465 ± 0.097 mg/L), San Carlos Park (0.300 ± 0.018 mg/L), and Summerwood Drive (0.608 ± 0.171 mg/L). However, of these sites, only Billy Creek, Heritage Farms, and Overlook Drive had significantly higher DIN concentrations than the Reference site (Mann-Whitney U, $p < 0.05$). Although Ligon Court had the highest mean DIN concentration of any site, the difference between the Reference site was not statistically significant (Mann-Whitney U, $p = 0.111$). There were no sites with a low mean DIN concentration (< 0.033 mg/L). Likewise, there were no sites with a significantly lower mean DIN concentration than the Reference site. DIN concentrations in the dry season (0.229 ± 0.025 mg/L) were significantly lower than in the wet season (0.259 ± 0.020 mg/L; t-test, $p = 0.04$).

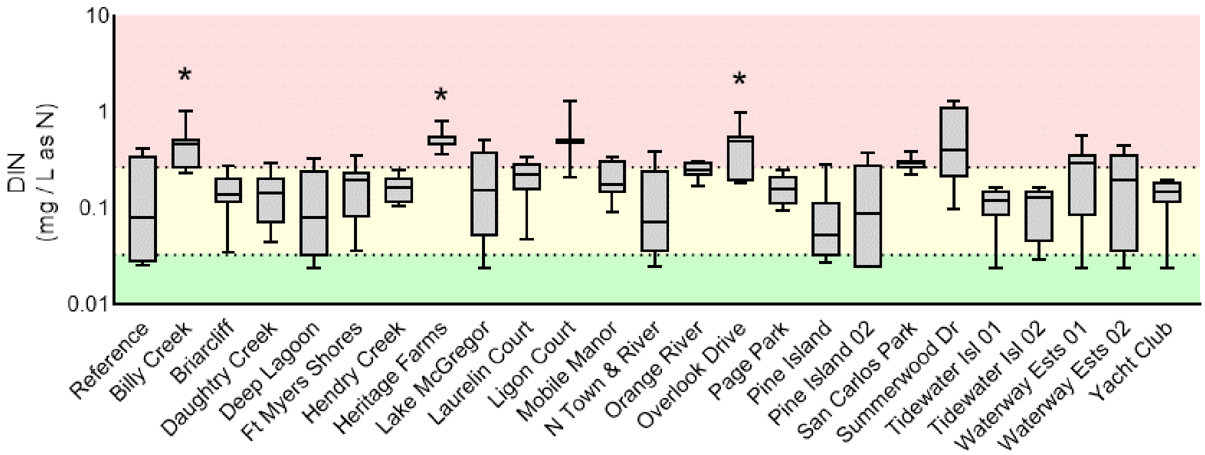

Figure 11. Dissolved inorganic nitrogen (DIN) concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median SRP concentration in surface water across all 25 sites was 0.045 mg/L, with some individual values ranging up to 0.32 mg/L (Figure 12; Table 4). The mean SRP concentration at the Reference site was 0.075 ± 0.013 mg/L. There were nine sites with high mean SRP concentrations (> 0.081 mg/L), including Billy Creek (0.127 ± 0.019 mg/L), Daughtry Creek (0.139 ± 0.027 mg/L), Laurelin Court (0.094 ± 0.021 mg/L), Mobile Manor (0.138 ± 0.035 mg/L), Orange River (0.104 ± 0.016 mg/L), Summerwood Drive (0.098 ± 0.019 mg/L), Waterway Estates 01 (0.127 ± 0.024 mg/L), Waterway Estates 02 (0.092 ± 0.016 mg/L), and Yacht Club (0.086 ± 0.016 mg/L). Of these sites, only Daughtry Creek had a significantly higher mean SRP concentration than the Reference site (Mann-Whitney U, $p = 0.043$). There were 11 sites with significantly lower mean SRP concentrations than the Reference site (Mann-Whitney U, $p < 0.05$). The mean wet season SRP concentration (0.075 ± 0.004 mg/L) was significantly higher than the mean dry season SRP concentration (0.047 ± 0.007 mg/L; Mann-Whitney U, $p = 0.01$).

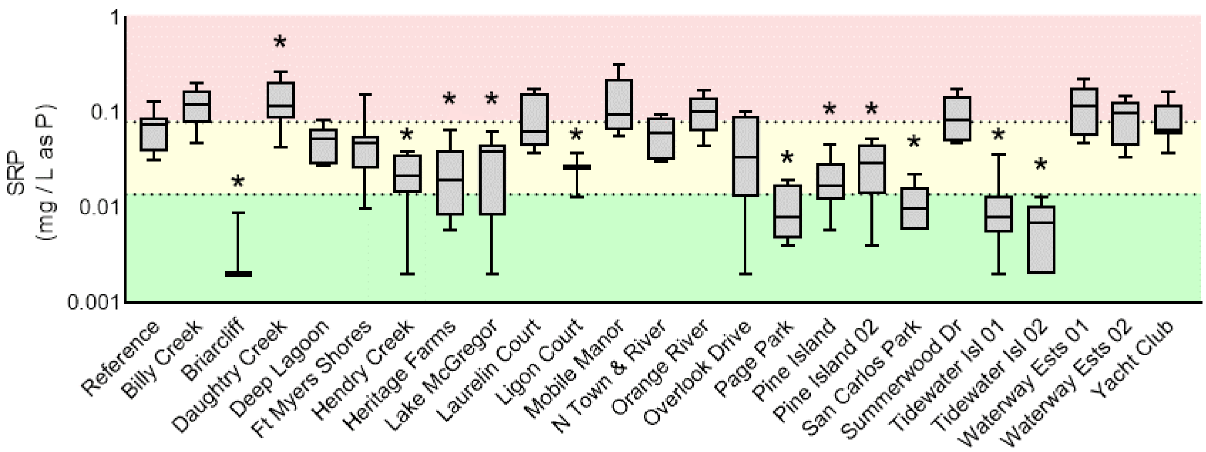

Figure 12. Soluble reactive phosphorus (SRP) concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median DIN:SRP in surface water across all 25 sites was 9.6, with individual values ranging from 0.4 to 240 (Figure 13; Table 4). The mean DIN:SRP in surface water at the Reference site was 5.2 ± 1.7. There were seven sites with a high mean DIN:SRP (> 32), including Briarcliff (140.4 ± 24.4), Heritage Farms (82.7 ± 19.2), Ligon Court (59.9 ± 21.6), Overlook Drive (54.5 ± 27.0), Page Park (44.3 ± 8.7), San Carlos Park (74.0 ± 13.7), and Tidewater Island 02 (38.3 ± 3.3). Additionally, the mean DIN:SRP ratio at all seven of these sites was significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$). Hendry Creek (30.6 ± 15.6), Lake McGregor (16.5 ± 4.4), and Tidewater Island 01 (29.7 ± 5.7) also had mean DIN:SRP that were significantly higher than the Reference site, but in the moderate range (8 – 32). Apart from the Reference site, there were nine other sites with a low mean DIN:SRP (< 8), including Daughtry Creek (3.0 ± 0.8), Deep Lagoon (4.3 ± 1.1), Laurelin Court (6.4 ± 1.2), Mobile Manor (5.1 ± 1.2), North Town & River (4.2 ± 1.3), Orange River (6.3 ± 0.9), Waterway Estates 01 (4.6 ± 0.9), Waterway Estates 02 (4.4 ± 1.1), and Yacht Club (4.3 ± 0.9). However, the differences between these sites and the Reference site were not statistically significant (Mann-Whitney U, $p > 0.05$). There were no significant differences in mean DIN:SRP between the dry season (29.1 ± 4.7) and wet season (23.5 ± 3.9; Mann-Whitney U, $p = 0.83$).

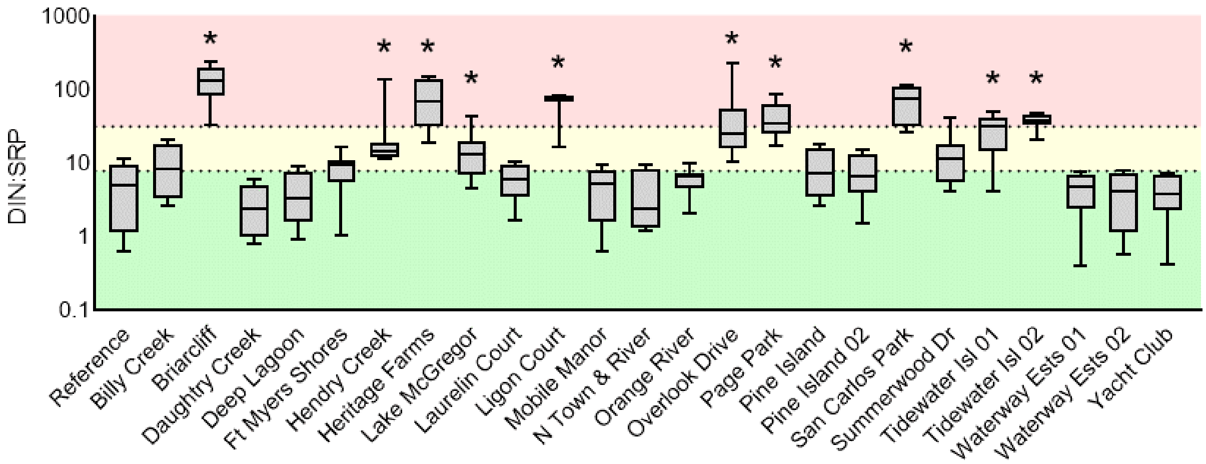

Figure 13. Dissolved inorganic nitrogen to soluble reactive phosphorus ratio (DIN:SRP) in Lee County surface water. Whiskers show the maximum and minimum measured ratio at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median TKN concentration in surface water across all 25 sites was 0.9 mg/L, with individual values ranging up to 3.3 mg/L (Figure 14; Table 4). The mean TKN concentration at the Reference site was 0.96 ± 0.10 mg/L. There were five sites with high mean TKN concentrations (> 1.10 mg/L). Of these sites, four had mean TKN concentrations that were significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$), including Heritage Farms (1.36 ± 0.07 mg/L), Ligon Court (2.67 ± 0.41 mg/L), Overlook Drive (2.08 ± 0.19 mg/L), and Summerwood Drive (1.60 ± 0.20 mg/L). Page Park (0.53 ± 0.03 mg/L) and San Carlos Park (0.59 ± 0.06) had significantly lower mean TKN concentrations than the Reference site (Mann-Whitney U, $p < 0.05$), although these concentrations were still in the moderate range. There were no sites with a low mean TKN concentration (< 0.52 mg/L). There were no significant differences in mean TKN concentrations between the dry season (0.94 ± 0.05 mg/L) and wet season (1.06 ± 0.04 mg/L; t-test, $p = 0.057$).

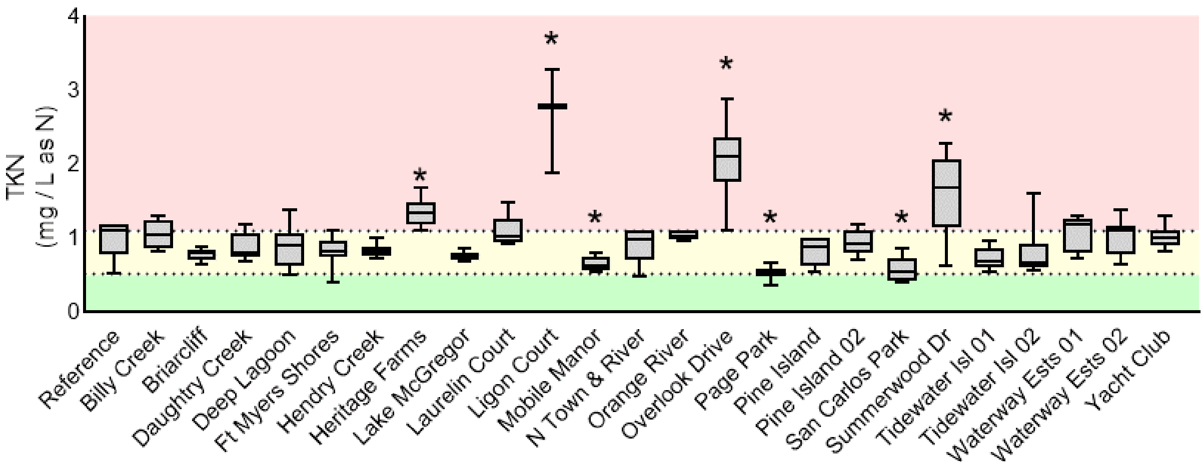

Figure 14. Total Kjeldahl nitrogen (TKN) concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median TN concentration in surface water across all 25 sites was 1.00 mg/L, with individual values ranging up to 3.4 mg/L (Figure 15; Table 4). Concentrations of TN exceeding the FDEP 62-302 ten percent threshold value for peninsular streams (> 1.54 mg/L) were detected in 12% of all samples collected during this study and at least once at 38% of sites with septic systems. The mean TN concentration at the Reference site was moderate (1.10 ± 0.14 mg/L). Four sites exhibited a high mean TN concentration which exceeded FDEP standards, including Heritage Farms (1.58 ± 0.07 mg/L), Ligon Court (2.73 ± 0.41 mg/L), Overlook Drive (2.19 ± 0.19 mg/L), and Summerwood Drive (1.60 ± 0.20 mg/L). Of these sites, all except Summerwood Drive had mean TN concentrations which were significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$). Mean TN concentrations at Page Park (0.60 ± 0.03 mg/L) were significantly lower than the Reference site (Mann-Whitney U, $p = 0.018$). Page Park was the only site with a low mean TN concentration (< 0.69 mg/L). The mean TN concentration in the dry season (1.05 ± 0.05 mg/L) was not significantly different from the wet season (1.17 ± 0.04 mg/L; t-test, $p = 0.088$).

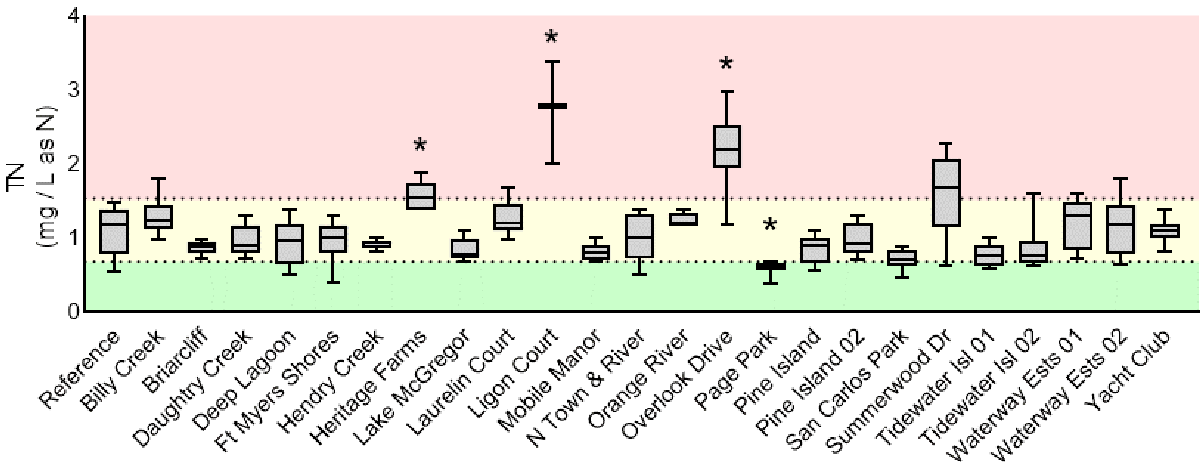

Figure 15. Total nitrogen (TN) concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median TP concentration in surface water across all 25 sites was 0.091 mg/L, with individual values ranging up to 0.380 mg/L (Figure 16; Table 4). Concentrations of TP exceeding the FDEP 62-302 ten percent threshold value for peninsular streams (> 0.12 mg/L) were detected in 35% of all samples collected during this study and at least once at 64% of all sites, including the Reference site. The mean TP concentration at the Reference site was 0.114 ± 0.016 mg/L. Mean TP concentrations that exceeded FDEP standards were observed at 11 sites. Of these, three sites had significantly higher mean TP concentrations than the Reference site (Mann-Whitney U, $p < 0.05$), including Billy Creek (0.233 ± 0.029 mg/L), Mobile Manor (0.219 ± 0.028 mg/L), and Overlook Drive (0.184 ± 0.019 mg/L). Eight sites had significantly lower mean TP concentrations than the Reference site (Mann-Whitney U, $p < 0.05$), including Briarcliff (0.032 ± 0.004 mg/L), Hendry Creek (0.064 ± 0.004 mg/L), Heritage Farms (0.065 ± 0.009 mg/L), Page Park (0.035 ± 0.005 mg/L), Pine Island (0.050 ± 0.004 mg/L), San Carlos Park (0.036 ± 0.004 mg/L), Tidewater Island 01 (0.031 ± 0.003 mg/L), and Tidewater Island 02 (0.048 ± 0.015 mg/L). The mean TP concentration in the dry season (0.096 ± 0.007 mg/L) was significantly lower than the mean TP concentration in the wet season (0.123 ± 0.008 mg/L; Mann-Whitney U, $p = 0.02$).

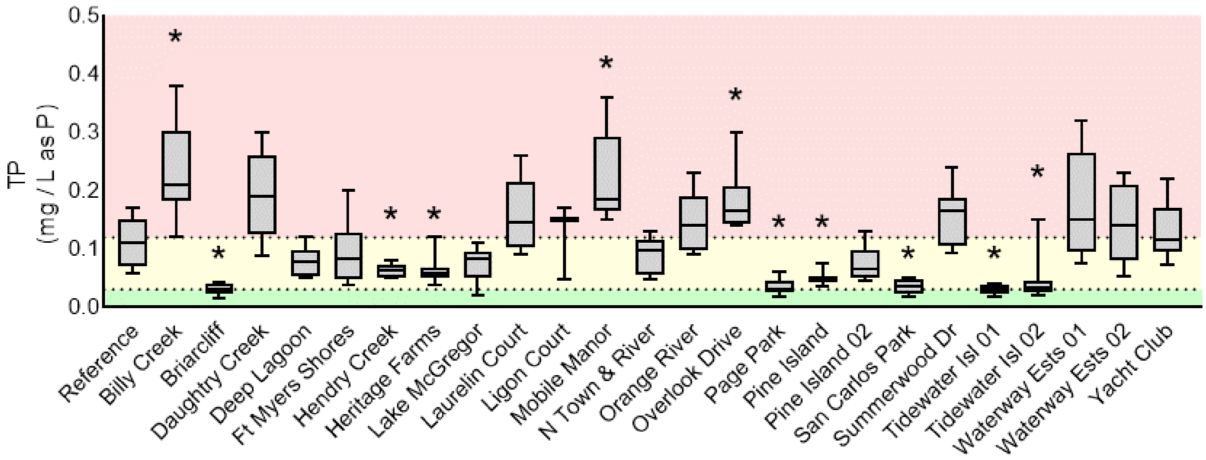

Figure 16. Total phosphorus (TP) concentrations in Lee County surface water. Whiskers show the maximum and minimum measured concentration at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median TN:TP in surface water across all 25 sites was 27, with individual values ranging from 5 to 115 (Figure 17; Table 4). The mean TN:TP at the Reference site was 22 ± 2. There were ten sites with a high mean TN:TP (> 32), of which nine were significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$), including Briarcliff (67 ± 8), Hendry Creek (32 ± 2), Heritage Farms (59 ± 6), Ligon Court (60 ± 17), Page Park (43 ± 6), Pine Island (39 ± 3), San Carlos Park (50 ± 7), Tidewater Island 01 (60 ± 7), and Tidewater Island 02 (51 ± 6). Pine Island 02 (32 ± 3) had a mean TN:TP that was significantly higher than the Reference site (Mann-Whitney U, $p = 0.023$), but in the moderate range (8 – 32). Although no sites had a low mean TN:TP (< 8), Billy Creek (14 ± 2), Daughtry Creek (13 ± 2), and Mobile Manor (9 ± 1) were all significantly lower than the Reference (Mann-Whitney U, $p < 0.05$). The mean TN:TP in the dry season (34 ± 2) was not significantly different from the wet season (30 ± 2; Mann-Whitney U, $p = 0.19$).

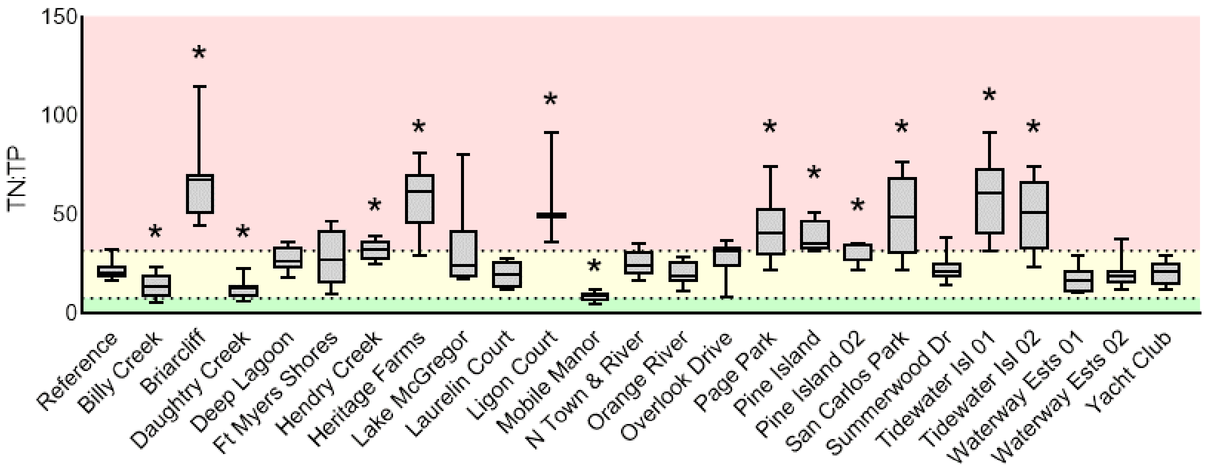

Figure 17. Total nitrogen to total phosphorus ratio (TN:TP) in Lee County surface water. Whiskers show the maximum and minimum measured ratio at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median $\delta^{15}N$-NO_3^- value in surface water across all 25 sites was +4.4‰, with individual values ranging from -6.5 to +14.5‰ (Figure 18; Table 4). $\delta^{15}N$-NO_3^- values in the range of human waste (> +7‰) were detected in 17% of all samples and at least once at 46% of sites with septic systems. The mean dissolved $\delta^{15}N$-NO_3^- value at the Reference site was +4.76 ± 0.80‰. There were three sites with a mean $\delta^{15}N$-NO_3^- value in the range of human waste (> +7‰), including Fort Myers Shores (+10.73 ± 1.76‰), Mobile Manor (+8.76 ± 1.01‰), and Page Park (+9.66 ± 0.21‰). Additionally, all these sites had a mean $\delta^{15}N$-NO_3^- value that was significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$). There were four sites with a low mean $\delta^{15}N$-NO_3^- value (< +2‰), including Overlook Drive (+0.39 ± 0.684‰), Pine Island (+1.01 ± 0.10‰), Pine Island 02 (+0.22 ± 0.98‰), and San Carlos Park (-2.19 ± 4.29‰). However, of these sites, only Overlook Drive had a mean $\delta^{15}N$-NO_3^- value that was significantly lower than the Reference site (Mann-Whitney U, $p = 0.03$). Only two of eight samples each for Pine Island, Pine Island 02, and San Carlos Park were analyzed for $\delta^{15}N$-NO_3^- because in all other samples from these sites the NO_x concentration was not high enough to meet the minimum threshold required for analysis (0.04 mg/L). No samples from Summerwood Drive were assessed for $\delta^{15}N$-NO_3 due to low NO_x concentrations and Ligon Court was not assessed for $\delta^{15}N$-NO_3^- due to no overlapping sampling dates. There were no significant differences in mean $\delta^{15}N$-NO_3^- values between the dry season (+4.27 ± 0.52‰) and wet season (+4.96 ± 0.44‰; t-test, $p = 0.312$).

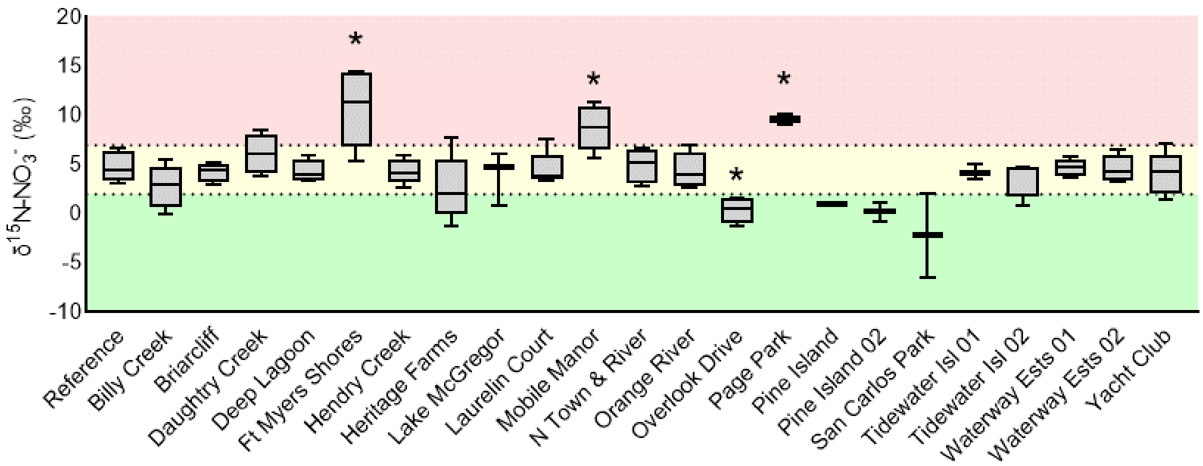

Figure 18. Nitrate isotope ($\delta^{15}N\text{-}NO_3^-$) values in Lee County surface water. Whiskers show the maximum and minimum measured value at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent high, moderate, and low classifications. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

Table 4. Nutrient concentrations and ratios, as well as nitrate isotope values (mean ± standard error) measured in surface water at each site within the Lee County study area. Green up arrows indicate a mean that is significantly higher than the Reference site. Red down arrows indicate a mean that is significantly lower than the Reference site (Mann-Whitney U, $p < 0.05$).

Site Name	NH_4^+ (mg/L as N)	NO_x (mg/L as N)	TKN (mg/L)	DIN (mg/L)	SRP (mg/L)	DIN:SRP	TN (mg/L)	TP (mg/L)	TN:TP	$\delta^{15}N\text{-}NO_3^-$ (‰)
Reference	0.05 ± 0.02	0.14 ± 0.05	0.96 ± 0.10	0.19 ± 0.07	0.07 ± 0.01	5 ± 2	1.10 ± 0.14	0.11 ± 0.02	22.0 ± 2.0	+4.8 ± 0.8
Billy Creek	0.24 ± 0.06 ↑	0.24 ± 0.05	1.05 ± 0.06	0.47 ± 0.09 ↑	0.13 ± 0.02	11 ± 3	1.30 ± 0.09	0.23 ± 0.03 ↑	14.2 ± 2.2 ↓	+2.8 ± 1.1
Briarcliff	0.06 ± 0.01	0.09 ± 0.02	0.79 ± 0.03	0.16 ± 0.03	0.00 ± 0.00 ↓	140 ± 24 ↑	0.88 ± 0.03	0.03 ± 0.004 ↓	67.4 ± 7.8 ↑	+4.3 ± 0.5
Daughtry Creek	0.06 ± 0.01	0.09 ± 0.02	0.89 ± 0.07	0.15 ± 0.03	0.14 ± 0.03 ↑	3 ± 1	0.97 ± 0.07	0.19 ± 0.03	12.7 ± 1.8 ↓	+6.1 ± 1.0
Deep Lagoon	0.04 ± 0.01	0.08 ± 0.04	0.89 ± 0.10	0.12 ± 0.04	0.05 ± 0.01	4 ± 1	0.96 ± 0.11	0.08 ± 0.01	27.2 ± 2.2	+4.4 ± 0.6
Ft Myers Shores	0.04 ± 0.01	0.14 ± 0.03	0.82 ± 0.07	0.18 ± 0.04	0.06 ± 0.02	9 ± 2	0.96 ± 0.10	0.09 ± 0.02	27.9 ± 4.8	+10.7 ± 1.8 ↑
Hendry Creek	0.10 ± 0.01 ↑	0.07 ± 0.01	0.84 ± 0.03	0.17 ± 0.02	0.02 ± 0.00 ↓	31 ± 16 ↑	0.91 ± 0.02	0.06 ± 0.004 ↓	32.5 ± 1.9 ↑	+4.3 ± 0.6
Heritage Farms	0.36 ± 0.05 ↑	0.19 ± 0.03	1.36 ± 0.07 ↑	0.56 ± 0.05 ↑	0.03 ± 0.01 ↓	83 ± 19 ↑	1.58 ± 0.07 ↑	0.06 ± 0.01 ↓	58.8 ± 5.9 ↑	+2.6 ± 1.5
Lake McGregor	0.13 ± 0.03	0.09 ± 0.04	0.77 ± 0.02	0.22 ± 0.07	0.03 ± 0.01 ↓	17 ± 4 ↑	0.86 ± 0.05	0.07 ± 0.01	32.4 ± 7.7	+3.9 ± 1.6
Laurelin Court	0.06 ± 0.01	0.16 ± 0.02	1.12 ± 0.07	0.22 ± 0.03	0.09 ± 0.02	6 ± 1	1.27 ± 0.09	0.16 ± 0.02	19.9 ± 2.3	+4.6 ± 0.8
Ligon Court	0.61 ± 0.30 ↑	0.07 ± 0.04	2.67 ± 0.41 ↑	0.68 ± 0.34	0.03 ± 0.01 ↓	60 ± 22 ↑	2.73 ± 0.41 ↑	0.12 ± 0.04	59.6 ± 16.8 ↑	No Data
Mobile Manor	0.04 ± 0.01	0.17 ± 0.03	0.65 ± 0.03 ↑	0.21 ± 0.03	0.14 ± 0.04	5 ± 1	0.82 ± 0.04	0.22 ± 0.03 ↑	9.0 ± 0.9 ↓	+8.8 ± 1.0 ↑
North Town	0.03 ± 0.01	0.10 ± 0.05	0.90 ± 0.08	0.13 ± 0.05	0.06 ± 0.01	4 ± 1	1.00 ± 0.11	0.09 ± 0.01	25.9 ± 2.3	+5.0 ± 0.9
Orange River	0.05 ± 0.01	0.20 ± 0.03	1.03 ± 0.02	0.25 ± 0.02	0.10 ± 0.02	6 ± 1	1.25 ± 0.03	0.14 ± 0.02	20.9 ± 2.2	+4.4 ± 0.9
Overlook Drive	0.36 ± 0.08 ↑	0.10 ± 0.02	2.08 ± 0.19 ↑	0.46 ± 0.10 ↑	0.05 ± 0.01	55 ± 27 ↑	2.19 ± 0.19 ↑	0.18 ± 0.02 ↑	28.5 ± 3.2	+0.4 ± 0.7 ↓
Page Park	0.09 ± 0.01 ↑	0.08 ± 0.02	0.53 ± 0.03 ↓	0.17 ± 0.02	0.01 ± 0.00 ↓	44 ± 9 ↑	0.60 ± 0.03 ↓	0.03 ± 0.00 ↓	43.2 ± 6.1 ↑	+9.7 ± 0.2 ↑
Pine Island	0.06 ± 0.02	0.03 ± 0.01	0.83 ± 0.07	0.09 ± 0.03	0.02 ± 0.00 ↓	9 ± 2	0.86 ± 0.07	0.05 ± 0.00 ↓	38.6 ± 2.8 ↑	+1.0 ± 0.1
Pine Island 02	0.10 ± 0.05	0.04 ± 0.02	0.95 ± 0.08	0.14 ± 0.07	0.03 ± 0.01 ↓	8 ± 2	0.99 ± 0.10	0.07 ± 0.01	31.6 ± 2.6 ↑	+0.2 ± 1.0
San Carlos Park	0.17 ± 0.05	0.12 ± 0.05	0.59 ± 0.06 ↓	0.30 ± 0.02	0.01 ± 0.00 ↓	74 ± 14 ↑	0.71 ± 0.05	0.04 ± 0.00 ↓	50.0 ± 7.3 ↑	-2.2 ± 4.3
Summerwood Dr	0.60 ± 0.17 ↑	0.01 ± 0.00 ↓	1.60 ± 0.20 ↑	0.61 ± 0.17	0.10 ± 0.02	15 ± 4	1.60 ± 0.20	0.16 ± 0.02	22.9 ± 2.6	No Data
Tidewater Isl 01	0.06 ± 0.01	0.05 ± 0.01	0.72 ± 0.05	0.11 ± 0.02	0.01 ± 0.00 ↓	30 ± 6 ↑	0.78 ± 0.05	0.03 ± 0.00 ↓	59.9 ± 7.1 ↑	+4.3 ± 0.4
Tidewater Isl 02	0.05 ± 0.01	0.06 ± 0.01	0.82 ± 0.12	0.11 ± 0.02	0.01 ± 0.00 ↓	38 ± 3 ↑	0.88 ± 0.11	0.05 ± 0.01 ↓	50.6 ± 6.5 ↑	+3.7 ± 0.9
Waterway Ests 01	0.12 ± 0.03	0.15 ± 0.04	1.08 ± 0.08	0.27 ± 0.07	0.13 ± 0.02	5 ± 1	1.22 ± 0.12	0.18 ± 0.03	17.5 ± 2.5	+4.7 ± 0.4
Waterway Ests 02	0.07 ± 0.02	0.14 ± 0.05	1.02 ± 0.09	0.21 ± 0.06	0.09 ± 0.02	4 ± 1	1.17 ± 0.14	0.14 ± 0.02	20.6 ± 2.8	+4.6 ± 0.6
Yacht Club	0.04 ± 0.01	0.10 ± 0.01	1.02 ± 0.05	0.14 ± 0.02	0.09 ± 0.02	4 ± 1	1.11 ± 0.06	0.13 ± 0.02	20.5 ± 2.2	+4.1 ± 1.0

3.6 Spearman correlations

Spearman correlations revealed many significant relationships between variables (Table 6). Enterococci concentrations were negatively correlated with salinity (Spearman r = -0.47, $p < 0.001$) and DO (Spearman r = -0.37, $p < 0.001$), but positively correlated with TN (Spearman r = 0.31, $p < 0.001$), TP (Spearman r = 0.33, $p < 0.001$), and NH_4^+ (Spearman r = 0.48, $p < 0.001$). Enterococci was additionally positively correlated with *E. coli*, NO_x, DIN, TKN, and SRP ($p < 0.05$). *E. coli* concentrations were negatively correlated with salinity (Spearman r = -0.24, p = 0.001) and DO (Spearman r = -0.24, p = 0.001), but positively correlated with TP (Spearman r = 0.154, p = 0.03) and NH_4^+ (Spearman r = 0.18, p = 0.01). HF183 was positively correlated with enterococci (Spearman r = 0.32, $p < 0.001$), *E. coli* (Spearman r = 0.29, $p < 0.001$), NH_4^+ (Spearman r = 0.24, p = 0.001), and NO_x (Spearman r = 0.15, p = 0.04). A negative correlation between HF183 and salinity was also observed (Spearman r = -0.22, p = 0.002). NH_4^+ exhibited a positive correlation with rainfall (Spearman r = 0.32, $p < 0.001$) and negative correlation with salinity (r = -0.31, $p < 0.001$). Additionally, NH_4^+ was positively correlated with BOD, NO_x, DIN, TKN, SRP, and TP, and negatively correlated with DO ($p < 0.05$). NO_3^- was positively correlated with DIN, TKN, TN, SRP, and TP, and negatively correlated with salinity ($p < 0.05$). TP exhibited a strong positive correlation with TN (Spearman r = 0.6, $p < 0.001$). Sucralose was positively correlated with BOD, NH_4^+, DIN, TKN, and TN ($p < 0.05$). HF183 was positively correlated with *E. coli*, enterococci, NH_4^+, NO_x, and DIN, and negatively correlated with DO, salinity, and temperature ($p < 0.05$).

Table 5. Matrix showing the Spearman Rank correlation coefficients (r) for water quality parameters tested.

	Sucralose	BOD	HF183	Entero-cocci	*E. coli*	NH_4^+	NO_x	SRP	TN	TP	Salinity	Rainfall	DO	Temp
BOD	0.14													
HF183	0.07	-0.05												
Enterococci	0.09	0.06	0.32											
E. coli	-0.04	-0.10	0.29	0.70										
NH_4^+	0.31	0.18	0.24	0.48	0.18									
NO_x	0.00	-0.19	0.15	0.23	0.08	0.21								
SRP	-0.12	0.01	-0.12	0.19	0.05	0.14	0.35							
TN	0.20	0.26	0.01	0.31	-0.03	0.50	0.45	0.45						
TP	0.00	0.17	-0.08	0.33	0.15	0.25	0.30	0.87	0.60					
Salinity	0.03	0.05	-0.22	-0.47	-0.24	-0.31	-0.46	-0.12	-0.23	-0.18				
Rainfall	0.05	0.27	-0.02	0.11	-0.06	0.32	0.03	0.19	0.27	0.19	-0.13			
DO	-0.10	-0.18	-0.34	-0.37	-0.24	-0.47	0.00	-0.04	-0.12	-0.10	0.27	-0.42		
Temp	-0.07	0.19	-0.14	-0.10	-0.13	0.05	-0.13	0.09	-0.01	0.08	0.05	0.72	-0.33	
$\delta^{15}N\text{-}NO_3^-$	0.06	-0.24	-0.05	0.11	0.25	-0.46	-0.16	-0.05	-0.53	-0.09	0.03	0.03	0.13	0.14

Negative Correlation | No Correlation | Positive Correlation

r = -1 | r = 0 | r = 1

3.7 Particulate organic matter stable isotopes and elemental composition

The overall median C:N ratio in POM collected from surface water across all 25 sites was 8.5, with individual values ranging from 2.4 to 27.7 (Figure 19). C:N ratios > 8 (indicating N-limitation) were detected in 55% of all samples and at least once at 96% of sites, including the Reference site. The mean C:N ratio at the Reference site was 8.3 ± 1.0. There were 15 sites with a mean C:N ratio > 8. The highest ratios were found at San Carlos Park (14.9 ± 2.8), Billy Creek (13.1 ± 0.7), and Mobile Manor (12.6 ± 1.1). Billy Creek and Mobile Manor were the only sites with a mean C:N ratio that was significantly higher than the Reference site (Mann-Whitney U, $p < 0.05$). C:N ratios < 6 (indicating C-limitation) were detected in 7% of all samples and at least once at 36% of sites, including the Reference site. There were no sites with a mean C:N ratio < 6. There were no sites with a mean C:N ratio that was significantly lower than the Reference site (Mann-Whitney U, $p > 0.05$). There were no significant differences in mean C:N ratios between the dry season (9.5 ± 0.4) and wet season (8.9 ± 0.3; t-test, $p = 0.23$).

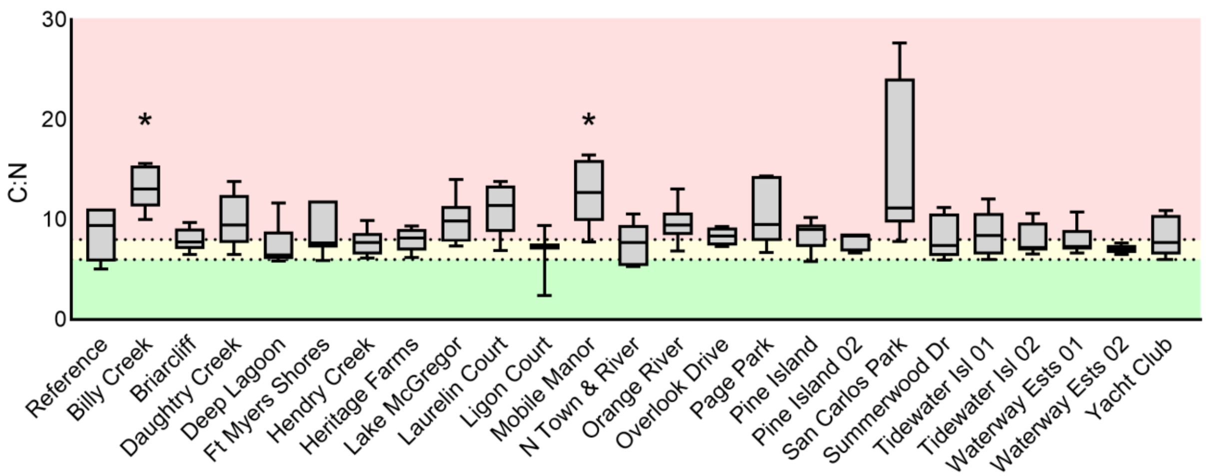

Figure 19. Particulate organic matter (POM) carbon to nitrogen elemental ratios (C:N) in Lee County surface water. Whiskers show the maximum and minimum measured value at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent N-limited, balanced, and C-limited nutrient regimes. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median N:P ratio in POM collected from surface water across all 25 sites was 14.9, with individual values ranging from 1.8 to 148 (Figure 21). N:P ratios > 30 (indicating P-limitation) were detected in 2% of all samples and at least once at 8% of sites with septic systems. The mean N:P ratio at the Reference site was 11.8 ± 1.7. There were four sites with a significantly higher mean N:P ratio than the Reference site, including Briarcliff (17.7 ± 0.8), Ligon Court (68.8 ± 40.3), Overlook Drive (19.3 ± 0.9), and Tidewater Island 02 (20.6 ± 4.1). Ligon Court was the only site with a mean N:P ratio > 30. N:P ratios < 15 (indicating N-limitation) were detected in 50.1% of all samples and at least once at 92% of sites, including the Reference site. Billy Creek (6.9 ± 2.7) and Mobile Manor (5.4 ± 0.8) were the only sites with a mean N:P ratio that was significantly lower than the Reference site (Mann-Whitney U, $p < 0.05$). There were no significant differences in mean N:P ratios between the dry season (16.2 ± 1.8) and wet season (14.1 ± 0.6; t-test, $p = 0.25$).

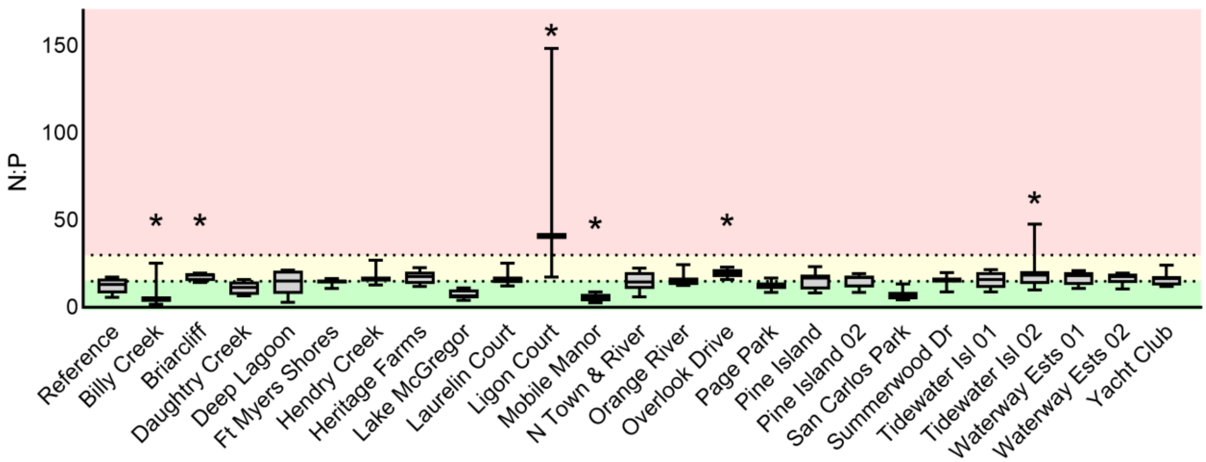

Figure 20. Particulate organic matter (POM) nitrogen to phosphorus elemental ratios (N:P) in Lee County surface water. Whiskers show the maximum and minimum measured value at each site. The line in each box shows the median concentration at each site. The red, yellow, and green shaded areas represent P-limited, balanced, and N-limited nutrient regimes. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median $\delta^{15}N$ value in POM collected from surface water across all 25 sites was +4.1‰, with individual values ranging from -3.8 to +16.0‰ (Figure 22). POM $\delta^{15}N$ values in the range of human waste (> +4.9‰) were detected in 39% of all samples and at least once at 88% of all sites, including the Reference site. There were 8 sites with a mean POM $\delta^{15}N$ value in the range of human waste, including Deep Lagoon (+6.0 ± 0.8‰), Fort Myers Shores (+6.9 ± 1.9‰), Lake McGregor (+5.3 ± 0.8‰), Laurelin Court (+6.8 ± 1.5‰), North Town & River (+8.2 ± 1.5‰), Orange River (+5.6 ± 1.0‰), Waterway Estates 02 (+5.7 ± 0.5‰), and the Reference site (+5.2 ± 0.9‰). There were no sites with a significantly higher mean POM $\delta^{15}N$ value than the Reference site. There were six sites with a mean POM $\delta^{15}N$ value that was significantly lower than the Reference site (Mann-Whitney U, $p < 0.05$), including Billy Creek (+2.5 ± 0.7‰), Briarcliff (+2.5 ± 0.7‰), Daughtry Creek (+2.6 ± 1.0‰), Hendry Creek (+2.8 ± 0.5‰), Heritage Farms (+1.4 ± 0.5‰), and Pine Island 02 (+1.6 ± 0.8‰). There were no significant differences in mean POM $\delta^{15}N$ values between the dry season (+3.9 ± 0.3‰) and wet season (+4.1 ± 0.3‰; t-test, $p = 0.62$).

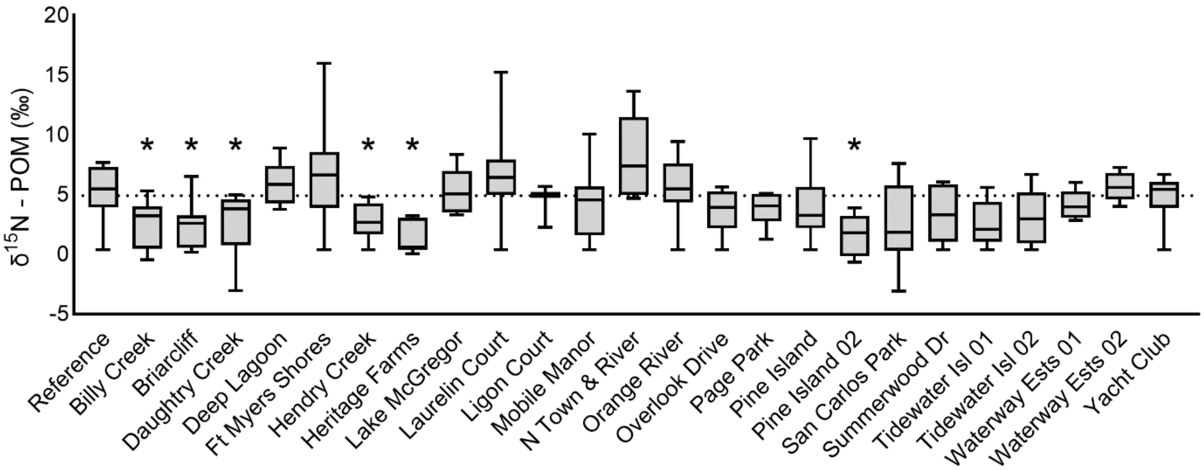

Figure 21. Particulate organic matter (POM) nitrogen stable isotopes ($\delta^{15}N$) in Lee County surface water. Whiskers show the maximum and minimum measured value at each site. The line in each box shows the median concentration at each site. Values above the dotted line indicate a human waste source. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

The overall median $\delta^{13}C$ value in POM collected from surface water across all 25 sites was -28.7‰, with individual values ranging from -39.2 to -18.0‰ (Figure 23). Depleted POM $\delta^{13}C$ values indicating a terrestrial origin (< -25‰) were detected in 90% of all samples and at least once at 96% of all sites, including the Reference site. The mean POM $\delta^{13}C$ value at the Reference site was -26.8 ± 0.4‰. Pine Island, Pine Island 02, and Ligon Court were the only sites with a mean POM $\delta^{13}C$ value > -25‰. In the case of Pine Island and Pine Island 02 this is likely reflective of a marine origin, whereas for Ligon Court this is indicative of increased relative abundance of C_4 plants (St. Augustine grass, *Stenotaphrum secundatum*). There were no significant differences in mean POM $\delta^{15}C$ values between the dry season (-28.8 ± 0.4‰) and wet season (-28.9 ± 0.3‰; t-test, $p = 0.88$).

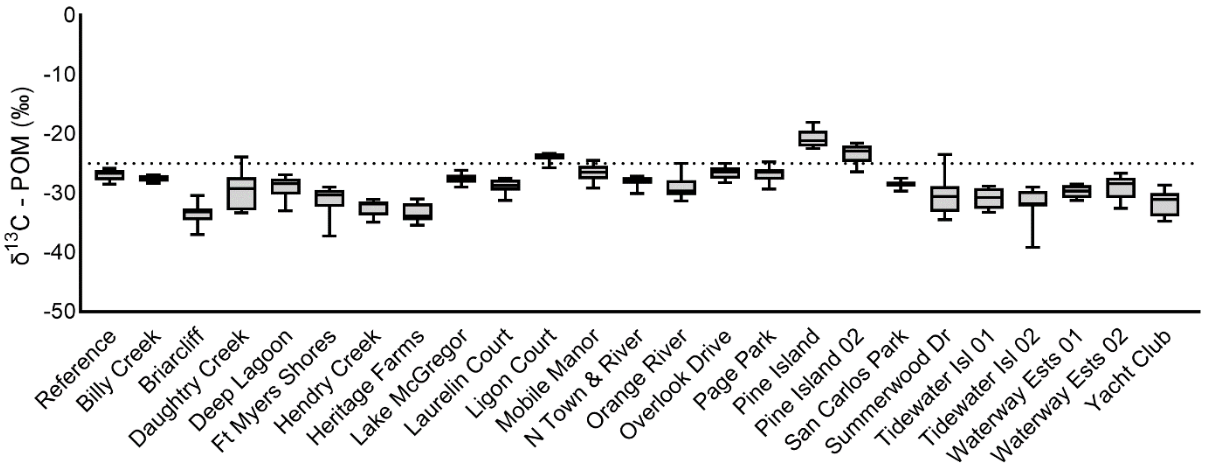

Figure 22. Particulate organic matter (POM) carbon stable isotopes ($\delta^{13}C$) in Lee County surface water. Whiskers show the maximum and minimum measured value at each site. The line in each box shows the median concentration at each site. Values below the dotted line indicate a terrestrial C source (C_3 plants) and values above the dotted line indicate a marine or C_4 plant C source. Asterisks (*) indicate a significant difference from the Reference site (Mann-Whitney U, $p < 0.05$).

3.8 Macrophyte stable isotopes and elemental composition

Elemental (C:N:P) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition of macrophyte tissue collected opportunistically from Lee County waterbodies is displayed in Table 5. Molar C:N ratios ranged from 11.3 to 32.5 with a median value of 14.8. Molar N:P ratios ranged from 12.5 to 64.4 with a median value of 21.9. There were no C-limited macrophytes (C:N < 6). N-limited macrophytes are identified by a C:N ratio > 8 and N:P ratio < 15. P-limited macrophytes are identified by a N:P ratio > 30. Stable carbon isotope values ($\delta^{13}C$) ranged from -37.6 to -11.7‰ with a median value of -24.0‰. Depleted POM $\delta^{13}C$ values < -25‰ indicate a terrestrial origin. Values > -25‰ likely indicate a marine origin for Fort Myers Beach. For all other sites (freshwater), POM $\delta^{13}C$ values > -25‰ indicate a C_4 plant origin (e.g., St. Augustine grass, *Stenotaphrum secundatum*). Stable nitrogen isotope values ($\delta^{15}N$) ranged from +3.9 to +18.4‰ with a median value of +8.8‰. Human waste influence is indicated by $\delta^{15}N$ values > 4.9‰.

Table 6. Macrophyte elemental (C:N:P) and isotopic ($\delta^{13}C$ and $\delta^{15}N$) composition (mean ± standard error). The algal mats found at several sites are nondescript filamentous algae.

Site	Species	C:N	N:P	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Briarcliff	Algal mat	25.2 ± 0.3	58.7 ± 3.2	-17.4 ± 0.2	+4.6 ± 0.0
Fort Myers Beach	*Hydropuntia secunda*	13.7 ± 0.2	17.0 ± 0.8	-23.9 ± 0.1	+4.5 ± 0.1
Fort Myers Beach	*Ulva lactuca*	31.9 ± 0.5	25.5 ± 0.7	-20.7 ± 0.4	+4.6 ± 0.1
Heritage Farms	Algal mat	16.8 ± 1.2	28.5 ± 2.9	-18.6 ± 0.6	+9.4 ± 0.1
Heritage Farms	*Pistia stratiotes*	14.2 ± 0.1	19.9 ± 0.2	-30.3 ± 0.1	+13.4 ± 0.2
Lake McGregor	*Cladophora prolifera*	13.8 ± 0.2	23.4 ± 0.5	-36.8 ± 0.4	+9.5 ± 0.2
Lake McGregor	*Hydrilla verticillata*	11.7 ± 0.1	17.0 ± 0.4	-37.3 ± 0.1	+8.0 ± 0.4
Mobile Manor	*Halodule wrightii*	12.0 ± 0.2	14.8 ± 1.4	-31.4 ± 0.1	+11.1 ± 0.0
Overlook Drive	Algal mat	18.2 ± 0.9	27.9 ± 5.5	-12.0 ± 0.2	+5.9 ± 0.2
Page Park	Algal mat	20.9 ± 1.8	34.9 ± 1.1	-18.8 ± 0.9	+7.8 ± 0.6
San Carlos Park	*Pistia stratiotes*	15.9 ± 0.4	21.9 ± 0.8	-29.1 ± 0.2	+8.4 ± 1.3
Summerwood Drive	Algal mat	15.4 ± 0.8	19.6 ± 2.6	-19.2 ± 0.5	+7.4 ± 1.3
Summerwood Drive	*Pistia stratiotes*	12.8 ± 0.1	13.8 ± 0.4	-28.3 ± 0.1	+9.0 ± 0.1
	Overall Average	**17.0 ± 0.6**	**25.0 ± 1.3**	**-24.8 ± 0.9**	**+8.0 ± 0.4**

4. Discussion

This study provides the first extensive evaluation of water quality parameters to assess human waste contamination in Lee County surface waters. Indicators of human waste influence, such as high fecal bacteria, HF183, chemical tracers, elevated NH_4^+ concentrations, and elevated $\delta^{15}N\text{-}NO_3^-$ values, were observed at many of the study sites. Many of these indicators were not found to be seasonally variable, which demonstrates the influence of human waste contamination throughout the year. A detailed analysis of the results is provided for each individual site in the Appendix of this report.

4.1 Fecal indicator bacteria

Fecal indicator bacteria concentrations exceeding the FDEP 62-302 ten percent threshold value for Class III waters were detected in 66% of samples collected during this study with no seasonal variability. The highest bacterial counts were found at Billy Creek, Lake McGregor, Ligon Court, and Overlook Drive. Bacteria at these sites often exceeded the upper limits of detection. Common sources of enterococci and *E. coli* include septic systems, aging sewer infrastructure, wastewater treatment plant effluent, animal waste, and other environmental sources (Byappanahalli et al., 2012). In this study, NH_4^+ significantly correlated with enterococci ($r = 0.48$) and *E. coli* ($r = 0.18$), suggesting a common source. Septic systems adjacent to Lake McGregor, Ligon Court, and Overlook Drive may contribute to the high bacterial counts observed at these sites. Enterococci and *E. coli* can survive wastewater treatment and can be found in wastewater treatment plant effluent and reclaimed water (Nagulapally et al., 2009; Carey et al., 2016). However, wastewater treatment plants that utilize chlorination in tertiary treatment have demonstrated effectiveness in reducing these bacteria to undetectable levels (Tree et al., 2003; Goldstein et al., 2014). Municipal wastewater treatment in Lee County utilizes chlorination. Therefore, it is unlikely that wastewater treatment plant effluent is a significant contributor of bacteria in the study area. By extension, reclaimed water application is also an unlikely source of bacteria in the study area because this too receives chlorination during treatment. Apart from the Reference site, other sites with low concentrations of fecal indicator bacteria include Briarcliff, Deep Lagoon, North Town & River, Pine Island, Pine Island 02, and Yacht Club. Of all sites sampled in this study, Deep Lagoon, North Town & River, Pine Island, and Pine Island 02 had the greatest marine influence, with mean salinities >10 ppt (mean salinity at the Reference site was 7.2). Negative correlations have been reported between salinity and fecal indicator bacteria (Lapointe et al., 2012; Price et al., 2021). Further, in the presence of ultraviolet radiation, *E. coli* survival rates have been shown to decline sharply as salinity increases above 10 ppt (Chan and Killick, 1995). This agrees with the results from this study, as we found that salinity was negatively correlated with enterococci and *E. coli* concentrations in surface water samples. This suggests that, although fecal indicator bacteria concentrations are low at these marine influenced sites, this may be explained by low survival rates rather than decreased loading. It has also been shown that dilution from tidal flushing plays a role (Coelho et al., 1999; Wilhelm et al., 2002; Mill et al., 2006). Enterococci and *E. coli* concentrations in surface water are often positively correlated with rainfall (Lipp et al., 2001; Stumpf et al., 2010; Mance et al., 2021). However, no such correlations between fecal indicator bacteria and rainfall were found in this study, even when sites with mean salinities >5 ppt were excluded from analysis. Further, there were no seasonal differences observed in fecal indicator bacteria concentrations. These observations may be attributed to a lack of sampling in May or June (due to COVID-19 travel restrictions). It is possible that if our sampling had captured the "first flush" of the wet season, we might have observed a relationship between fecal indicator bacteria and rainfall. Fecal indicator bacteria populations also tend to increase in the environment where P concentrations are high (Lebaron et al., 1999; Ishii et al., 2006; He et al., 2007; Toothman et al., 2009; Surbeck et al., 2010; Chudoba et al., 2013; Mallin and Cahoon, 2020). Therefore, at sites with high P, enterococci and *E. coli* populations may not be indicative of recent human waste pollution; rather, these populations are sustained by P inputs that allow them to persist and grow in the environment. The results from this study show a similar relationship, as enterococci and *E. coli* were positively correlated with TP. The relationship between fecal indicator bacteria and the environment is demonstrably complex, as many factors can affect concentrations in surface water. This highlights the importance of using additional parameters to assess water quality.

HF183 was detected in half of the samples collected in this study and did not vary seasonally, indicating year-round influence of human waste. Most sites had isolated or sporadic detections of HF183 in high concentrations, but two sites had consistently high concentrations: Heritage Farms and San Carlos Park. The San Carlos Park site was located in a residential neighborhood with high densities of septic systems. Therefore, it is likely that the high concentrations of HF183 observed at this location originated from septic system leachate. The Heritage Farms site was also near septic systems that may have been the source of HF183 at this location. However, septic systems adjacent to the Heritage Farms site are relatively sparse. Reclaimed water application on the adjacent golf course represents another potential HF183 source. The highest concentrations of HF183 were detected at Tidewater Island 02 (23,700 copies/100mL, September 2020) and Summerwood Drive (10,700 copies/mL, January 2021). 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. HF183 was positively correlated with enterococci and *E. coli*, confirming that a significant portion of these bacterial detections originate from a human waste source. Positive correlations with HF183 were also observed for NH_4^+, further connecting these detections and N loading to human waste, as NH_4^+ concentrations in untreated sewage and septic system effluent are generally high (Heaton et al., 2012). A negative correlation between HF183 and salinity was also observed. This is in alignment with other studies (Mika et al., 2014) and may be explained by higher dilution at sites with tidal flushing rather than decreased loading from human waste sources.

4.2 Using chemical tracers to identify human waste sources

Sucralose concentrations in Lee County surface waters were generally high and positively correlated with NH_4^+ (Spearman $r = 0.31$, $p < 0.001$). Prior studies have found a similar relationship between sucralose and NH_4^+ that was attributed to non-functioning, poorly sited septic systems (Lapointe et al., 2017; Herren et al., 2021). High concentrations of sucralose (>380 ng/L) were found in 54% of samples and at least once at every site, except Billy Creek, Daughtry Creek, Pine Island, Pine Island 02, and Yacht Club. The highest and most consistent sucralose concentrations were found at Heritage Farms, Ligon Court, Mobile Manor, and Overlook Drive. The Mobile Manor site is in a drainage ditch adjacent to a residential community with very high densities of septic systems, thus the sucralose observed here is likely attributable to human waste contamination from septic systems. Heritage Farms, Ligon Court, and Overlook Drive are all also adjacent to septic systems, but the density is lower. Additional sources of sucralose may also exist at these sites, including reclaimed water application on nearby golf courses or leaky municipal sewer infrastructure. Therefore, sucralose concentrations alone may not be enough to determine the exact human waste source(s) affecting these sites.

Other chemical tracers of human waste were found in surface waters throughout Lee County. Carbamazepine was detected at least once at every site, except Billy Creek, Briarcliff, Fort Myers Shores, Lake McGregor, Pine Island, Pine Island 02, and Summerwood Drive. The highest concentrations were observed at Page Park (12 ng/L). Septic system leachate is a likely source of carbamazepine at many of these sites, but alternative sources also are possible, such as reclaimed water runoff, leaky sewer infrastructure, and background concentrations in the Caloosahatchee River from upstream sources, such as wastewater treatment plant effluent. Carbamazepine concentrations in wastewater are usually around 1 mg/L (Fenz et al., 2005). Accordingly, carbamazepine is a useful indicator of human waste contamination, but it is not useful for differentiating between septic system effluent and treated municipal wastewater sources. Prior studies have found carbamazepine in both septic system and municipal wastewater treatment effluent (Godfrey et al., 2007; Luo et al., 2014). Carbamazepine detection rates of 23% are reported in soil pore water contaminated by septic system effluent (Yang et al., 2017), whereas detections in treated wastewater are much higher due to low removal efficiencies (<10%; Ternes, 1998; Zhang et al., 2008). By contrast, wastewater treatment removal efficiencies >90% are reported for acetaminophen and ibuprofen

(Clara et al., 2005; Joss et al., 2006; Santos et al., 2007; Kasprzyk-Hordern et al., 2009; Du et al. 2014). Acetaminophen removal in septic systems is much lower, as the reported detection rate in septic system effluent is in the range of 53 – 83% (James et al., 2016; Yang et al., 2017). This makes acetaminophen a useful tracer of septic system contamination, especially where multiple wastewater pollution sources exist. Unfortunately, its usefulness is limited because dilution in surface water causes concentrations to rapidly fall below detection limits. Even in the highly impaired Hudson River acetaminophen detection rates are low and, of those detections that are found nearest human waste sources, concentrations are extremely low (< 18 ng/L; Cantwell et al., 2018). These limitations are reflected in the results of this study, as acetaminophen was only detected in 2% of samples. These detections occurred at Lake McGregor (33 ng/L), Briarcliff (21 ng/L), Hendry Creek (9 ng/L), and Daughtry Creek (9 ng/L). Thus, these acetaminophen detections provide strong evidence of human waste contamination via septic system effluent at these sites.

4.3 Nutrients and stable isotopes

Moderate to high concentrations of TN were observed throughout Lee County with no seasonal variability. Consistently high TN concentrations occurred at Heritage Farms, Ligon Court, and Overlook Drive. Nearby septic systems may be a contributing source of TN, as septic system effluent typically averages ~60 mg/L (Robertson et al., 2012). However, septic system densities at Heritage Farms, Ligon Court, and Overlook Drive are not high when compared to other sites in this study which have lower TN concentrations. For example, San Carlos Park and Mobile Manor have very high septic density and no nearby application of reclaimed water, but only moderate concentrations of TN (0.46 – 1.00 mg/L). Improperly functioning septic systems directly adjacent to the Heritage Farms, Ligon Court, and Overlook Drive sites may account for this discrepancy. Stormwater runoff is another potential source of TN, as other studies in Florida have found TN concentrations in urban stormwater runoff typically average between 1 – 3 mg/L (Badruzzaman et al., 2012; Jani et al., 2020). Notably, all three of the sites with high TN concentrations are adjacent to golf courses on which fertilizers and/or reclaimed water are applied. Monthly average TN concentrations in reclaimed water from the Fiesta Village Wastewater Treatment Plant that services this area range from 0.6 to 1.3 mg/L. Although nutrient uptake by turfgrass greatly reduces the amount of nitrogen that reaches surface water through runoff (40 – 90% uptake; Sartain, 1985; Bowman et al. 2002; Wherley et al., 2009; Stiegler et al., 2011), fertilizer and reclaimed water nitrogen sources may still contribute to the high TN concentrations observed at these sites. Overall, mean TN concentrations were below the FDEP 62-302 ten percent threshold value for Class III waters. However, this study finds that there are limitations to monitoring for only TN concentrations, as this ignores important N species such as NH_4^+.

Moderate to high NH_4^+ concentrations were also observed throughout Lee County with no seasonal variability. Although no differences were observed between the wet and dry seasons, NH_4^+ was positively correlated with rainfall and negatively correlated with salinity, indicating increased loading following rain events. Nitrogen in septic system effluent is primarily in the form of NH_4^+ and prior studies showed that this reduced N form was 6.5-fold higher than NO_3^- in North Fort Myers groundwater impacted by septic systems (Lapointe et al., 2021). Improperly located septic systems in areas with high water tables and porous sandy soils facilitate rapid transport of NH_4^+ enriched groundwaters to nearby surface waters (LeBlanc, 1985; Ptacek, 1998). In this study, we identified eight NH_4^+ rich sites, including Heritage Farms, Lake McGregor, Ligon Court, Overlook Drive, Pine Island, Pine Island 02, San Carlos Park, and Summerwood Drive. Note that the three sites identified as having high TN (Heritage Farms, Ligon Court, and Overlook Drive) are all included here, but examination of N speciation data revealed five additional sites with potential human waste influence. This highlights the limitations of TN monitoring and the advantage of including N speciation data (e.g. NH_4^+) in water quality analyses. Pine Island, Pine Island 02, and San Carlos Park have high septic system density with no municipal sewer infrastructure nearby; therefore, septic systems are likely the primary contributor of NH_4^+ at these sites. While Pine Island and Pine Island 02 were enriched in NH_4^+, the overall concentrations were lower compared to the other sites listed above. This may be attributed to a dilution effect rather than reduced N loading, since these two sites experience a greater influence of tidal pumping (Lapointe et al. 1990). The areas surrounding Heritage Farms, Lake McGregor, Ligon Court, Overlook Drive, and Summerwood Drive are primarily serviced by municipal sewer, but some septic systems are adjacent to these sites as well. The highest concentrations of NH_4^+ were detected at Summerwood Drive (up to 1.34 mg/L). Assuming proper functioning of municipal sewer infrastructure, the most likely source of NH_4^+ is nearby septic systems. While homes in the Summerwood area are on relatively large lots resulting in low septic system density, placement of drainfields close to surface water or high groundwater levels could explain the higher NH_4^+ at this site. Alternatively, the high NH_4^+ observed here may be from another source, such as aging sewer infrastructure. At other sites with high NH_4^+, such as Heritage Farms, Lake McGregor, Ligon Court, and Overlook Drive, adjacent golf courses may also contribute NH_4^+ to surface water through the application of fertilizers. Depending on the level of treatment prior to application, secondary effluent that is used as reclaimed water can be rich in NH_4^+ and poor in NO_3^- (NH_4^+ = 9.5 mg/L, NO_3^- = 1.4 mg/L; Asano et al., 2007). However, reclaimed water from the Fiesta Village Wastewater Treatment Plant is unlikely to serve as a significant source of NH_4^+ because it is treated to surface water discharge standards.

TP concentrations were moderate across all sites, but the highest concentrations approached 0.4 mg/L, which is 3 times greater than the FDEP threshold for impaired waterbodies. Billy Creek, Mobile Manor, and Overlook Drive were identified as sites with consistently high TP concentrations and septic system effluent is a likely P source at these sites. Mobile Manor has high density septic systems directly adjacent and upstream of the site with no municipal sewer infrastructure. The areas surrounding Billy Creek and Overlook Drive are primarily serviced by municipal sewer, but some septic systems are present directly adjacent to both sites. Septic system effluent is P rich (Corbett et al., 2002; Efroymson et al., 2007) and although soil adsorption may significantly reduce the amount of P that reaches groundwater and adjacent surface water, improperly located septic systems in areas with high water tables and porous sandy soils may facilitate rapid transport of unadsorbed P to nearby waterbodies. Further, installation of septic tanks in high densities may cause the subsurface soil environment to transition from a P sink to a P source over long periods of time as the soil becomes P saturated (Robertson et al., 1998; Robertson, 2008). Other potential sources of P include fertilizers or natural weathering of soil minerals. A significant portion (if not the majority) of dissolved phosphorus detected in this study may be derived from phosphate bearing geological formations in or upstream from the Caloosahatchee River watershed (Odum, 1953). Nearby fertilizer application on a golf course adjacent to Overlook Drive may also contribute to high TP. A quantitative analysis by Baris et al. (2010) found that 86% of surface water samples collected from waterbodies adjacent to golf courses throughout the US were in exceedance of local water quality standards for P. Water quality reports from the Fiesta Village Wastewater Treatment Plant that provides reclaimed water for this area indicate that while their alum treatment for P removal is generally effective, resulting in effluent TP concentrations < 0.1 mg/L, extended periods of high flow can yield concentrations up to 0.26 mg/L. TP exhibited a strong positive correlation with TN, which is unsurprising given that these nutrients often originate from a common source.

Stable nitrogen isotope values ($\delta^{15}N$) in dissolved NO_3^-, POM, and macrophytes suggest multiple nitrate sources, including human waste, soil organics, and synthetic fertilizers (Kendall, 1998; Hinkle et al., 2008; Aravena and Mayer, 2009; Zhang et al., 2018). The highest $\delta^{15}N$ values were detected at Fort Myers Shores (up to +14.5‰ in NO_3^- and +16.0‰ in POM), Heritage Farms (up to 13.7‰ in macrophytes), Laurelin Court (up to +15.2‰ in POM) Mobile Manor (up to +11.3‰ in NO_3^-, +10.1‰ in POM, and 11.1‰ in macrophytes), North Town & River (up to +13.7‰ in POM), Page Park (up to +10.1‰ in NO_3^-), San Carlos Park (up to 18.4‰ in macrophytes), and Summerwood Drive (up to 11.2‰ in macrophytes). Based on published N-isotope ranges, human waste is likely the primary source of N at these locations. This data is in alignment with the land use directly upstream of many of these sites, which is mostly residential communities serviced by septic systems. Other sources could include manure from livestock on upstream pastureland or municipal sewer infrastructure. Although various bacterial and chemical human waste tracers were detected at every site in this study, many of these sites exhibited moderate dissolved $\delta^{15}N$ values in the range of soil N. A few sites in this study exhibited low $\delta^{15}N$ values < +2‰ in POM and NO_3^-, suggesting fertilizers as the primary nitrate source. Due to frequently overlapping isotopic signals, $\delta^{15}N$ data must be analyzed in the context of other human waste indicator data to obtain a holistic picture of contaminant sources at each site. Depleted $\delta^{13}C$ values in POM (< -25‰) revealed that the primary C source for most sites is 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 $\delta^{13}C$ values (-18 to -26‰). Ligon Court also had somewhat enriched $\delta^{13}C$ 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 $\delta^{13}C$ (about -13‰; Peterson and Fry, 1987).

Fertilizers have previously been implicated as a significant contributor to HABs in Florida. Consequently, policy makers have imposed residential fertilizer ordinances in several counties throughout the state (Hochmuth et al., 2009), including Lee County. Unfortunately, in some coastal areas these ordinances have not yielded the desired nutrient loading reductions, and water quality and HABs continue to worsen (Phlips et al., 2015; Lapointe et al., 2020; Krimsky et al., 2021). Septic systems and other urban human waste sources have been increasingly identified as another important factor contributing to HABs in Florida (Lapointe et al., 2012, 2015, 2021). The present study finds that human waste sources contribute to nutrient and bacteria loading in surface water throughout Lee County. This warrants close monitoring as these inputs may drive HABs and hazardous conditions within the Caloosahatchee River Estuary and nearby coastal waters. Overreliance on septic systems can result in elevated NH_4^+ loading to groundwater and nearby surface water (Lapointe et al., 2012, 2017, 2021); and because many primary producers prefer NH_4^+ over other N sources (Blomqvist et al., 1994; Beversdorf et al., 2015), this can play a critical role in the development of HABs.

Nutrient loading from human waste sources may also cause stoichiometric shifts in C:N:P. Elemental C:N ratios in POM samples were generally between 7 to 10 at most sites. The highest C:N values were found at Billy Creek (up to 15.6 in POM), Mobile Manor (up to 16.5 in POM), and San Carlos Park (up to 27.7). These elevated values (> 8) indicate N-limiting conditions. The highest DIN:SRP ratios were observed in urban drainage ditches, while lower ratios were usually observed at sites within the Caloosahatchee River Estuary. High DIN:SRP and TN:TP were observed in nearly a quarter of samples, specifically at Briarcliff, Heritage Farms, Ligon Court, Page Park, San Carlos Park, Tidewater Island 01, and Tidewater Island 02. Briarcliff had the highest DIN:SRP (up to 240) and TN:TP (up to 115) values. Notably, N:P ratios in POM and macroalgae rarely exceeded 30, indicating that these samples are not P-limited. This is expected since the geology in Lee County is P-rich. These data indicate that nitrogen is the most limiting nutrient in Lee County surface waters. The high NH_4^+ concentrations in conjunction with low N:P ratios observed in this study provide favorable conditions for the development of harmful algal blooms (Lapointe et al., 2012, 2015). This has local relevance, as blooms of *M. aeruginosa* have occurred in the Caloosahatchee River Estuary in 2005, 2016, and 2018 (Lapointe et al., 2006, 2020). Coastal red tides can also be stimulated by high NH_4^+ inputs. This was demonstrated in January 2005 when a severe *K. brevis* bloom developed off southwest Florida. This red tide event occurred after significant rain events from hurricanes Charley, Frances, and Jeanne, which drove a six-fold increase in coastal NH_4^+ concentrations in October 2004 (Lapointe and Bedford, 2007). The bloom persisted for much of the year and resulted in widespread hypoxia and fish mortalities. This shows the importance of reducing local N inputs, especially NH_4^+ from untreated domestic wastewater sources.

5. Conclusions

This MST study showed widespread human waste contamination throughout Lee County to varying degrees of severity. Accordingly, improvements to wastewater infrastructure may yield improved surface water quality and diminished risks to human health. This report will aid Lee County in the development of a focused master wastewater plan as described in Florida Senate Bill 712, also referred to as "The Clean Waterways Bill." A detailed site analysis is included in the Appendix of this report. The conclusions of this study are summarized below.

- High levels of fecal indicator bacteria were present year-round in surface water at 76% of sites in Lee County.
- The human fecal marker HF183 was present in surface water year-round at 52% of sites in Lee County and was positively correlated with fecal indicator bacteria, indicating that a significant portion of these bacteria originated from a human waste source.
- The widespread detection of sucralose and pharmaceuticals in surface water throughout Lee County further demonstrated the presence of human waste.
- High concentrations of NH_4^+ were observed throughout Lee County and positively correlated with HF183, suggesting that a significant fraction originated from a human waste source.
- Dissolved nitrate isotope values showed that nitrate sources included wastewater, soil, and fertilizer.
- At sites with clear evidence of human waste pollution, infrastructure upgrades may improve water quality in local and downstream waterbodies.

- At sites where the primary contaminant source remains unclear due to the presence of multiple potential sources, additional fine scale sampling may be necessary to identify those sources.

6. Acknowledgements

We would like to thank the Lee County Department of Natural Resources, especially Lisa Kreiger, David Reycraft, Bruce Westberry, and Roland Ottolini. We also thank the staff of the Lee County Environmental Lab, including Rick Armstrong and Mishel Mullan. We appreciate the Florida Department of Environmental Protection, including Julie Espy, David Whiting, John Watts, Kathy Lurding, and Cheryl Swanson for supporting the MST analyses for this project, as well as providing insightful comments and guidance.

7. References

Ahmed, W., Stewart, J., Powell, D., and Gardner, T. (2008). Evaluation of *Bacteroides* markers for the detection of human faecal pollution. *Letters in Applied Microbiology*, 46(2), 237-242.

Ahmed, W., Hughes, B., and Harwood, V.J. (2016). Current status of marker genes of Bacteroides and related taxa for identifying sewage pollution in environmental waters. *Water*, 8(6), p.231.

Ahmed, W., Gyawali, P., Feng, S., and McLellan, S.L. (2019). Host specificity and sensitivity of established and novel sewage-associated marker genes in human and nonhuman fecal samples. *Applied and Environmental Microbiology*, 85(14).

Al-Gheethi, A., Efaq, A., Bala, J., Norli, I., Abdel-Monem, M., and Kadir, M.A. (2018). Removal of pathogenic bacteria from sewage-treated effluent and biosolids for agricultural purposes. *Applied Water Science*, 8(2), 1-25.

Albertin, A.R., Sickman, J.O., Pinowska, A., and Stevenson, R.J. (2012). Identification of nitrogen sources and transformations within karst springs using isotope tracers of nitrogen. *Biogeochemistry*, 108(1), 219-232.

Altabet, M.A., Wassenaar, L.I., Douence, C., and Roy, R. (2019). A Ti (III) reduction method for one-step conversion of seawater and freshwater nitrate into N_2O for stable isotopic analysis of 15N/14N, 18O/16O and 17O/16O. *Rapid Communications in Mass Spectrometry*, 33(15), 1227-1239.

American Public Health Association, American Water Works Association, Water Pollution Control Federation and Water Environment Federation. (1912). *Standard Methods for the Examination of Water and Wastewater* (Vol. 2). American Public Health Association.

Anderson, D.L. (1998). March. Natural denitrification in groundwater impacted by onsite wastewater treatment systems. In *Proceedings of the eighth national symposium on individual and small community sewage systems* (pp. 336-345).

Aravena, R., Evans, M., and Cherry, J. (1993). Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. *Groundwater*, 31(2), 180-186.

Aravena, R. and Mayer, B. (2009). Isotopes and processes in the nitrogen and sulfur cycles. *Environmental Isotopes in Biodegradation and Bioremediation*, 203-246.

Arnade, L.J. (1999). Seasonal correlation of well contamination and septic tank distance. *Groundwater*, 37(6), 920-923.

Asano, T., Burton, F., and Leverenz, H. (2007). *Water Reuse: Issues, Technologies, and Applications*. McGraw-Hill Education.

Atkinson, M. and Smith, S. (1983). C: N: P ratios of benthic marine plants 1. *Limnology and Oceanography*, 28(3), 568-574.

Badruzzaman, M., Pinzon, J., Oppenheimer, J., and Jacangelo, J.G. (2012). Sources of nutrients impacting surface waters in Florida: A review. *Journal of Environmental Management*, 109, 80-92.

Bailey, N., Magley, W., Mandrup-Poulsen, J., O'Donnell, K., and Peets, R. (2009). Final TMDL Report Nutrient TMDL for the Caloosahatchee Estuary (WBIDs 3240A, 3240B, and 3240C). Florida Department of Environmental Protection.

Baris, R.D., Cohen, S.Z., Barnes, N.L., Lam, J., and Ma, Q. (2010). Quantitative analysis of over 20 years of golf course monitoring studies. *Environmental Toxicology and Chemistry*, 29(6), 1224-1236.

Bateman, A.S., and Kelly, S.D. (2007). Fertilizer nitrogen isotope signatures. *Isotopes in Environmental and Health Studies*, 43(3), 237-247.

Bernhard, A.E. and Field, K.G. (2000). A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. *Applied and Environmental Microbiology*, 66(10), 4571-4574.

Beversdorf, L.J., Miller, T.R., and McMahon, K.D. (2015). Long-term monitoring reveals carbon–nitrogen metabolism key to microcystin production in eutrophic lakes. *Frontiers in Microbiology*, 6, 456.

Blomqvist, P., Pettersson, A., and Hyenstrand, P. (1994). Ammonium-nitrogen: a key regulatory factor causing dominance of non-nitrogen-fixing cyanobacteria in aquatic systems. *Archiv für Hydrobiologie*, 141-164.

Boehm, A.B., Van De Werfhorst, L.C., Griffith, J.F., Holden, P.A., Jay, J.A., Shanks, O.C., Wang, D., and Weisberg, S.B. (2013). Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research*, 47(18), 6812-6828.

Boehm, A.B. and Soller, J.A. (2020). Refined ambient water quality thresholds for human-associated fecal indicator HF183 for recreational waters with and without co-occurring gull fecal contamination. *Microbial Risk Analysis*, 16, 100139.

Bowman, D.C., Cherney, C.T., and Rufty Jr, T.W. (2002). Fate and transport of nitrogen applied to six warm-season turfgrasses. *Crop Science*, 42(3), 833-841.

Brand, L.E. and Compton, A. (2007). Long-term increase in *Karenia brevis* abundance along the Southwest Florida Coast. *Harmful Algae*, 6(2), 232-252.

Braun, M. (2015). Water warning issued at North Shore Park due to bacteria. *News-Press*. https://www.news-press.com/story/news/2015/11/24/water-warning-issued-north-shore-park-due-bacteria/76326046/

Braun, M. (2016). North Shore Park in North Fort Myers tests high for bacteria. *News-Press*. https://www.press-citizen.com/story/news/2016/06/09/north-shore-park-north-fort-myers-tests-high-bacteria/85651776/

Buxton, M., Carey, R., and Phelan, K. (2016). The role of peri-urban land use planning in resilient urban agriculture: A case study of Melbourne, Australia. In *Balanced Urban Development: Options and Strategies for Liveable Cities* (153-170). Springer, Cham.

Byappanahalli, M.N., Nevers, M.B., Korajkic, A., Staley, Z.R., and Harwood, V.J. (2012). Enterococci in the environment. *Microbiology and Molecular Biology Reviews*, 76(4), 685-706.

Cantwell, M.G., Katz, D.R., Sullivan, J.C., Shapley, D., Lipscomb, J., Epstein, J., Juhl, A.R., Knudson, C., and O'Mullan, G.D. (2018). Spatial patterns of pharmaceuticals and wastewater tracers in the Hudson River Estuary. *Water Research*, 137, 335-343.

Cao, Y., Raith, M.R., and Griffith, J.F. (2015). Droplet digital PCR for simultaneous quantification of general and human-associated fecal indicators for water quality assessment. *Water Research*, 70, 337-349.

Carey, S.A., Goldstein, R.E.R., Gibbs, S.G., Claye, E., He, X., and Sapkota, A.R. (2016). Occurrence of vancomycin-resistant and-susceptible Enterococcus s in reclaimed water used for spray irrigation. *Environmental Research*, 147, 350-355.

Carr, D.L., Morse, A.N., Zak, J.C., and Anderson, T.A. (2011). Biological degradation of common pharmaceuticals and personal care products in soils with high water content. *Water, Air, and Soil Pollution*, 217(1), 127-134.
Casciotti, K.L., Sigman, D.M., Hastings, M.G., Böhlke, J., and Hilkert, A. (2002). Measurement of the oxygen isotopic composition of nitrate in seawater and freshwater using the denitrifier method. *Analytical Chemistry*, 74(19), 4905-4912.
Chan, Y.Y. and Killick, E.G. (1995). The effect of salinity, light and temperature in a disposal environment on the recovery of E. coli following exposure to ultraviolet radiation. *Water Research*, 29(5), 1373-1377.
Chen, M. (1988). Pollution of ground water by nutrients and fecal coliforms from lakeshore septic tank systems. *Water, Air, and Soil Pollution*, 37(3), 407-417.
Chudoba, E.A., Mallin, M.A., Cahoon, L.B., and Skrabal, S.A. (2013). Stimulation of fecal bacteria in ambient waters by experimental inputs of organic and inorganic phosphorus. *Water Research*, 47(10), 3455-3466.
Clara, M., Strenn, B., Gans, O., Martinez, E., Kreuzinger, N., and Kroiss, H. (2005). Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water Research*, 39(19), 4797-4807.
Coelho, M.P., Marques, M.E., and Roseiro, J.C. (1999). Dynamics of microbiological contamination at a marine recreational site. *Marine Pollution Bulletin*, 38(12), 1242-1246.
Corbett, D.R., Dillon, K., Burnett, W., and Schaefer, G. (2002). The spatial variability of nitrogen and phosphorus concentration in a sand aquifer influenced by onsite sewage treatment and disposal systems: a case study on St. George Island, Florida. *Environmental Pollution*, 117(2), 337-345.
Costanzo, S., O'donohue, M., Dennison, W., Loneragan, N., and Thomas, M. (2001). A new approach for detecting and mapping sewage impacts. *Marine Pollution Bulletin*, 42(2), 149-156.
Cox, A.H., Loomis, G.W., and Amador, J.A. (2019). Preliminary evidence that rising groundwater tables threaten coastal septic systems. *Journal of Sustainable Water in the Built Environment*, 5(4), 04019007.
Cox, A.H., Surabian, D., Loomis, G.W., Turenne, J.D., and Amador, J.A. (2020). Temporal Variability in the Vertical Separation Distance of Septic System Drainfields Along the Southern Rhode Island Coast. *Water, Air, and Soil Pollution*, 231(3), 1-17.
DeFlorio-Barker, S., Wing, C., Jones, R.M., and Dorevitch, S. (2018). Estimate of incidence and cost of recreational waterborne illness on United States surface waters. *Environmental Health*, 17(1), 1-10.
Drake, V.M. and Bauder, J.W. (2005). Ground water nitrate-nitrogen trends in relation to urban development, Helena, Montana, 1971–2003. *Groundwater Monitoring and Remediation*, 25(2), 118-130.
Du, B., Price, A.E., Scott, W.C., Kristofco, L.A., Ramirez, A.J., Chambliss, C.K., Yelderman, J.C., and Brooks, B.W. (2014). Comparison of contaminants of emerging concern removal, discharge, and water quality hazards among centralized and on-site wastewater treatment system effluents receiving common wastewater influent. *Science of the Total Environment*, 466, 976-984.
Efroymson, R.A., Jones, D.S., and Gold, A.J. (2007). An ecological risk assessment framework for effects of onsite wastewater treatment systems and other localized sources of nutrients on aquatic ecosystems. *Human and Ecological Risk Assessment*, 13(3), 574-614.
EPA. (2000a). *Decentralized Systems Technology Fact Sheet: Septic Tank Systems for Large Flow Applications*.
EPA. (2000b). *Guidelines for Management of Onsite/Decentralized Wastewater Systems*.
EPA. (2002). *Onsite Wastewater Treatment Systems Manual*.
EPA. (2008). *Septic Systems Fact Sheet*.
EPA. (2017). *National Water Quality Inventory: Report to Congress*.

FDOH. (2018). *Onsite Sewage Disposal (Septic Systems)*. Retrieved 02/12/2021 from http://lee.floridahealth.gov/programs-and-services/environmental-health/onsite-sewage-disposal/index.html

FDOH. (2020). *Onsite Sewage Programs*. Florida Department of Health. Retrieved 02/12/2021 from http://www.floridahealth.gov/environmental-health/onsite-sewage/index.html

Fenz, R., Blaschke, A.P., Clara, M., Kroiss, H., Mascher, D., and Zessner, M. (2005). Monitoring of carbamazepine concentrations in wastewater and groundwater to quantify sewer leakage. *Water Science and Technology*, 52(5), 205-213.

Ferguson, C., Husman, A.M.D.R., Altavilla, N., Deere, D., and Ashbolt, N. (2003). Fate and transport of surface water pathogens in watersheds.

Foreman, R.K., Segura-Noguera, M., and Karl, D.M. (2016). Validation of Ti (III) as a reducing agent in the chemiluminescent determination of nitrate and nitrite in seawater. *Marine Chemistry*, 186, 83-89.

France, R., Holmquist, J., Chandler, M., and Cattaneo, A. (1998). $\delta^{15}N$ evidence for nitrogen fixation associated with macroalgae from a seagrass-mangrove-coral reef system. *Marine Ecology Progress Series*, 167, 297-299.

Geider, R.J., and La Roche, J. (2002). Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *European Journal of Phycology*, 37(1), pp.1-17.

Gerardi, M.H. (2006). *Wastewater Bacteria* (Vol. 5). John Wiley & Sons.

Gerardi, M.H., and Zimmerman, M.C. (2004). *Wastewater Pathogens*. John Wiley & Sons.

Gobler, C.J., and Sañudo-Wilhelmy, S.A. (2001). Temporal variability of groundwater seepage and brown tide blooms in a Long Island embayment. *Marine Ecology Progress Series*, 217, 299-309.

Godfrey, E., Woessner, W.W., and Benotti, M.J. (2007). Pharmaceuticals in on-site sewage effluent and ground water, western Montana. *Groundwater*, 45(3), 263-271.

Goldstein, R.E.R., Micallef, S.A., Gibbs, S.G., George, A., Claye, E., … & Sapkota, A.R. (2014). Detection of vancomycin-resistant enterococci (VRE) at four US wastewater treatment plants that provide effluent for reuse. *Science of the Total Environment*, 466, 404-411.

Graves, G.A., Wan, Y. and Fike, D.L. (2004). Water quality characteristics of storm water from major land uses in South Florida 1. *Journal of the American Water Resources Association*, 40(6), 1405-1419.

Green, H.C., and Field, K.G. (2012). Sensitive detection of sample interference in environmental qPCR. *Water Research*, 46(10), 3251-3260.

Griffin, D.W., Gibson, C.J., Lipp, E.K., Riley, K., Paul, J.H., and Rose, J.B. (1999). Detection of viral pathogens by reverse transcriptase PCR and of microbial indicators by standard methods in the canals of the Florida Keys. *Applied and Environmental Microbiology*, 65(9), 4118-4125.

Hagedorn, C., Blanch, A.R., and Harwood, V.J. (2011). *Microbial Source Tracking: Methods, Applications, and Case Studies*. Springer Science & Business Media.

Haile, R.W., Witte, J.S., Gold, M., Cressey, R., McGee, C., Millikan, R.C., Glasser, A., Harawa, N., Ervin, C., and Harmon, P. (1999). The health effects of swimming in ocean water contaminated by storm drain runoff. *Epidemiology*, 355-363.

He, L.M., Lu, J., and Shi, W. (2007). Variability of fecal indicator bacteria in flowing and ponded waters in southern California: Implications for bacterial TMDL development and implementation. *Water Research*, 41(14), 3132-3140.

He, Z., Han, D., Song, X., Yang, L., Zhang, Y., Ma, Y., Bu, H., Li, B., and Yang, S. (2021). Variations of Groundwater Dynamics in Alluvial Aquifers with Reclaimed Water Restoring the Overlying River, Beijing, China. *Water*, 13(6), p.806.

Heaton, T.H. (1986). Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review. *Chemical Geology: Isotope Geoscience Section*, 59, 87-102.

Heaton, T.H., Stuart, M.E., Sapiano, M., and Sultana, M.M. (2012). An isotope study of the sources of nitrate in Malta's groundwater. *Journal of Hydrology*, 414, 244-254.

Heisler, J., Glibert, P.M., Burkholder, J.M., Anderson, D.M., Cochlan, W., Dennison, W.C., Dortch, Q., Gobler, C.J., Heil, C.A., and Humphries, E. (2008). Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae*, 8(1), 3-13.

Herren, L.W., Brewton, R.A., Wilking, L.E., Tarnowski, M.E., Vogel, M.A., and Lapointe, B.E. (2021). Septic systems drive nutrient enrichment of groundwaters and eutrophication in the urbanized Indian River Lagoon, Florida. *Marine Pollution Bulletin*, 172, 112928.

Hinkle, S.R., Böhlke, J.K., and Fisher, L.H. (2008). Mass balance and isotope effects during nitrogen transport through septic tank systems with packed-bed (sand) filters. *Science of the total environment*, 407(1), pp.324-332.

Hochmuth, G.J., Nell, T., Sartain, J., Unruh, J.B., Martinez, C., Trenholm, L., and Cisar, J. (2009). Urban water quality and fertilizer ordinances: Avoiding unintended consequences: A review of the scientific literature. *EDIS*, 2009(2).

Hossain, F., Chang, N.B., Wanielista, M., Xuan, Z., and Daranpob, A. (2010). Nitrification and denitrification in a passive on-site wastewater treatment system with a recirculation filtration tank. *Water Quality, Exposure and Health*, 2(1), 31-46.

Howarth, R.W., Anderson, D., Cloern, J.E., Elfring, C., Hopkinson, C.S., Lapointe, B., Malone, T., Marcus, N., McGlathery, K., and Sharpley, A.N. (2000). Nutrient pollution of coastal rivers, bays, and seas. *Issues in Ecology* (7), 1-16.

Ishii, S., Ksoll, W.B., Hicks, R.E. and Sadowsky, M.J. (2006). Presence and growth of naturalized Escherichia coli in temperate soils from Lake Superior watersheds. *Applied and Environmental Microbiology*, 72(1), 612-621.

James, C.A., Miller-Schulze, J.P., Ultican, S., Gipe, A.D., and Baker, J.E. (2016). Evaluating contaminants of emerging concern as tracers of wastewater from septic systems. *Water Research*, 101, 241-251.

Jani, J., Yang, Y.Y., Lusk, M.G., and Toor, G.S. (2020). Composition of nitrogen in urban residential stormwater runoff: Concentrations, loads, and source characterization of nitrate and organic nitrogen. *PloS One*, 15(2), p.e0229715.

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., and Siegrist, H. (2006). Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme. *Water Research*, 40(8), 1686-1696.

Kanakaraju, D., Motti, C.A., Glass, B.D., and Oelgemöller, M. (2015). TiO2 photocatalysis of naproxen: effect of the water matrix, anions and diclofenac on degradation rates. *Chemosphere*, 139, 579-588.

Kaown, D., Hyun, Y., Bae, G.O., Oh, C.W., and Lee, K.K. (2012). Evaluation of spatio-temporal trends of groundwater quality in different land uses using Kendall test. *Geosciences Journal*, 16(1), 65-75.

Kasprzyk-Hordern, B., Dinsdale, R.M., and Guwy, A.J. (2009). The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Research*, 43(2), 363-380.

Kendall, C. (1998). Tracing nitrogen sources and cycling in catchments. In *Isotope Tracers in Catchment Hydrology* (519-576). Elsevier.

Krimsky, L.S., Lusk, M.G., Abeels, H., and Seals, L. (2021). Sources and concentrations of nutrients in surface runoff from waterfront homes with different landscape practices. *Science of the Total Environment*, 750, 142320.

Lapointe, B.E., O'Connell, J.D., and Garrett, G.S. (1990). Nutrient couplings between on-site sewage disposal systems, groundwaters, and nearshore surface waters of the Florida Keys. *Biogeochemistry*, 10(3), 289-307.

Lapointe, B., and Krupa, S. (1995). Jupiter Creek septic tank water quality investigation. *Final Report to the Loxahatchee River Environmental Control District, Jupiter, FL*, 96.

Lapointe, B.E., Bedford, B., Brand, L., and Yentsch, C.S. (2006). Harmful Algal Blooms in Coastal Waters of Lee County, FL: Bloom Dynamics and Identification of Land-Based Nutrient Sources. *Final Report to Lee County.*

Lapointe, B.E., and Bedford, B.J. (2007). Drift rhodophyte blooms emerge in Lee County, Florida, USA: evidence of escalating coastal eutrophication. *Harmful Algae*, 6(3), 421-437.

Lapointe, B.E., Herren, L.W., and Bedford, B.J. (2012). Effects of hurricanes, land use, and water management on nutrient and microbial pollution: St. Lucie Estuary, Southeast Florida. *Journal of Coastal Research*, 28(6), 1345-1361.

Lapointe, B.E., Herren, L.W., Debortoli, D.D., and Vogel, M.A. (2015). Evidence of sewage-driven eutrophication and harmful algal blooms in Florida's Indian River Lagoon. *Harmful Algae*, 43, 82-102.

Lapointe, B.E., Herren, L.W., Paule, A.L., Sleeman, A., and Brewton, R. (2016). Charlotte County Water Quality Assessment. Final Report to the Charlotte County Board of County Commissioners and the Charlotte County Utilities Department.

Lapointe, B.E., Herren, L.W., and Paule, A.L. (2017). Septic systems contribute to nutrient pollution and harmful algal blooms in the St. Lucie Estuary, Southeast Florida, USA. *Harmful Algae*, 70, 1-22.

Lapointe, B., Brewton, R., and Baladi, D. (2020). Septic system – groundwater – surface water couplings in the North Fork of the St. Lucie River: MST Phase II. Final Report to the City of Port St. Lucie Utilities Department.

Lapointe, B.E., Herren, L.W., Brewton, R.A., and Alderman, P.K. (2020). Nutrient over-enrichment and light limitation of seagrass communities in the Indian River Lagoon, an urbanized subtropical estuary. *Science of the Total Environment*, 699, 134068.

Lapointe, B.E., Brewton, R.A., Tyre, K.N., Baladi, D., Wilking, L.E., and Herren, L.W. (2021). *Caloosahatchee River - North Fort Myers Nutrient and Bacteria Source Identification Study: Year 2*. Final Report to the Lee County Commission.

Lapointe, B.E., Tewfik, A., and Phillips, M. (2021). Macroalgae reveal nitrogen enrichment and elevated N: P ratios on the Belize Barrier Reef. *Marine Pollution Bulletin*, 171, 112686.

Lebaron, P., Servais, P., Troussellier, M., Courties, C., Vives-Rego, J., Muyzer, G., Bernard, L., Guindulain, T., Schäfer, H. and Stackebrandt, E. (1999). Changes in bacterial community structure in seawater mesocosms differing in their nutrient status. *Aquatic Microbial Ecology*, 19(3), 255-267.

LeBlanc, D.R. (1985). Sewage plume in a sand and gravel aquifer, Cape Cod, Massachusetts (Vol. 2218). Department of the Interior, US Geological Survey.

Lee, M.S., Qin, G., Nakanishi, K., and Zagorski, M.G. (1989). Biosynthetic studies of brevetoxins, potent neurotoxins produced by the dinoflagellate *Gymnodinium breve*. *Journal of the American Chemical Society*, 111(16), 6234-6241.

Li, Y., Zhang, H., Tu, C., Fu, C., Xue, Y., and Luo, Y. (2016). Sources and fate of organic carbon and nitrogen from land to ocean: Identified by coupling stable isotopes with C/N ratio. *Estuarine, Coastal and Shelf Science*, 181, 114-122.

Li, C., Li, B., and Bi, E. (2019). Characteristics of hydrochemistry and nitrogen behavior under long-term managed aquifer recharge with reclaimed water: A case study in north China. *Science of the Total Environment*, 668, 1030-1037.

Lipp, E.K., Kurz, R., Vincent, R., Rodriguez-Palacios, C., Farrah, S.R., and Rose, J.B. (2001). The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary. *Estuaries*, 24(2), 266-276.

Lipp, E.K., Farrah, S.A., and Rose, J.B. (2001). Assessment and impact of microbial fecal pollution and human enteric pathogens in a coastal community. *Marine Pollution Bulletin*, 42(4), 286-293.

Liu, Z., Choudhury, S.H., Xia, M., Holt, J., Wallen, C.M., Yuk, S., and Sanborn, S.C. (2009). Water quality assessment of coastal Caloosahatchee River watershed, Florida. *Journal of Environmental Science and Health Part A*, 44(10), 972-984.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., and Wang, X.C. (2014). A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment*, 473, 619-641.

Luu, T.N.M., Do, T.N., Matiatos, I., Panizzo, V.N., and Trinh, A.D. (2020). Stable isotopes as an effective tool for N nutrient source identification in a heavily urbanized and agriculturally intensive tropical lowland basin. *Biogeochemistry*, 149(1), 17-35.

Mallin, M.A. (2013). Septic systems in the coastal environment: multiple water quality problems in many areas. *Monitoring Water Quality, Quality—Pollution Assessment, Analysis and Remediation*, 81-102.

Mallin, M.A. and Cahoon, L.B. (2020). The hidden impacts of phosphorus pollution to streams and rivers. *BioScience*, 70(4), 315-329.

Mance, D., Mance, D., and Vukić Lušić, D. (2021). Environmental and anthropogenic factors affecting coastal bathing water quality: preliminary study for Primorje-Gorski Kotar County (Croatia). *Pomorstvo*, 35(1), 57-68.

McQuaig, S.M., Scott, T.M., Harwood, V.J., Farrah, S.R., and Lukasik, J.O. (2006). Detection of human-derived fecal pollution in environmental waters by use of a PCR-based human polyomavirus assay. *Applied and Environmental Microbiology*, 72(12), 7567-7574.

Medina, M., Huffaker, R., Jawitz, J.W., and Muñoz-Carpena, R. (2020). Seasonal dynamics of terrestrially sourced nitrogen influenced *Karenia brevis* blooms off Florida's southern Gulf Coast. *Harmful Algae*, 98, 101900.

Meeroff, D.E., Bloetscher, F., Bocca, T., and Morin, F. (2008). Evaluation of water quality impacts of on-site treatment and disposal systems on urban coastal waters. *Water, Air, and Soil Pollution*, 192(1), 11-24.

Meybeck, M. (2004). The global change of continental aquatic systems: dominant impacts of human activities. *Water Science and Technology*, 49(7), 73-83.

Mhuka, V., Dube, S., and Nindi, M.M. (2020). Occurrence of pharmaceutical and personal care products (PPCPs) in wastewater and receiving waters in South Africa using LC-Orbitrap™ MS. *Emerging Contaminants*, 6, 250-258.

Mika, K.B., Ginsburg, D.W., Lee, C.M., Thulsiraj, V., and Jay, J.A. (2014). Fecal indicator bacteria levels do not correspond with incidence of human-associated HF183 Bacteroides 16S rRNA genetic marker in two urban southern California watersheds. *Water, Air, and Soil Pollution*, 225(6), 1-11.

Mill, A., Schlacher, T. and Katouli, M. (2006). Tidal and longitudinal variation of faecal indicator bacteria in an estuarine creek in south-east Queensland, Australia. *Marine Pollution Bulletin*, 52(8), 881-891.

Nagulapally, S.R., Ahmad, A., Henry, A., Marchin, G.L., Zurek, L., and Bhandari, A. (2009). Occurrence of ciprofloxacin-, trimethoprim-sulfamethoxazole-, and vancomycin-resistant bacteria in a municipal wastewater treatment plant. *Water Environment Research*, 81(1), 82-90.

NRC. (2000). *Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution*. National Academies Press.

Nshimyimana, J.P., Ekklesia, E., Shanahan, P., Chua, L.H.C., and Thompson, J.R. (2014). Distribution and abundance of human-specific Bacteroides and relation to traditional indicators in an urban tropical catchment. *Journal of Applied Microbiology*, 116(5), 1369-1383.

Odum, H.T. (1953). Dissolved phosphorus in Florida waters. *Florida Geological Survey Report of Investigation*, 9(Pt 1), pp.1-40.

Olsen, Y.S., Fox, S.E., Kinney, E.L., Teichberg, M., and Valiela, I. (2010). Differences in urbanization and degree of marine influence are reflected in $\delta^{13}C$ and $\delta^{15}N$ of producers and consumers in seagrass habitats of Puerto Rico. *Marine Environmental Research*, 69(3), 198-206.

Oppenheimer, J., Eaton, A., Badruzzaman, M., Haghani, A.W., and Jacangelo, J.G. (2011). Occurrence and suitability of sucralose as an indicator compound of wastewater loading to surface waters in urbanized regions. *Water Research*, 45(13), 4019-4027.

Paerl, H. and Fogel, M. (1994). Isotopic characterization of atmospheric nitrogen inputs as sources of enhanced primary production in coastal Atlantic Ocean waters. *Marine Biology*, *119*(4), 635-645.

Peterson, B.J., and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual review of ecology and systematics*, 18(1), 293-320.

Phlips, E.J., Badylak, S., Lasi, M.A., Chamberlain, R., Green, W.C., Hall, L.M., Hart, J.A., Lockwood, J.C., Miller, J.D., Morris, L.J., and Steward, J.S. (2015). From red tides to green and brown tides:

bloom dynamics in a restricted subtropical lagoon under shifting climatic conditions. *Estuaries and Coasts*, 38(3), 886-904.

Price, M.T., Blackwood, A.D., and Noble, R.T. (2021). Integrating culture and molecular quantification of microbial contaminants into a predictive modeling framework in a low-lying, tidally-influenced coastal watershed. *Science of The Total Environment*, p.148232.

Ptacek, C.J. (1998). Geochemistry of a septic-system plume in a coastal barrier bar, Point Pelee, Ontario, Canada. *Journal of Contaminant Hydrology*, 33(3-4), 293-312.

Redfield, A.C. (1958). The biological control of chemical factors in the environment. *American Scientist*, 46(3), 230A-221.

Risk, M.J., Lapointe, B.E., Sherwood, O.A., and Bedford, B.J. (2009). The use of $\delta^{15}N$ in assessing sewage stress on coral reefs. *Marine Pollution Bulletin*, 58(6), 793-802.

Robertson, W.D., Schiff, S.L., and Ptacek, C.J. (1998). Review of phosphate mobility and persistence in 10 septic system plumes. *Groundwater*, 36(6), 1000-1010.

Robertson, W.D. (2008). Irreversible phosphorus sorption in septic system plumes? *Groundwater*, 46(1), 51-60.

Robertson, W.D., Moore, T.A., Spoelstra, J., Li, L., Elgood, R.J., Clark, I.D., Schiff, S.L., Aravena, R., and Neufeld, J.D. (2012). Natural attenuation of septic system nitrogen by anammox. *Groundwater*, 50(4), 541-553.

Sadowsky, M.J., and Whitman, R.L. (2011). *The Fecal Bacteria*. Wiley Online Library.

Santos, J.L., Aparicio, I., and Alonso, E. (2007). Occurrence and risk assessment of pharmaceutically active compounds in wastewater treatment plants. A case study: Seville city (Spain). *Environment International*, 33(4), 596-601.

Sartain, J.B. (1985). Effect of Acidity and N Source on the Growth and Thatch Accumulation of Tifgreen Bermudagrass and on Soil Nutrient Retention 1. *Agronomy Journal*, 77(1), 33-36.

Sauer, E.P., VandeWalle, J.L., Bootsma, M.J., and McLellan, S.L. (2011). Detection of the human specific Bacteroides genetic marker provides evidence of widespread sewage contamination of stormwater in the urban environment. *Water Research*, 45(14), 4081-4091.

Savoye, N., Aminot, A., Tréguer, P., Fontugne, M., Naulet, N., and Kérouel, R. (2003). Dynamics of particulate organic matter $\delta^{15}N$ and $\delta^{13}C$ during spring phytoplankton blooms in a macrotidal ecosystem (Bay of Seine, France). *Marine Ecology Progress Series*, 255, 27-41.

Scheurer, M., Brauch, H.J., and Lange, F.T. (2009). Analysis and occurrence of seven artificial sweeteners in German wastewater and surface water and in soil aquifer treatment (SAT). *Analytical and Bioanalytical Chemistry*, 394(6), 1585-1594.

Seurinck, S., Defoirdt, T., Verstraete, W., and Siciliano, S.D. (2005). Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. *Environmental Microbiology*, 7(2), 249-259.

Seurinck, S., Verdievel, M., Verstraete, W., and Siciliano, S.D. (2006). Identification of human fecal pollution sources in a coastal area: a case study at Oostende (Belgium). *Journal of Water and Health*, 4(2), 167-175.

Silvanima, J., Woeber, A., Sunderman-Barnes, S., Copeland, R., Sedlacek, C., and Seal, T. (2018). A synoptic survey of select wastewater-tracer compounds and the pesticide imidacloprid in Florida's ambient freshwaters. *Environmental Monitoring and Assessment*, 190(7), 1-22.

Sivaganesan, M., Siefring, S., Varma, M., and Haugland, R.A. (2011). MPN estimation of qPCR target sequence recoveries from whole cell calibrator samples. *Journal of Microbiological Methods*, 87(3), 343-349.

South Florida Water Management District. (2020). 2019 Estimated Water Use Report. https://www.sfwmd.gov/sites/default/files/documents/2019_Water_Use_Estimation_Report_Final_0.pdf.

Stauber, J.L., Chariton, A., and Apte, S. (2016). Global change. In *Marine Ecotoxicology* (pp. 273-313). Academic Press.

Stiegler, J.C., Richardson, M.D., and Karcher, D.E. (2011). Foliar nitrogen uptake following urea application to putting green turfgrass species. *Crop Science*, 51(3), 1253-1260.

Stoner, B., Howard, B., Noel, S., Arrington, D.A., and District, L.R. (2016). Loxahatchee river water quality monitoring task 2: final report assessment of 2014-2015 Loxahatchee River water quality.

Stumpf, C.H., Piehler, M.F., Thompson, S., and Noble, R.T. (2010). Loading of fecal indicator bacteria in North Carolina tidal creek headwaters: hydrographic patterns and terrestrial runoff relationships. *Water Research*, 44(16), 4704-4715.

Surbeck, C.Q., Jiang, S.C., and Grant, S.B. (2010). Ecological control of fecal indicator bacteria in an urban stream. *Environmental Science and Technology*, 44(2), 631-637.

Swartz, C.H., Reddy, S., Benotti, M.J., Yin, H., Barber, L.B., Brownawell, B.J., and Rudel, R.A. (2006). Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. *Environmental Science and Technology*, 40(16), 4894-4902.

Ternes, T.A. (1998). Occurrence of drugs in German sewage treatment plants and rivers. *Water Research*, 32(11), 3245-3260.

Toor, G.S., Lusk, M., and Obreza, T. (2011). Onsite sewage treatment and disposal systems: Nitrogen. *EDIS*, 2011(4).

Toothman, B.R., Cahoon, L.B., and Mallin, M.A. (2009). Phosphorus and carbohydrate limitation of fecal coliform and fecal enterococcus within tidal creek sediments. *Hydrobiologia*, 636(1), 401-412.

Torres, C.I., Ramakrishna, S., Chiu, C.A., Nelson, K.G., Westerhoff, P., and Krajmalnik-Brown, R. (2011). Fate of sucralose during wastewater treatment. *Environmental Engineering Science*, 28(5), 325-331.

Tran, N.H., Gin, K.Y.H., and Ngo, H.H. (2015). Fecal pollution source tracking toolbox for identification, evaluation and characterization of fecal contamination in receiving urban surface waters and groundwater. *Science of the Total Environment*, 538, 38-57.

Tran, N.H., Li, J., Hu, J., and Ong, S.L. (2014). Occurrence and suitability of pharmaceuticals and personal care products as molecular markers for raw wastewater contamination in surface water and groundwater. *Environmental Science and Pollution Research*, 21(6), 4727-4740.

Tree, J.A., Adams, M.R., & Lees, D.N. (2003). Chlorination of indicator bacteria and viruses in primary sewage effluent. *Applied and Environmental Microbiology*, 69(4), 2038-2043.

Urakawa, H., Hancock, T.L., Steele, J.H., Dahedl, E.K., Urakawa, H.E., Ndungu, L.K., Krausfeldt, L.E., Rosen, B.H., and Lopez, J.V. (2020). Complete Genome Sequence of *Microcystis aeruginosa* FD4, Isolated from a Subtropical River in Southwest Florida. *Microbiology Resource Announcements*, 9(38).

Viso, E., and Zachariadis, G. (2018). Method development of phosphorus and boron determination in fertilizers by ICP-AES. *Separations*, 5(3), 36.

W. Dexter Bender and Associates, I. (1995). North Fort Myers surface and ground water quality survey. *Final Report to Lee County Department of Utilities*.

Wade, T.J., Pai, N., Eisenberg, J.N., and Colford Jr, J.M. (2003). Do US Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis. *Environmental Health Perspectives*, 111(8), 1102-1109.

Walters, S.P., and Field, K.G. (2009). Survival and persistence of human and ruminant-specific faecal Bacteroidales in freshwater microcosms. *Environmental Microbiology*, 11(6), 1410-1421.

Wedderburn, L.A., Knapp, M., Waltz, D., and Burns, W. (1982). Hydrogeologic reconnaissance of Lee County. *Florida: South Florida Water Management District Technical Publication*, 82-81.

Wen, Q., Tutuka, C., Keegan, A., and Jin, B. (2009). Fate of pathogenic microorganisms and indicators in secondary activated sludge wastewater treatment plants. *Journal of Environmental Management*, 90(3), 1442-1447.

Wherley, B.G., Shi, W., Bowman, D.C., and Rufty, T.W. (2009). Fate of 15N-nitrate applied to a bermudagrass system: Assimilation profiles in different seasons. *Crop science*, 49(6), 2291-2301.

White, D., Lapworth, D.J., Civil, W., and Williams, P. (2019). Tracking changes in the occurrence and source of pharmaceuticals within the River Thames, UK; from source to sea. *Environmental Pollution*, 249, 257-266.

Wilhelm, S.W., Brigden, S.M., and Suttle, C.A. (2002). A dilution technique for the direct measurement of viral production: a comparison in stratified and tidally mixed coastal waters. *Microbial Ecology*, 168-173.

Wojcieszyńska, D., and Guzik, U. (2020). Naproxen in the environment: its occurrence, toxicity to nontarget organisms and biodegradation. *Applied Microbiology and Biotechnology*, 104(5), 1849-1857.

Wolf, L., Zwiener, C., and Zemann, M. (2012). Tracking artificial sweeteners and pharmaceuticals introduced into urban groundwater by leaking sewer networks. *Science of the Total Environment*, 430, 8-19.

Xu, L., Ma, X., Niu, J., Chen, J., and Zhou, C. (2019). Removal of trace naproxen from aqueous solution using a laboratory-scale reactive flow-through membrane electrode. *Journal of Hazardous Materials*, 379, 120692.

Xue, J., Schmitz, B.W., Caton, K., Zhang, B., Zabaleta, J., Garai, J., Taylor, C.M., Romanchishina, T., Gerba, C.P., Pepper, I.L., and Sherchan, S.P. (2019). Assessing the spatial and temporal variability of bacterial communities in two Bardenpho wastewater treatment systems via Illumina MiSeq sequencing. *Science of the Total Environment*, 657, 1543-1552.

Yang, Y.Y., Toor, G.S., Wilson, P.C., and Williams, C.F. (2017). Micropollutants in groundwater from septic systems: Transformations, transport mechanisms, and human health risk assessment. *Water Research*, 123, 258-267.

Yates, M.V. (1985). Septic tank density and ground-water contamination. *Groundwater*, *23*(5), 586-591.

Yentsch, C., Lapointe, B., Poulton, N., and Phinney, D. (2008). Anatomy of a red tide bloom off the southwest coast of Florida. *Harmful Algae*, 7(6), 817-826.

Yuan, X., Hu, J., Li, S., and Yu, M. (2020). Occurrence, fate, and mass balance of selected pharmaceutical and personal care products (PPCPs) in an urbanized river. *Environmental Pollution*, *266*, 115340.

Zhang, Y., Geißen, S.U., and Gal, C. (2008). Carbamazepine and diclofenac: removal in wastewater treatment plants and occurrence in water bodies. *Chemosphere*, 73(8), 1151-1161.

Zhang, Y., Shi, P., Song, J., and Li, Q. (2018). Application of nitrogen and oxygen isotopes for source and fate identification of nitrate pollution in surface water: a review. *Applied sciences*, 9(1), p.18.

Zmirou, D., Pena, L., Ledrans, M., and Letertre, A. (2003). Risks associated with the microbiological quality of bodies of fresh and marine water used for recreational purposes: summary estimates based on published epidemiological studies. *Archives of Environmental Health: An International Journal*, 58(11), 703-711.

Appendix

Lee County MST Site Analysis

Table S1. Lee County MST site coordinates.

Site Name	Site #	Latitude	Longitude
Billy Creek	1	26.663949	-81.81077
Briarcliff	2	26.506229	-81.849549
Daughtry Creek	3	26.7058	-81.8376
Deep Lagoon	4	26.5374	-81.92432
Fort Myers Shores	5	26.719939	-81.716584
Hendry Creek	6	26.51716	-81.879125
Heritage Farms	7	26.509791	-81.890957
Lake McGregor	8	26.541574	-81.91176
Laurelin Court	9	26.710209	-81.782493
Ligon Court	10	26.503397	-81.906561
Mobile Manor	11	26.691481	-81.858887
North Town & River	12	26.548333	-81.917888
Orange River	13	26.685743	-81.788933
Overlook Drive	14	26.544275	-81.895575
Page Park	15	26.575423	-81.867376
Pine Island	16	26.491923	-82.081931
Pine Island 02	16B	26.495158	-82.078043
San Carlos Park	17	26.478219	-81.813646
Summerwood Drive	18	26.513918	-81.963984
Tidewater Island 01	19	26.47887	-81.861963
Tidewater Island 02	20	26.480076	-81.862079
Waterway Estates 01	21	26.6499717	-81.8975495
Waterway Estates 02	22	26.634393	-81.913725
Yacht Club	23	26.694755	-81.832244
Reference	24	26.566226	-81.909797

Reference Site

The Reference site was located in a residential finger canal serviced by municipal sewer (Figure S1). There were no direct septic system or reclaimed water influences at this site. Total nitrogen and total phosphorus were found in moderate to high concentrations (Figure S2a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S2b) and soluble reactive phosphorus comprised 65% of total phosphorus on average. Bacteria concentrations were predominantly low and HF183 was detected in three out of seven total samples (Figure S2c). Sucralose was consistently found in moderate to high concentrations and carbamazepine was detected in one sample (Figure S2d). Dissolved nitrate isotope values ($\delta^{15}N\text{-}NO_3^-$) were primarily in the range of wastewater and soil nitrogen, and to a lesser extent fertilizer nitrogen (Figure S2e).

The presence of sucralose, carbamazepine, and HF183 indicates a human wastewater influence at the Reference Site. However, HF183 detections were sporadic, and concentrations of ammonium and bacteria were low. Assuming proper functioning of municipal sewer infrastructure, this suggests that the wastewater influence at this location is transient and may reflect background concentrations originating upstream in the Caloosahatchee River Estuary from other sources, such as wastewater treatment plant discharge. Stable isotope data show that the primary nitrate source is likely a combination of soil and wastewater, although fertilizers may also contribute.

Figure S1. The Reference site located in a residential finger canal serviced by municipal sewer.

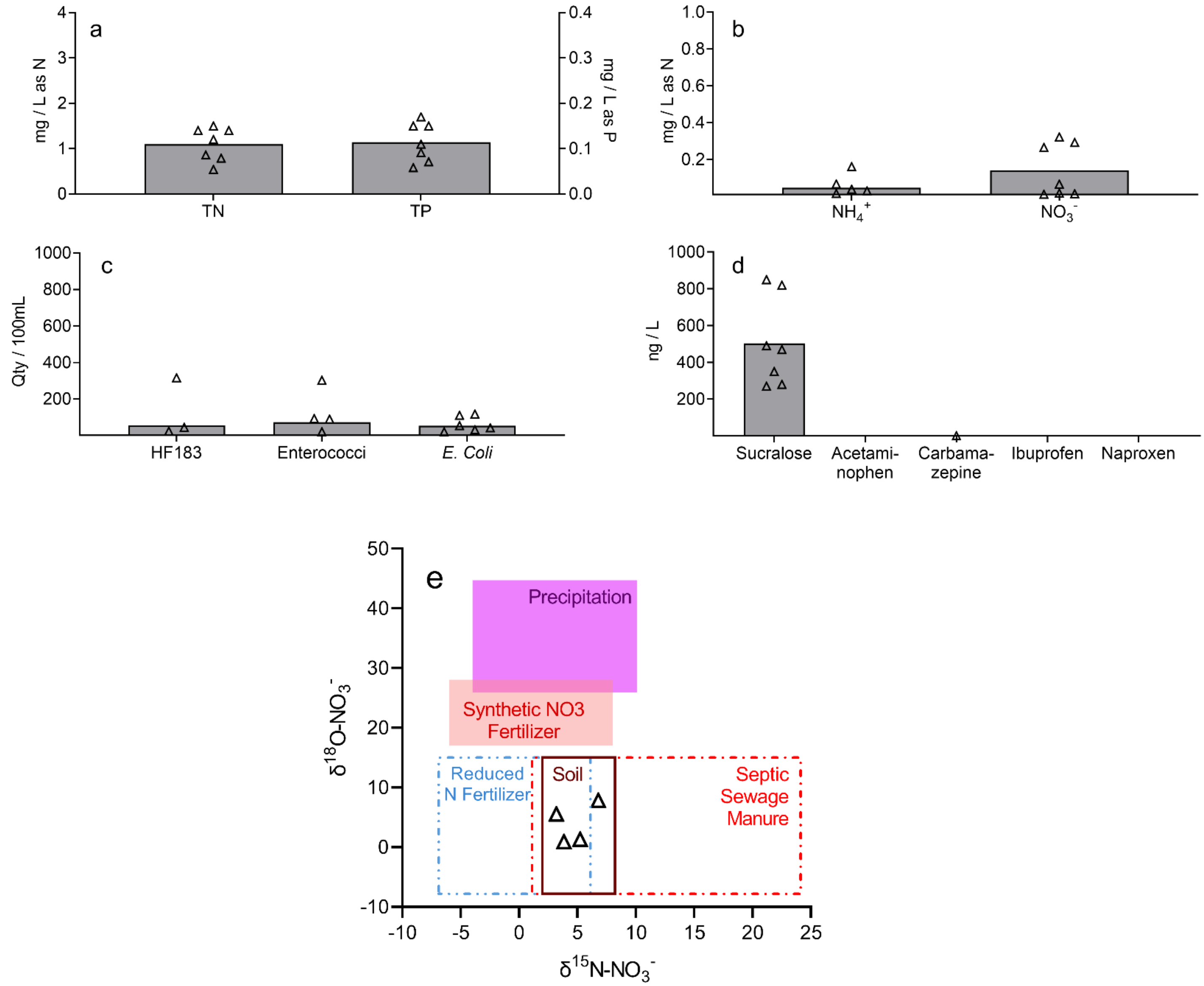

Figure S2. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Reference Site. Bars show mean values and triangles show individual detections.

Billy Creek

The Billy Creek site was located in a fast-flowing drainage ditch at the entrance of a small residential neighborhood. The surrounding and upstream area is largely serviced by municipal sewer but the homes adjacent to the Billy Creek site are serviced by septic systems (Figure S3). There are no reclaimed water influences at this site. Total nitrogen was found in moderate concentrations, whereas total phosphorus concentrations were very high (Figure S4a). Dissolved inorganic nitrogen was evenly comprised of ammonium and nitrate (Figure S4b) and soluble reactive phosphorus comprised 54% of total phosphorus on average. Bacteria concentrations were very high, frequently exceeding the upper limits of detection, and HF183 was detected in five out of eight total samples (Figure S4c). Sucralose was consistently found in moderate concentrations, but no other chemical tracers were detected (Figure S4d). $\delta^{15}N\text{-}NO_3^-$ values were primarily in the range of fertilizer nitrogen, and to a lesser extent soil and wastewater nitrogen (Figure S4e).

Very high phosphorus and bacteria concentrations at Billy Creek are concerning. Incomplete ammonium oxidation was observed, suggesting rapid transport of nutrients to surface water. The detection of HF183 and sucralose in most samples indicates human wastewater influence. Assuming proper functioning of municipal sewer infrastructure, the most likely source of these contaminants is localized septic system effluent, but upstream sources may also contribute. Stable isotope data suggest that the primary nitrate source is likely fertilizer, although soil organics and wastewater may also contribute.

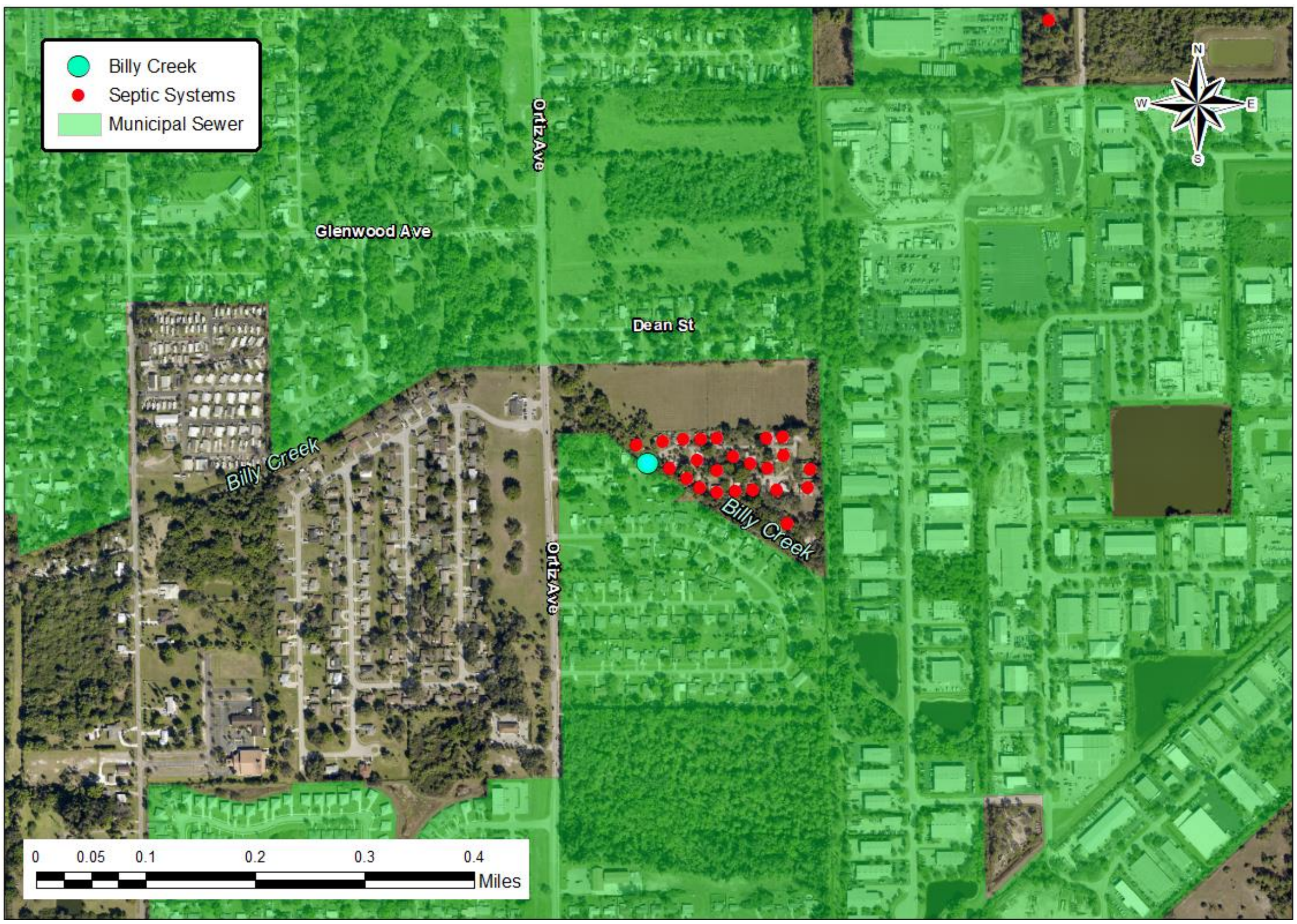

Figure S3. The Billy Creek site is located at the entrance of a small residential neighborhood.

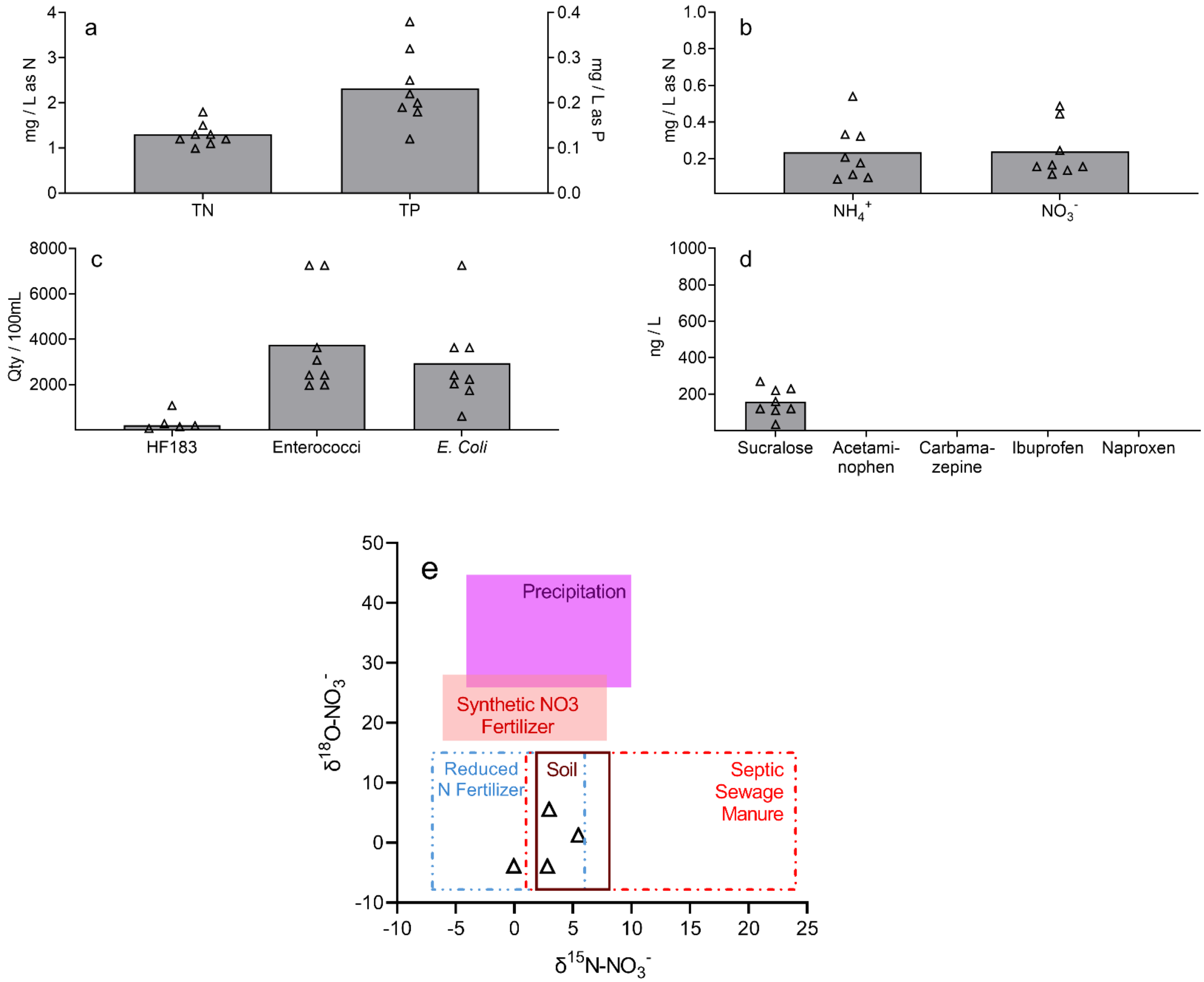

Figure S4. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Billy Creek site. Bars show mean values and triangles show individual detections.

Briarcliff

The Briarcliff site was located in a large drainage canal with sparsely placed septic systems servicing homes on large lots to the north of the canal and pastureland to the south (Figure S5). There is no municipal sewer infrastructure or reclaimed water influences at this site. Total nitrogen was found in moderate concentrations, whereas total phosphorus concentrations were low (Figure S6a). Dissolved inorganic nitrogen was evenly comprised of ammonium and nitrate (Figure S6b) and soluble reactive phosphorus comprised 10% of total phosphorus on average. Bacteria concentrations were low and HF183 was detected in one out of eight total samples (Figure S6c). Sucralose was consistently found in moderate to high concentrations and acetaminophen was detected in one sample (Figure S6d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of wastewater, soil organics, and fertilizer nitrogen (Figure S6e).

The presence of sucralose at elevated concentrations, in addition to isolated detections of HF183 and acetaminophen, provides evidence of human wastewater contamination. The most likely source of these contaminants is nearby septic systems, but upstream sources may also exist. However, these sources appear to contribute minimally to nutrient and bacteria pollution, as evidenced by the low concentrations of nitrogen, phosphorus, and bacteria found in surface water at this location. Nitrate stable isotope data show that nitrate sources may include soil organics, wastewater, and fertilizers.

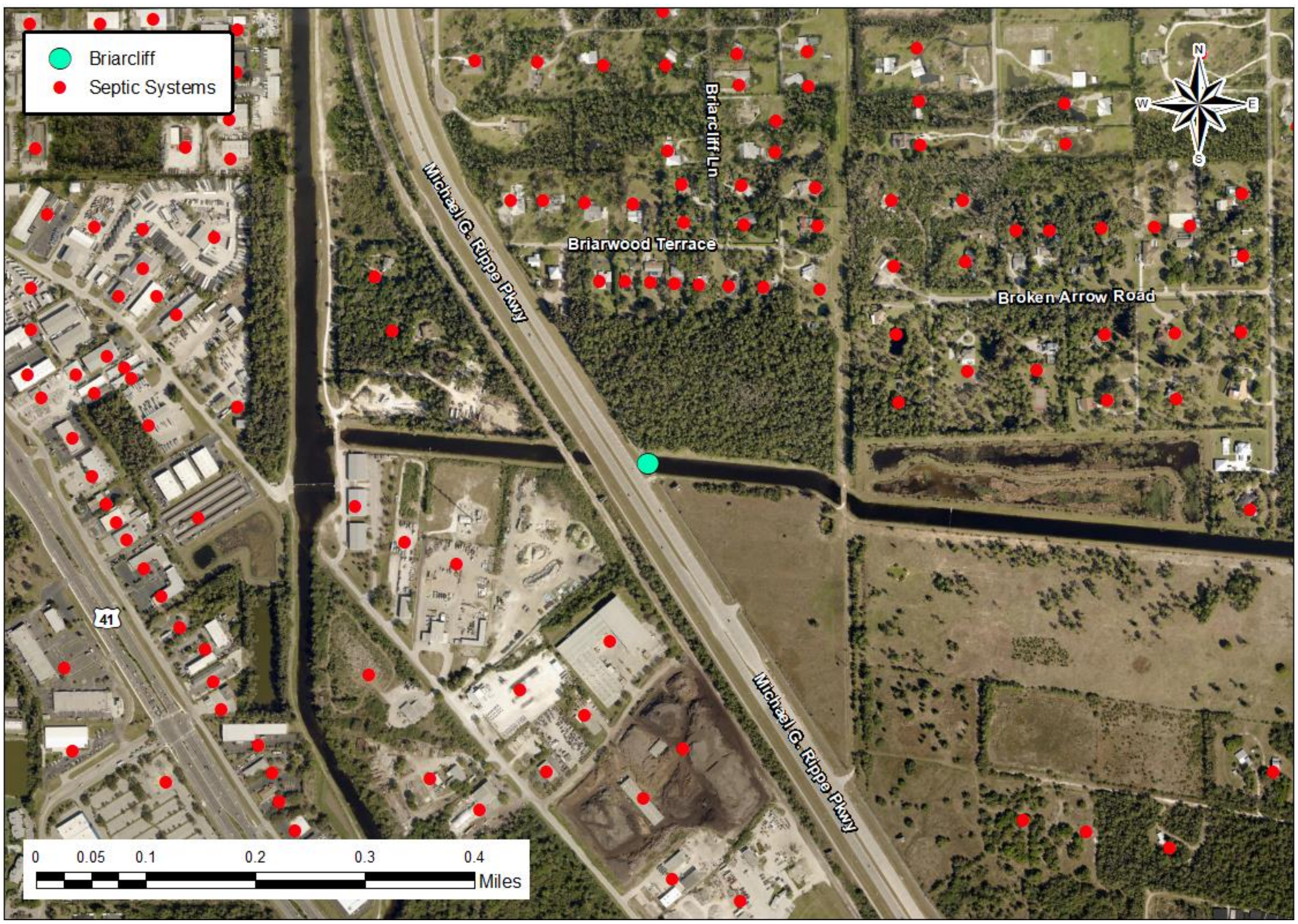

Figure S5. The Briarcliff site in a large drainage canal with homes on large lots to the North of the canal and pastureland to the South.

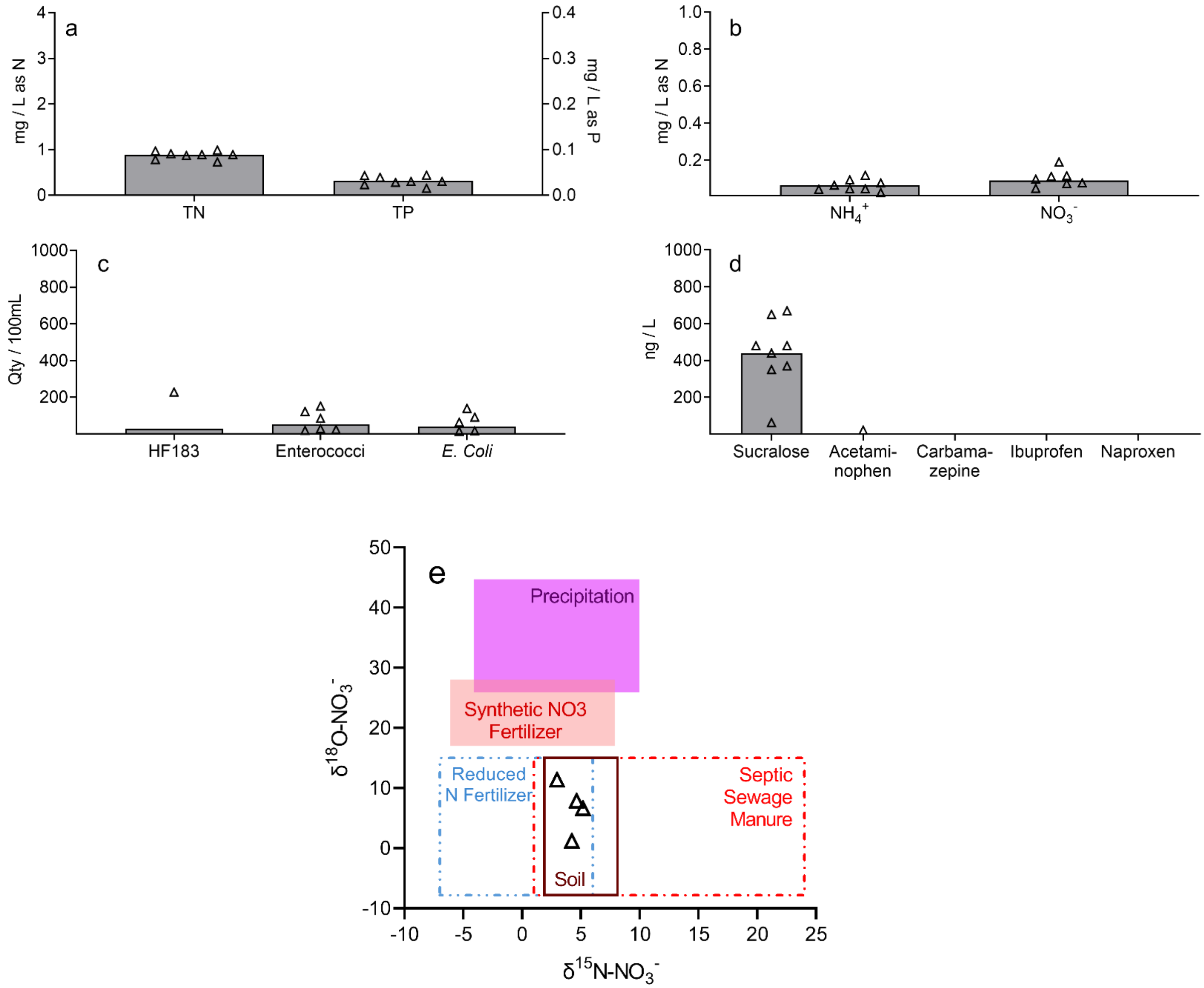

Figure S6. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Briarcliff site. Bars show mean values and triangles show individual detections.

Daughtry Creek

The Daughtry Creek site was located in a residential neighborhood serviced by septic systems. Land use in the surrounding and upstream area includes residential, forest, and pastureland (Figure S7). There is no municipal sewer infrastructure or reclaimed water influences at this site. Total nitrogen was found in moderate concentrations, whereas total phosphorus concentrations were high (Figure S8a). Dissolved inorganic nitrogen was evenly comprised of ammonium and nitrate (Figure S8b) and soluble reactive phosphorus comprised 69% of total phosphorus on average. Bacteria concentrations ranged from low to high and HF183 was only detected in two of eight total samples (Figure S8c). Sucralose was consistently found in moderate concentrations, carbamazepine was detected in two samples, and acetaminophen was detected in one sample (Figure S8d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of human waste but may also include fertilizers and soil organics (Figure S8e).

While bacteria concentrations varied widely between samples, the periodic detections of HF183, acetaminophen, and carbamazepine provide evidence of contamination from septic system effluent at Daughtry Creek. Although nitrogen loading does not appear to be excessive, stable isotope data show that human waste is a nitrate source. Soil organic matter and fertilizers may also contribute. High phosphorus concentrations may possibly be explained by leaching from phosphorus saturated soils where septic systems are present, but upstream sources of phosphorus may also exist.

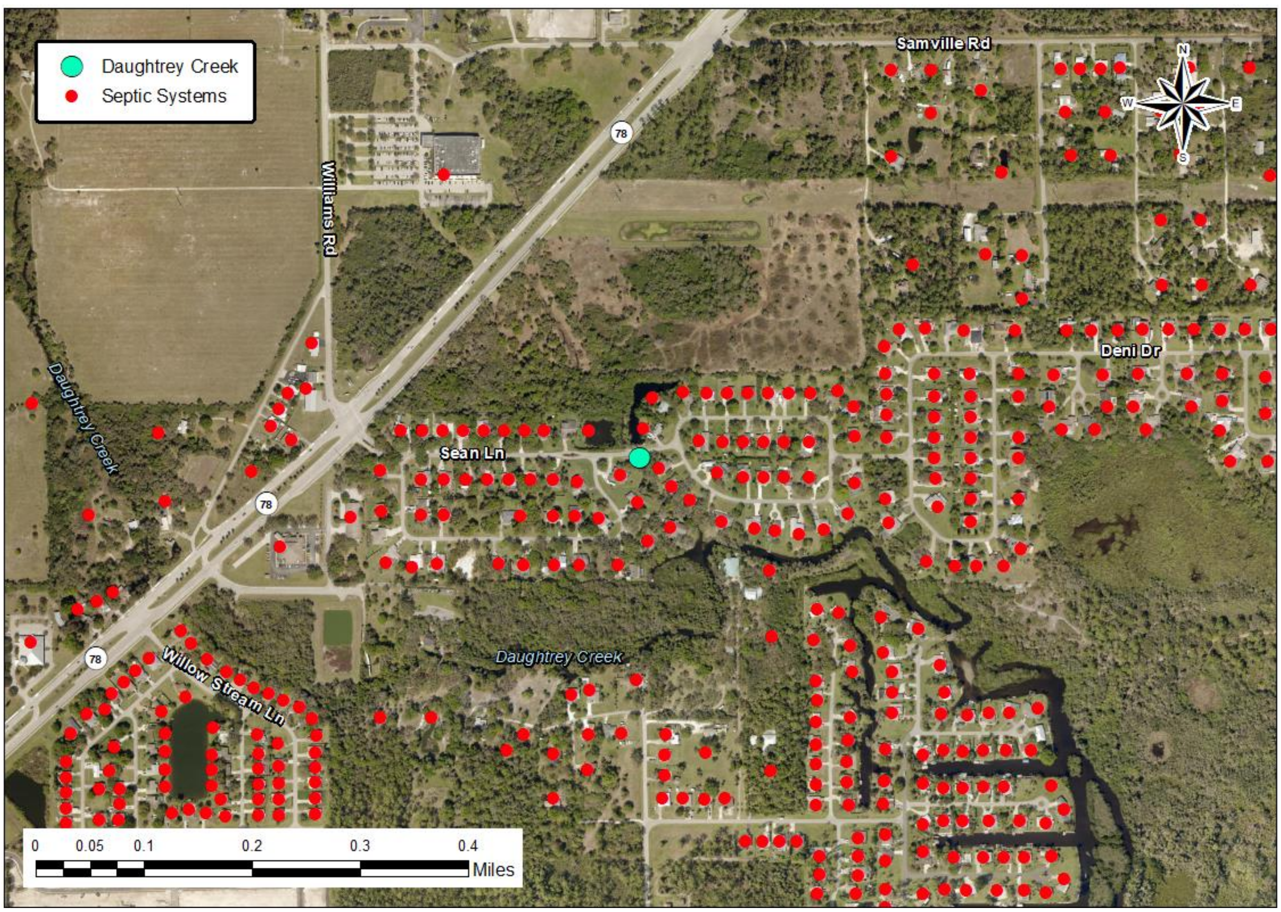

Figure S7. The Daughtry Creek site in a residential neighborhood serviced by septic systems.

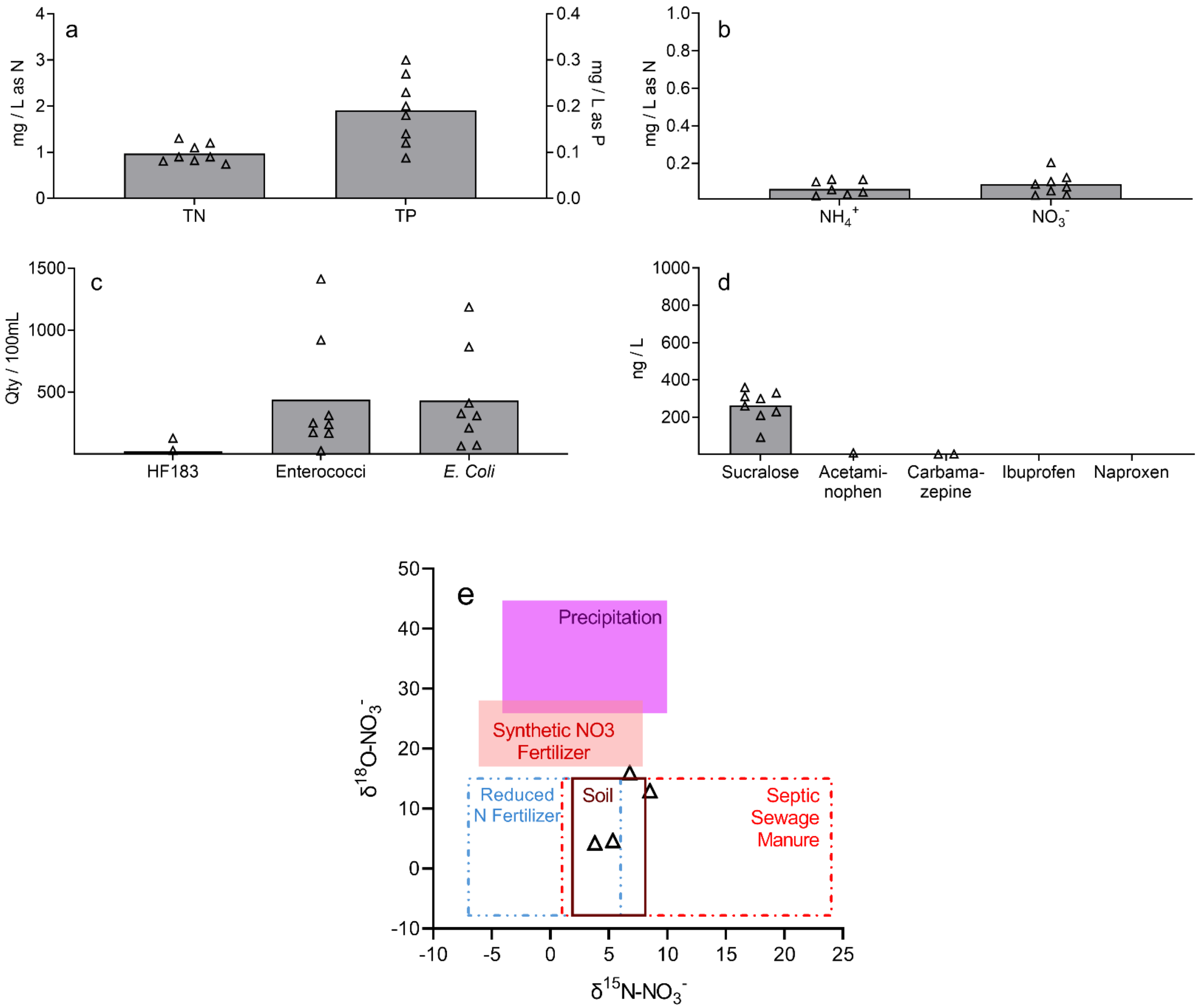

Figure S8. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Daughtry Creek site. Bars show mean values and triangles show individual detections.

Deep Lagoon

The Deep Lagoon site was located in a residential finger canal serviced by septic systems, but the surrounding area is primarily serviced by municipal sewer (Figure S9). There are no reclaimed water influences at this site. Total nitrogen and total phosphorus were found in moderate concentrations (Figure S10a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S10b) and soluble reactive phosphorus comprised 65% of total phosphorus on average. Bacteria concentrations were predominantly low and HF183 was detected in one of eight total samples (Figure S10c). Sucralose was consistently found in high concentrations and carbamazepine was detected in 3 of 8 samples (Figure S10d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of soil, wastewater, and fertilizer nitrogen (Figure S10e).

Although carbamazepine and HF183 were detected periodically and sucralose was found in high concentrations, these values are very similar to those found at the Reference Site. Further, moderate nutrient concentrations and near-complete ammonium oxidation are not indicative of localized septic system contamination. Inconclusive stable isotope data did not allow for identification of specific nitrate sources. Therefore, we find that the presence of human wastewater indicators in surface water at this site may be explained by background concentrations originating upstream in the Caloosahatchee River Estuary. Septic systems may have an influence at this site, but this is not apparent in the data. Dilution may also play an important role, as this site is tidally influenced.

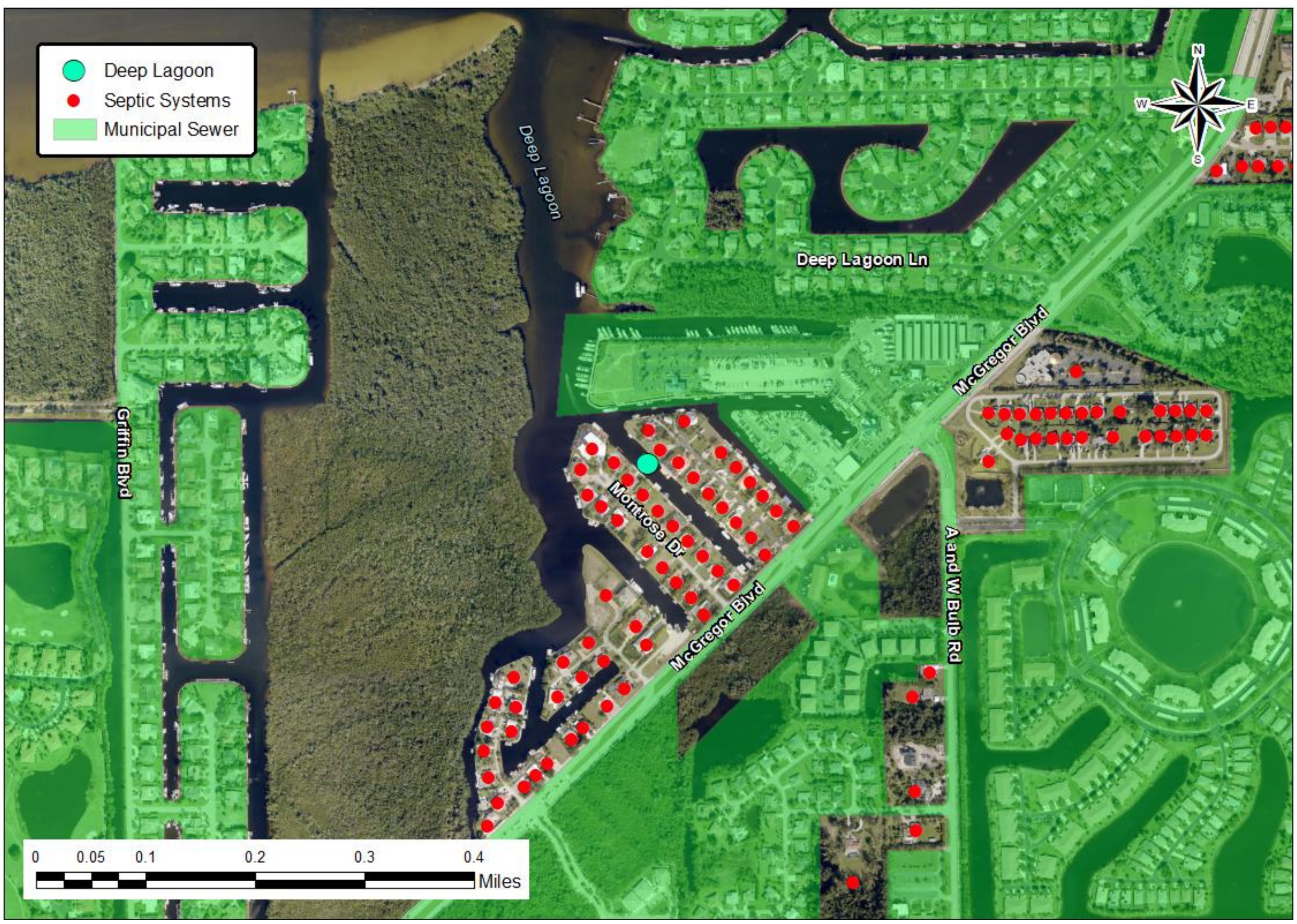

Figure S9. The Deep Lagoon site in a tidally influenced residential finger canal within the Caloosahatchee River Estuary.

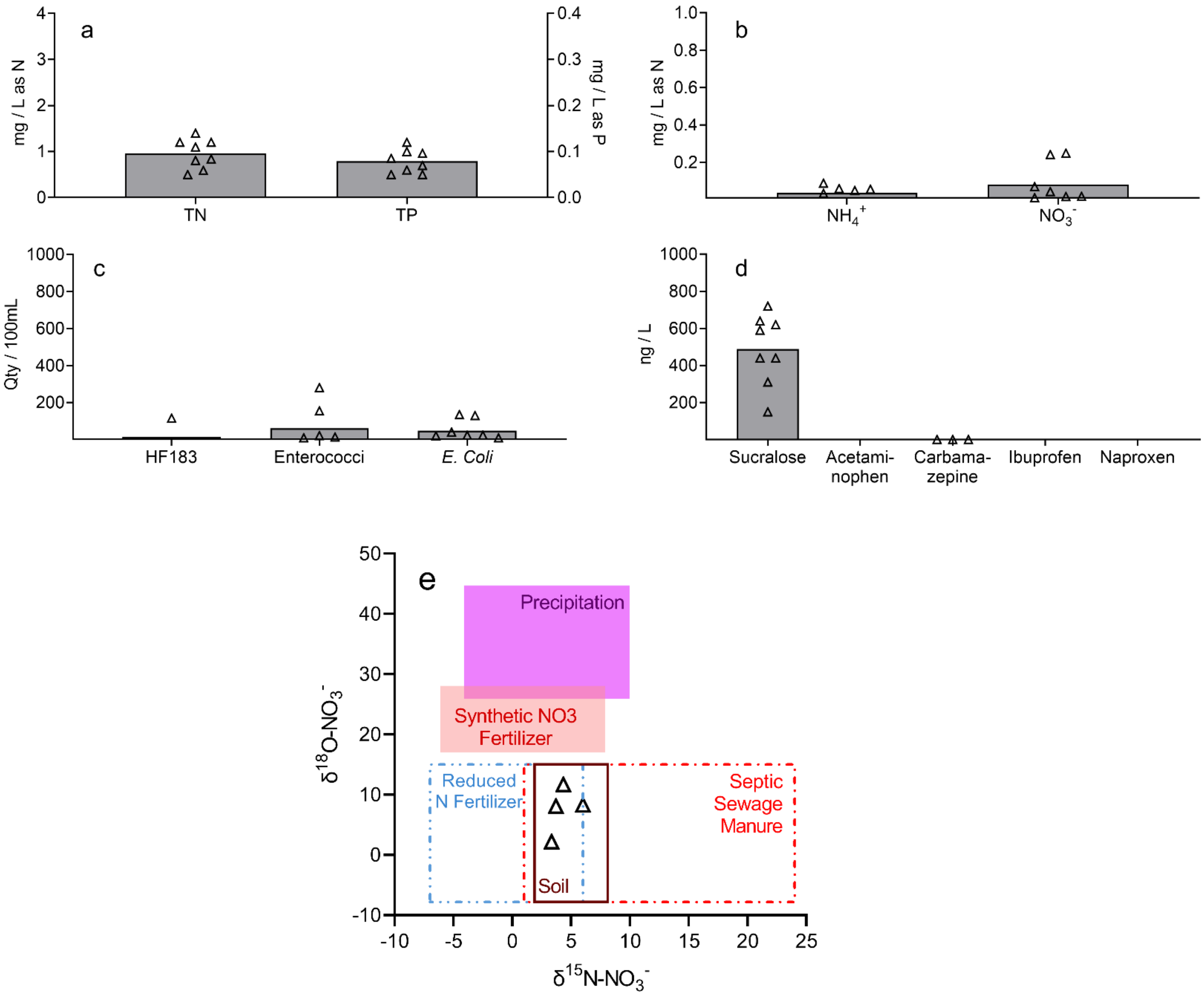

Figure S10. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Deep Lagoon site. Bars show mean values and triangles show individual detections.

Fort Myers Shores

The Fort Myers Shores site was located in a residential finger canal serviced by septic systems (Figure S11). There are no reclaimed water influences at this site. Total nitrogen and total phosphorus were found in moderate concentrations (Figure S12a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S12b) and soluble reactive phosphorus comprised 55% of total phosphorus on average. Bacteria concentrations were high and HF183 was detected in six of eight samples (Figure S12c). Sucralose was found in mostly moderate concentrations, but no other chemical tracers were detected (Figure S12d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of wastewater nitrogen (Figure S12e).

Although neither sucralose nor nutrients were detected in high concentrations, and no other chemical wastewater tracers were detected, very high concentrations of bacteria in conjunction with frequent detections of HF183 indicate a human wastewater influence. Since there is no reclaimed water application or municipal sewer infrastructure at this site, septic systems are likely the source of this contamination. This is further supported by stable isotope data which suggests that the primary nitrate source is human waste.

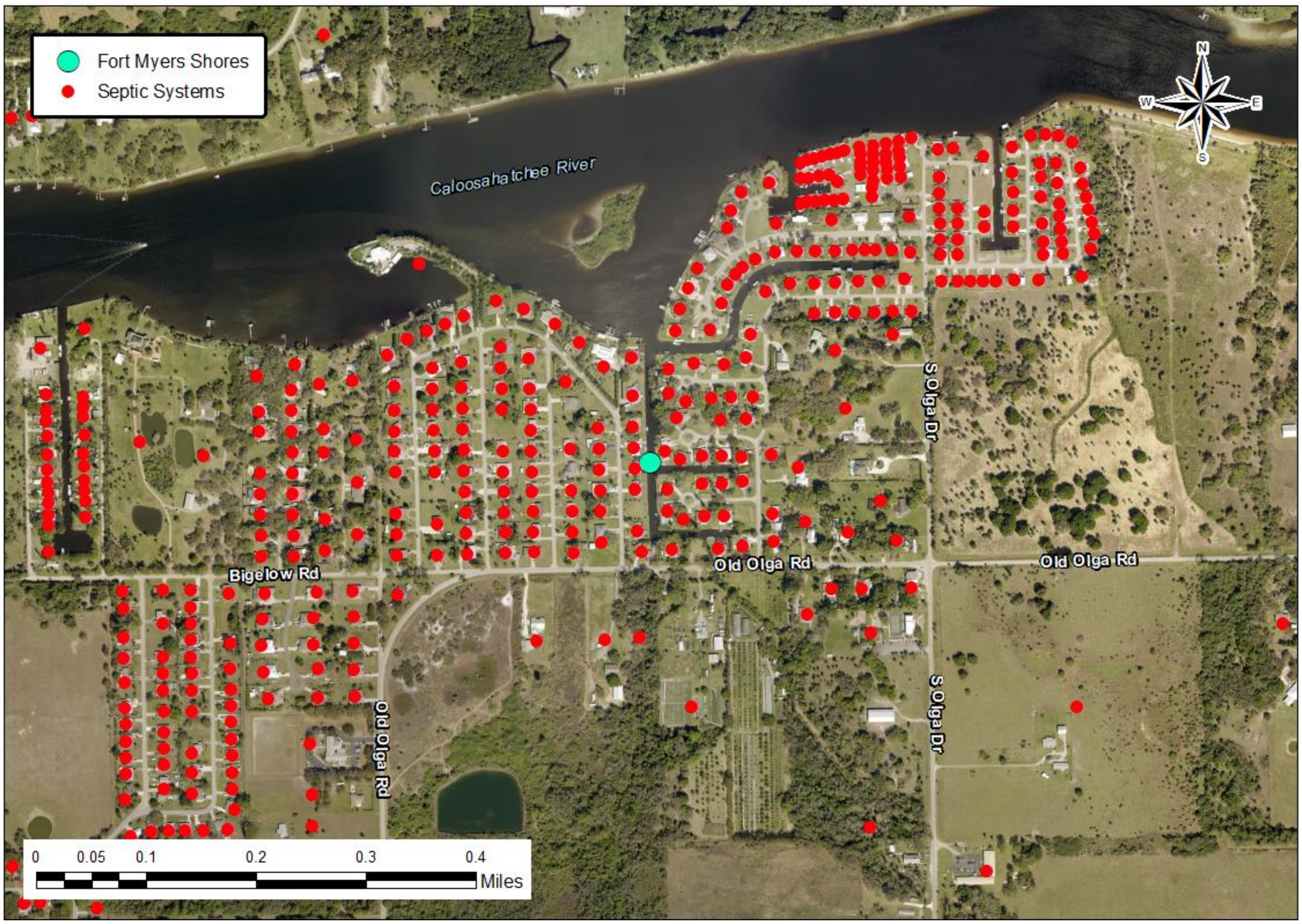

Figure S11. The Fort Myers Shores site located in a residential finger canal serviced by septic systems within the Caloosahatchee River Estuary.

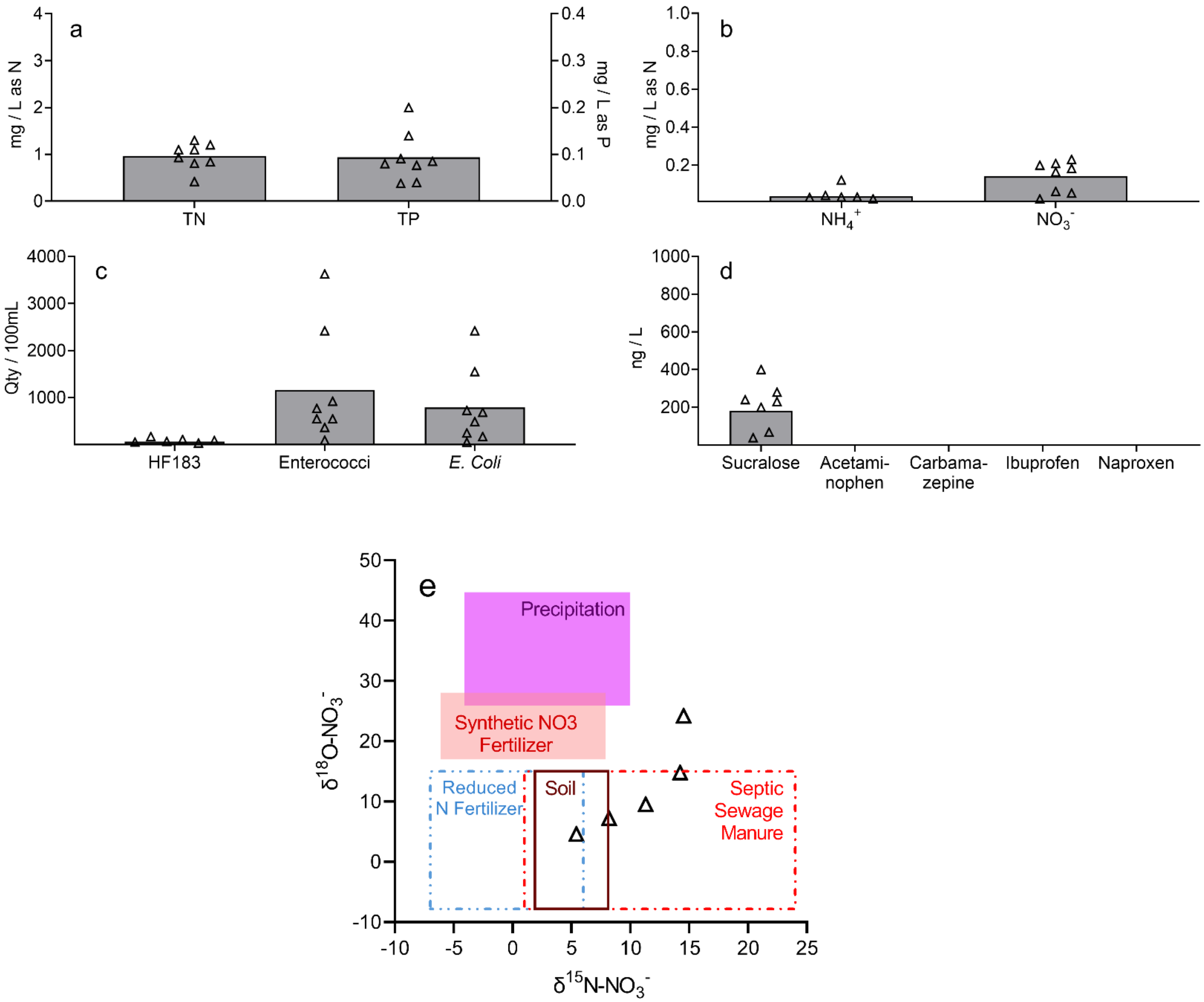

Figure S12. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Fort Myers Shores site. Bars show mean values and triangles show individual detections.

Hendry Creek

The Hendry Creek site was located in a meandering brackish tidal creek adjacent to several waterfront communities serviced by septic systems (Figure S13). Some nearby upstream communities are serviced municipal sewer. There are no direct reclaimed water influences at this site. Total nitrogen and total phosphorus were found in moderate concentrations (Figure S14a). Dissolved inorganic nitrogen was evenly comprised of ammonium and nitrate (Figure S14b) and soluble reactive phosphorus comprised 37% of total phosphorus on average. Bacteria concentrations ranged from moderate to high and HF183 was detected in all samples (Figure S14c). Very high concentrations of HF183 were observed in January 2021. Sucralose was consistently found in high concentrations, carbamazepine was detected in four of eight samples, and acetaminophen was detected in 1 sample (Figure S14d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of wastewater, fertilizer, and soil organic nitrogen (Figure S14e).

The detection of HF183 in all samples provides clear evidence of human waste contamination at Hendry Creek. Septic systems located along the creek are the most probable source of this contamination. High concentrations of sucralose in conjunction with frequent detections of carbamazepine and acetaminophen provides further evidence of this. Although the stable isotope data shows that multiple potential nitrate sources may exist, we can conclude based on the prevalence of other wastewater indicators that human wastewater is a significant contributor.

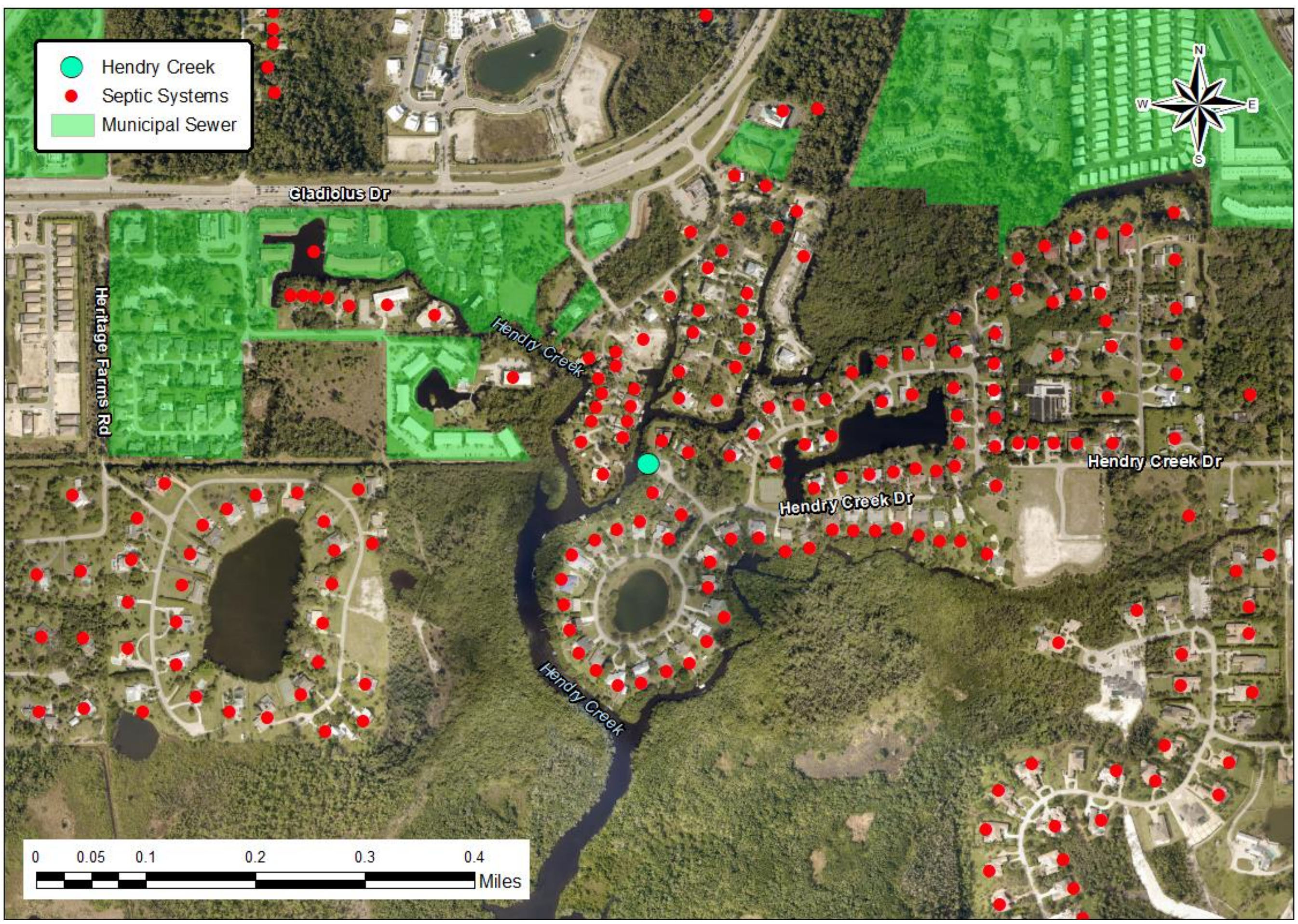

Figure S13. The Hendry Creek site located in a meandering brackish creek adjacent to several waterfront communities serviced by septic systems.

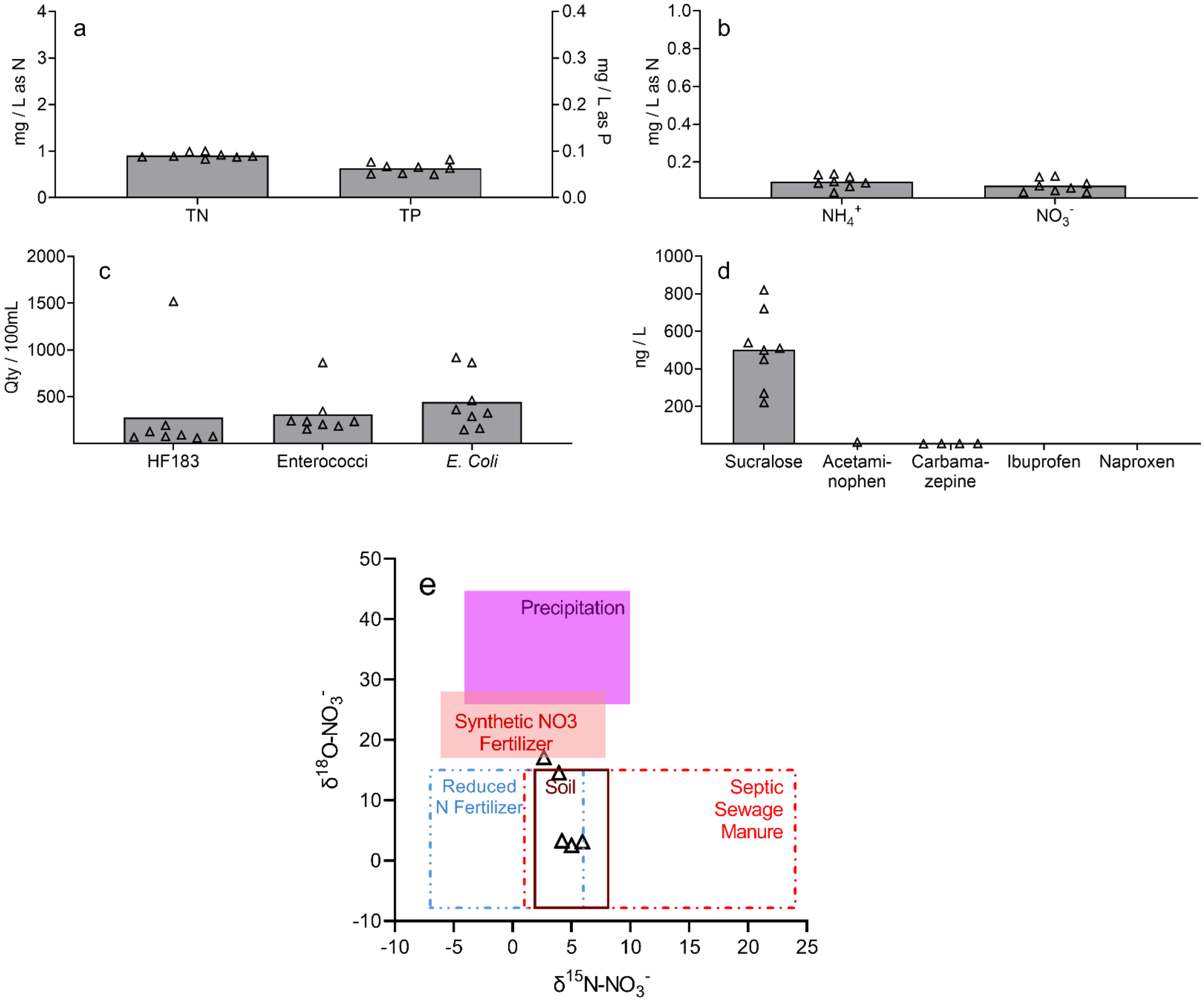

Figure S14. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Hendry Creek site. Bars show mean values and triangles show individual detections.

Heritage Farms

The Heritage Farms site was located in a slow-flowing drainage ditch between a residential community serviced by septic systems to the north and a golf course upon which reclaimed water is applied to the south (Figure S15). Total nitrogen was found in moderate to high concentrations and phosphorus was found in moderate concentrations (Figure S16a). Dissolved inorganic nitrogen was primarily in the form of ammonium (Figure S16b) and soluble reactive phosphorus comprised 36% of total phosphorus on average. Bacteria concentrations were high for enterococci and low to moderate for *E. coli*. HF183 was detected in seven of eight samples and concentrations ranged from low to very high (Figure S16c). High sucralose concentrations were found in all samples, carbamazepine was also detected in all samples, and naproxen was detected in one sample (Figure S16d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of soil and fertilizer nitrogen (Figure S16e).

Human waste contamination at Heritage Farms is evidenced by very high concentrations of sucralose, enterococci, and HF183, as well as by detections of carbamazepine and naproxen. Incomplete ammonium oxidation suggests rapid transport of these contaminants to the surface water. Assuming proper functioning of municipal sewer infrastructure, these contaminants likely originate from either nearby septic systems and/or reclaimed water application. Stable isotope data show that nitrate sources may include soil, fertilizer, and wastewater. However, based on the prevalence of other human waste indicators, we can conclude that human wastewater is a significant contributor.

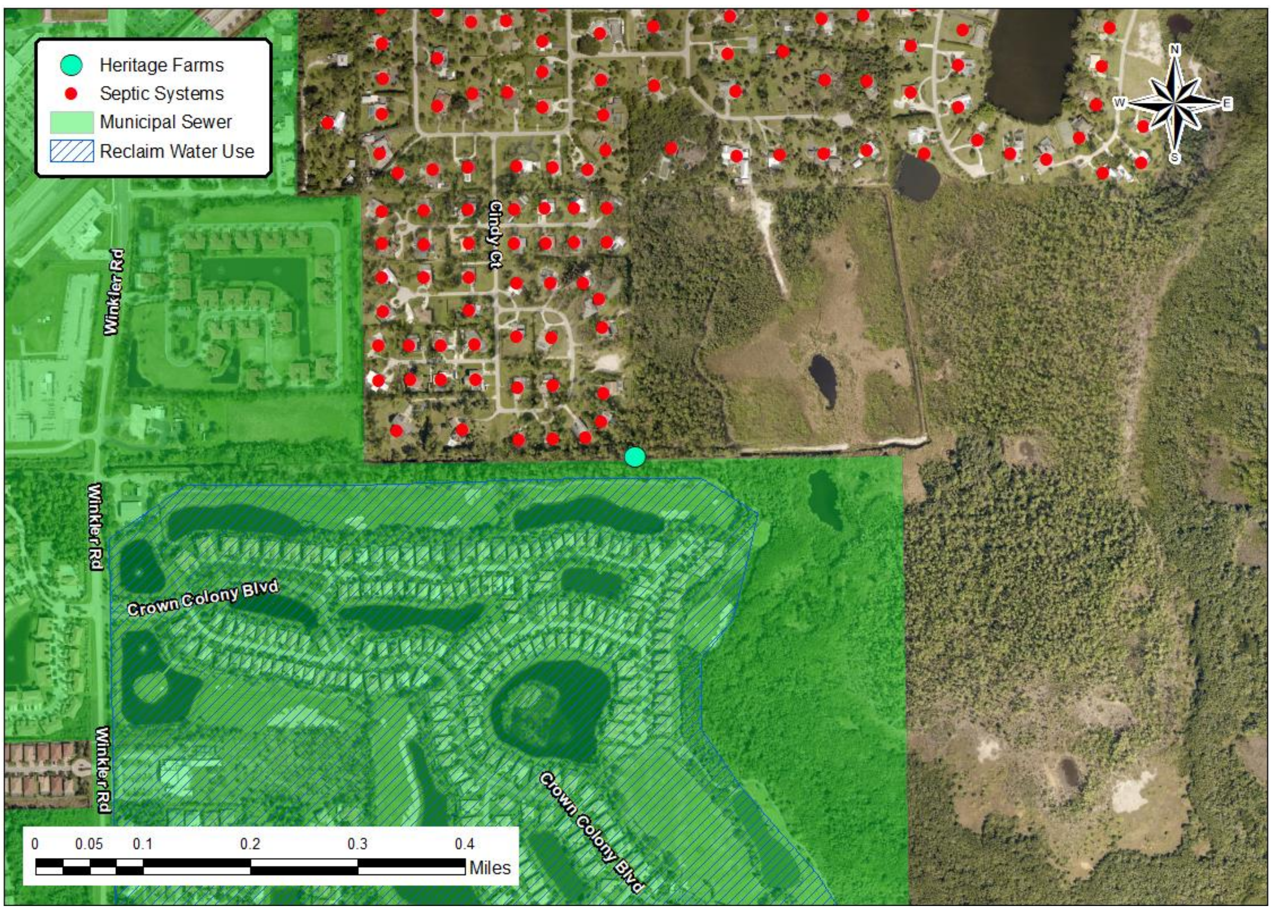

Figure S15. The Heritage Farms site located in a drainage ditch adjacent to a residential community and golf course.

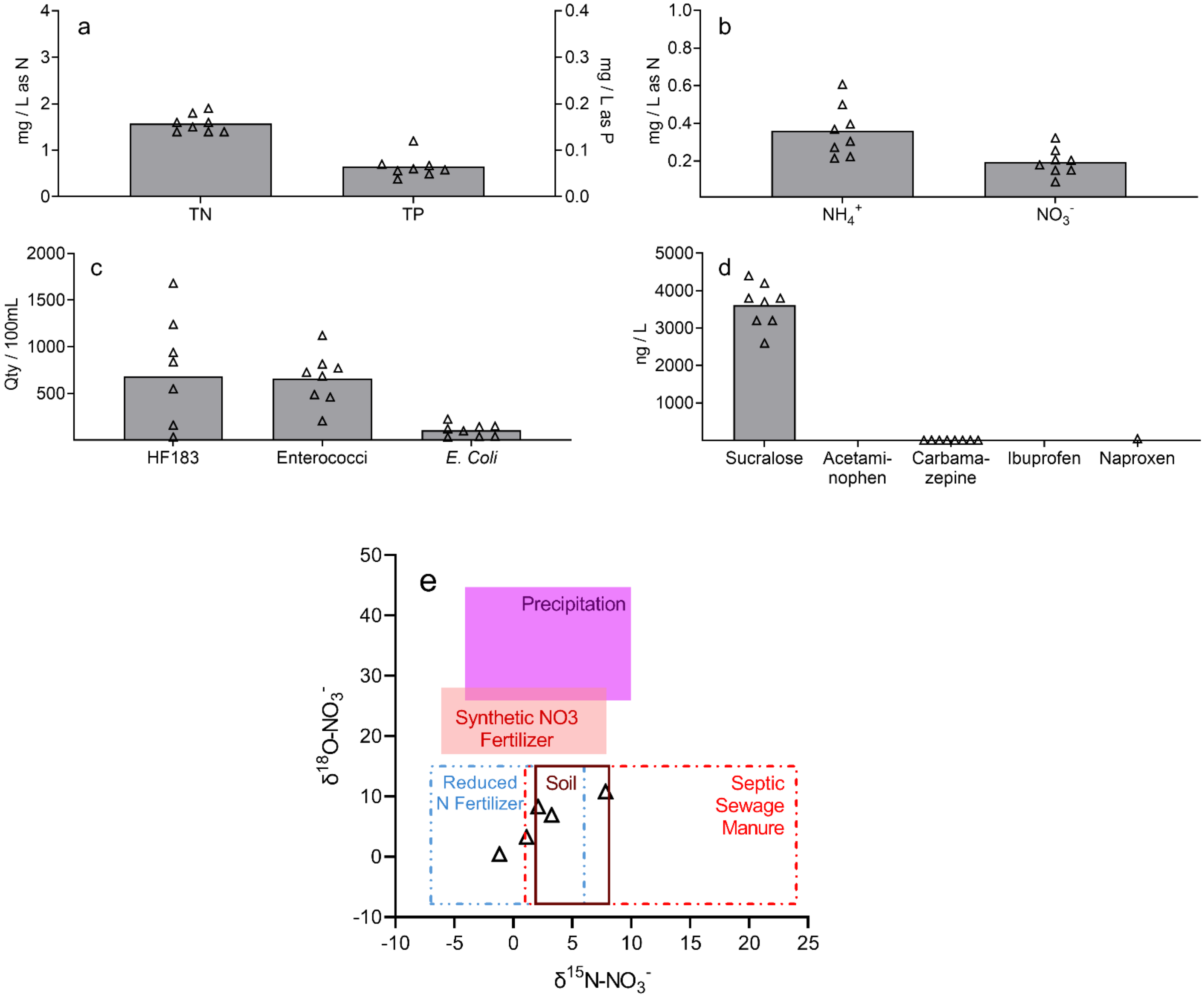

Figure S16. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Heritage Farms site. Bars show mean values and triangles show individual detections.

Lake McGregor

The Lake McGregor site was located in a drainage ditch in a residential area serviced primarily by municipal sewer (Figure S17). A small block of homes serviced by septic systems is located adjacent to the site and reclaimed water is applied on a golf course directly upstream of the site. Total nitrogen and total phosphorus were found in moderate concentrations (Figure S18a). Dissolved inorganic nitrogen was primarily in the form of ammonium (Figure S18b) and soluble reactive phosphorus comprised 39% of total phosphorus on average. Bacteria concentrations were predominantly high, especially enterococci which was found in very high concentrations, and HF183 was detected in seven of eight samples (Figure S18c). Sucralose was consistently found in moderate concentrations and acetaminophen was detected in one sample (Figure S18d). $\delta^{15}N$-NO_3^- values were in the range of soil and fertilizer nitrogen (Figure S18e).

Although nutrient concentrations at Lake McGregor were moderate, the prevalence of ammonium may be indicative of human waste influence. Recurring detections of chemical tracers and HF183 in conjunction with high concentrations of fecal indicator bacteria confirm the presence of human waste at this site. Assuming proper functioning of municipal sewer infrastructure, the source of these contaminants is likely either septic systems directly adjacent to the site or from reclaimed water application on golf courses directly upstream. However, stable isotope data indicate that nitrate sources may also include soil and fertilizer.

Figure S17. The Lake McGregor site located in a drainage ditch in a residential area.

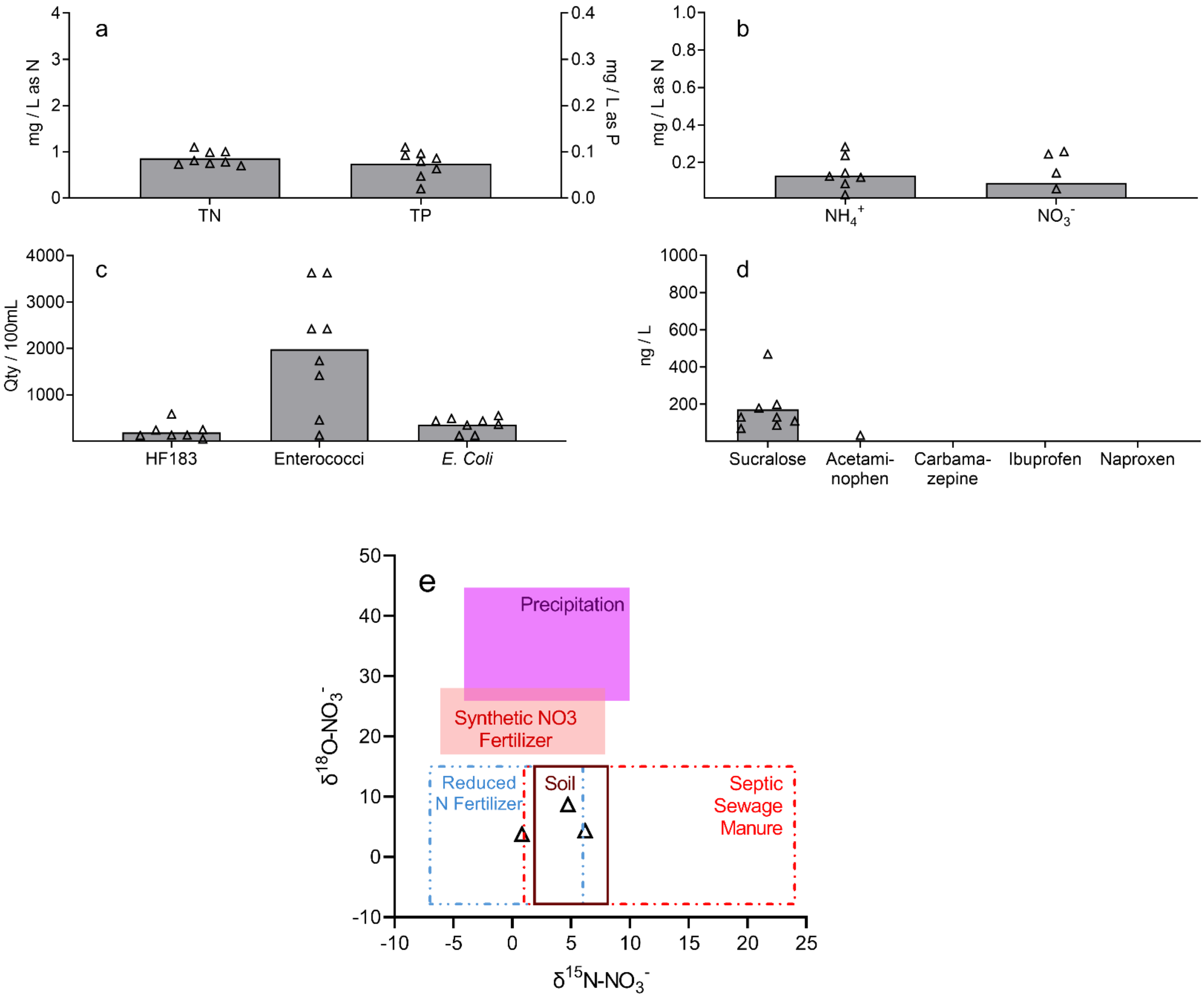

Figure S18. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Lake McGregor site. Bars show mean values and triangles show individual detections.

Laurelin Court

The Laurelin Court site was located in a tidally influenced finger canal in North Fort Myers serviced by septic systems within the Caloosahatchee River Estuary (Figure S19). Total nitrogen was found in moderate concentrations, whereas concentrations of total phosphorus ranged from moderate to high (Figure S20a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S20b) and soluble reactive phosphorus comprised 53% of total phosphorus on average. Bacteria concentrations were predominantly moderate but were sometimes found in high concentrations for enterococci. HF183 was not detected in any of the samples collected at this site (Figure S20c). Sucralose was detected in all samples at mostly moderate concentrations and carbamazepine was detected in one sample (Figure S20d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of both soil and human waste nitrogen (Figure S20e).

Detections of sucralose and carbamazepine are indicators of human waste pollution, but the concentrations observed here are similar to those observed at the Reference site where no septic systems are present. This may indicate that sucralose and carbamazepine originate upstream and are present in the Caloosahatchee River Estuary at background levels. High total phosphorus and enterococci may possibly be explained by leaching from septic systems. However, if septic systems were significantly contributing to waste contamination at this site, higher prevalence of ammonium and HF183 would be expected. Further, isotope data suggests that soil or sediment may be a significant source of nitrate. High $\delta^{15}N\text{-}NO_3^-$ (>7‰) observed in one sample shows that human waste may periodically dominate as a nitrate source.

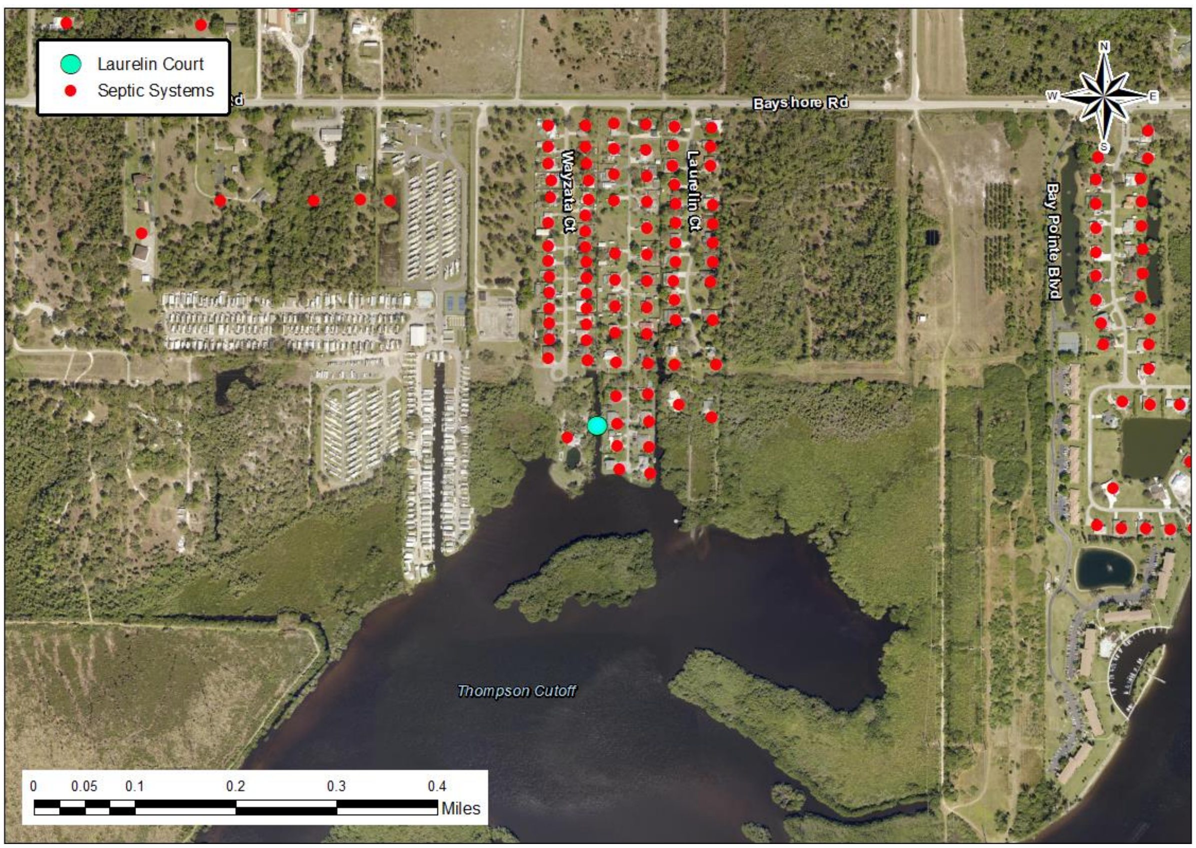

Figure S19. The Laurelin Court site located in a tidally influenced finger canal in North Fort Myers serviced by septic systems within the Caloosahatchee River Estuary.

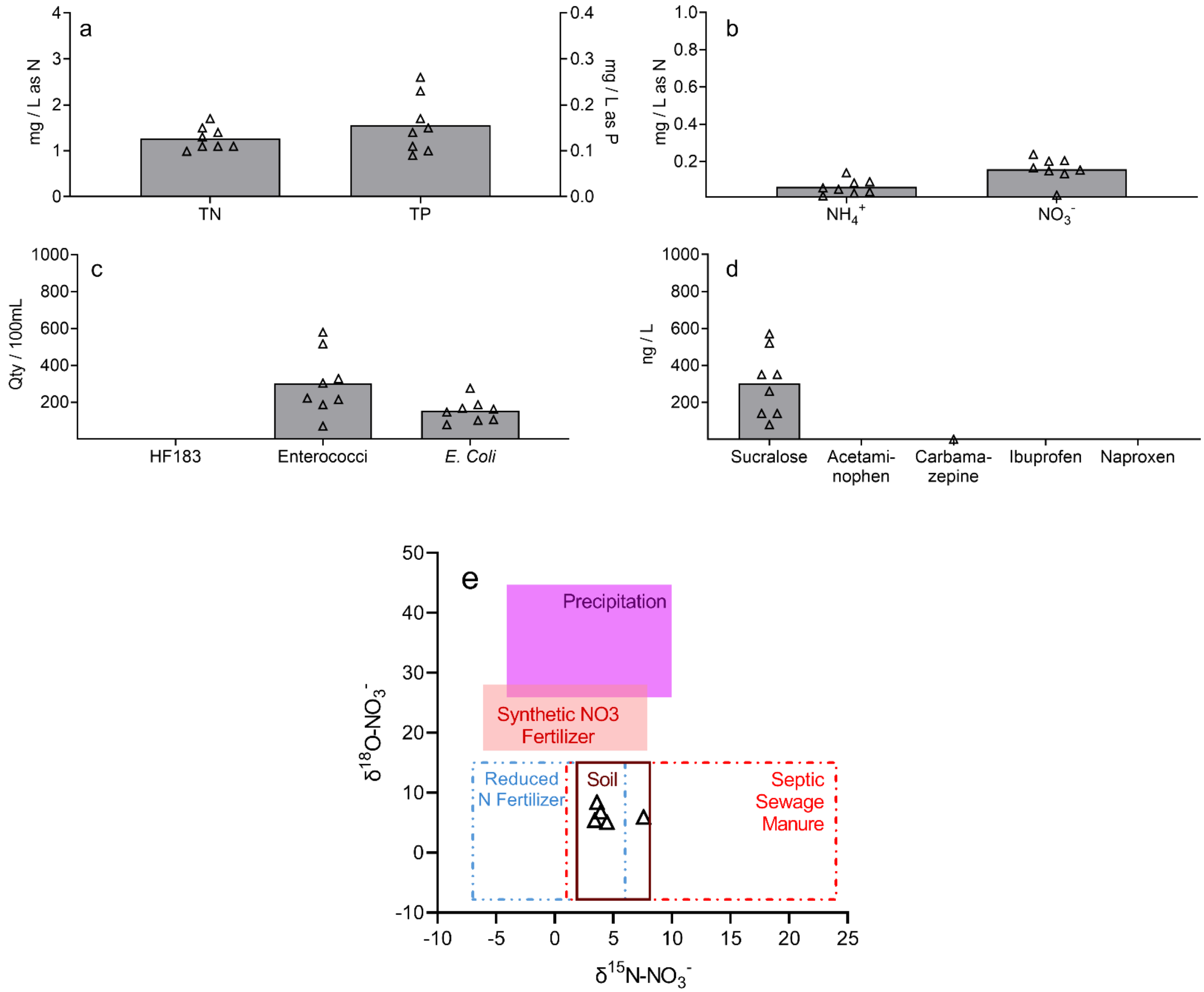

Figure S20. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Laurelin Court site. Bars show mean values and triangles show individual detections.

Ligon Court

The Ligon Court site was located in a shallow, seasonally wet drainage ditch in a residential neighborhood serviced by septic systems (Figure S21). The area surrounding this neighborhood is serviced by municipal sewer and reclaimed water is applied on a nearby golf course. This site was sampled three times before being discontinued due to the ditch being seasonally dry. Total nitrogen was found in very high concentrations and total phosphorus concentrations were mostly high (Figure S22a). Dissolved inorganic nitrogen was primarily in the form of ammonium (Figure S22b) and soluble reactive phosphorus comprised 23% of total phosphorus on average. Bacteria concentrations were predominantly high, especially for enterococci, yet HF183 was not detected in any samples (Figure S22c). Sucralose was consistently found in very high concentrations and carbamazepine was detected in all three samples (Figure S22d). Samples were not analyzed for $\delta^{15}N$-NO_3^- values at this site.

Although HF183 was not detected, several other parameters provide evidence of human waste contamination at Ligon Court, including high concentrations of ammonium, sucralose, fecal indicator bacteria, and frequent detections of carbamazepine. Assuming proper functioning of municipal sewer infrastructure, septic systems are a likely source for these contaminants. Nearby reclaimed water application may also contribute as a contaminant source.

Figure S21. The Ligon Court site located in a shallow, seasonally wet drainage ditch in a residential neighborhood serviced by septic systems.

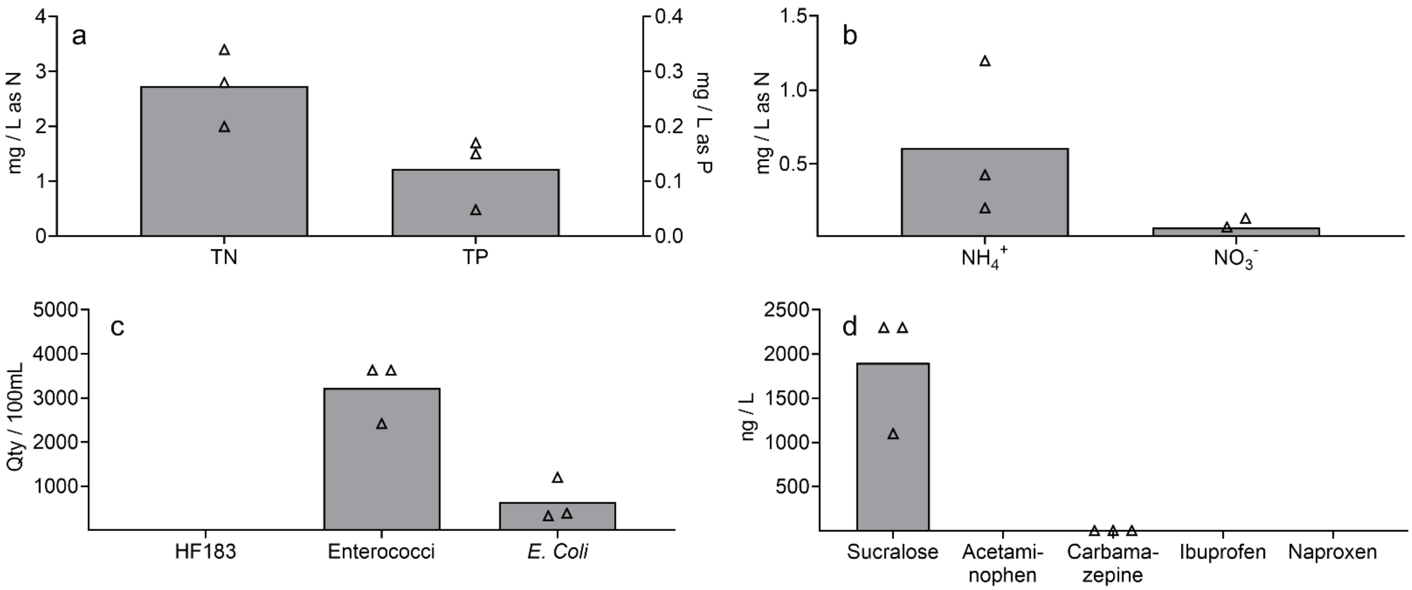

Figure S22. Total nutrients (a), nitrogen speciation (b), bacteria (c), and chemical tracers (d) measured in surface water samples collected from the Ligon Court site. Bars show mean values and triangles show individual detections.

Mobile Manor

The Mobile Manor site was located in a residential drainage ditch serviced by septic systems (Figure S23). There are no municipal sewer or reclaimed water influences at this site. Total nitrogen was found in moderate concentrations whereas total phosphorus concentrations were very high (Figure S24a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S24b) and soluble reactive phosphorus comprised 58% of total phosphorus on average. Bacteria concentrations were high and HF183 was detected in six of eight samples (Figure S24c). Sucralose was consistently found in high concentrations and carbamazepine was detected in all eight samples (Figure S24d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of human waste (Figure S24e).

Human waste influence at Mobile Manor is evidenced by high concentrations of sucralose and fecal indicator bacteria, as well as by frequent detections of carbamazepine and HF183. Further, high total phosphorus is indicative of phosphorus-saturated soils. High density septic systems directly adjacent to the study site are a likely source of these contaminants. This is supported by high $\delta^{15}N\text{-}NO_3^-$ values, indicating that the primary nitrate source is human waste.

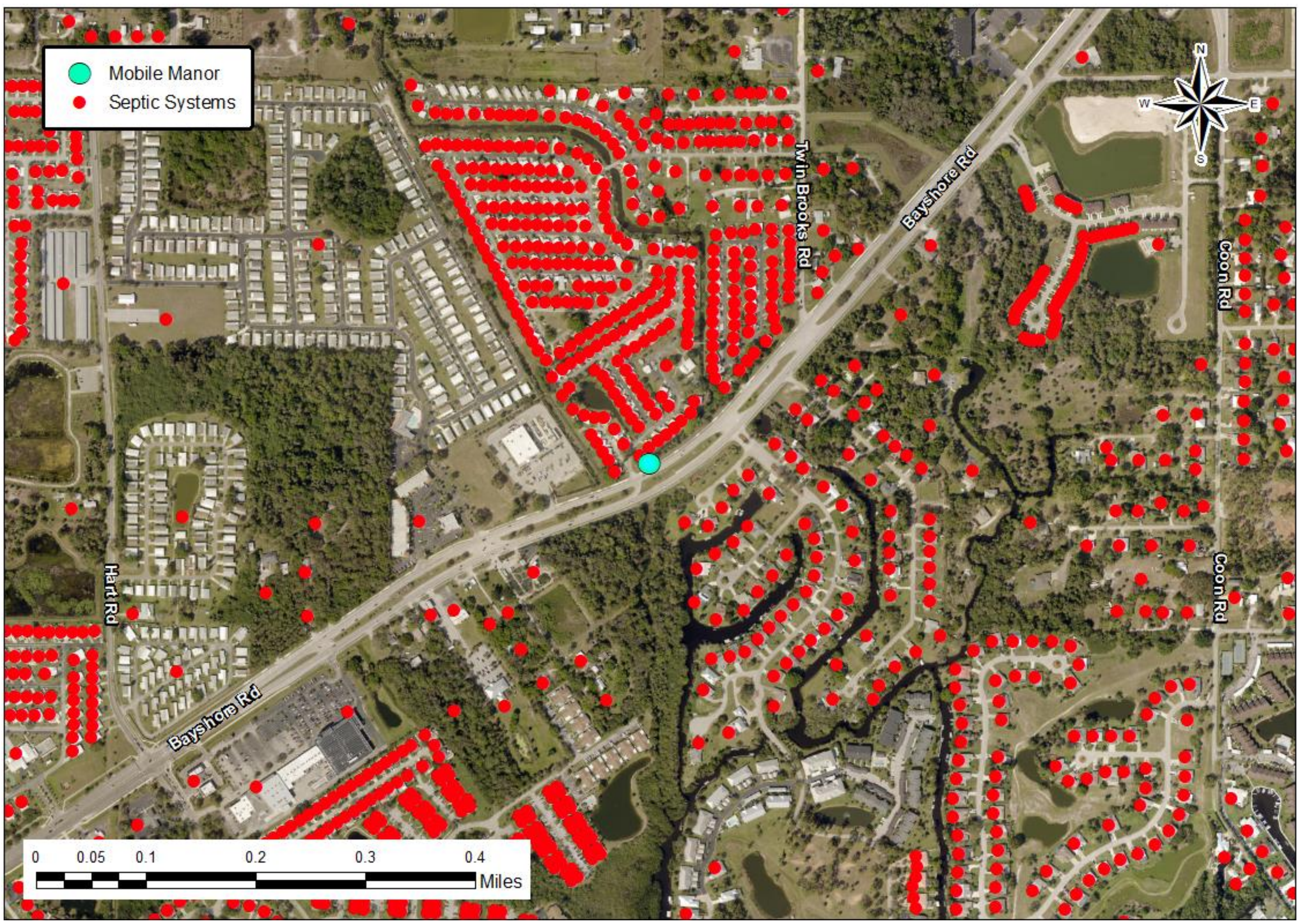

Figure S23. The Mobile Manor site located in a residential drainage ditch serviced by septic systems.

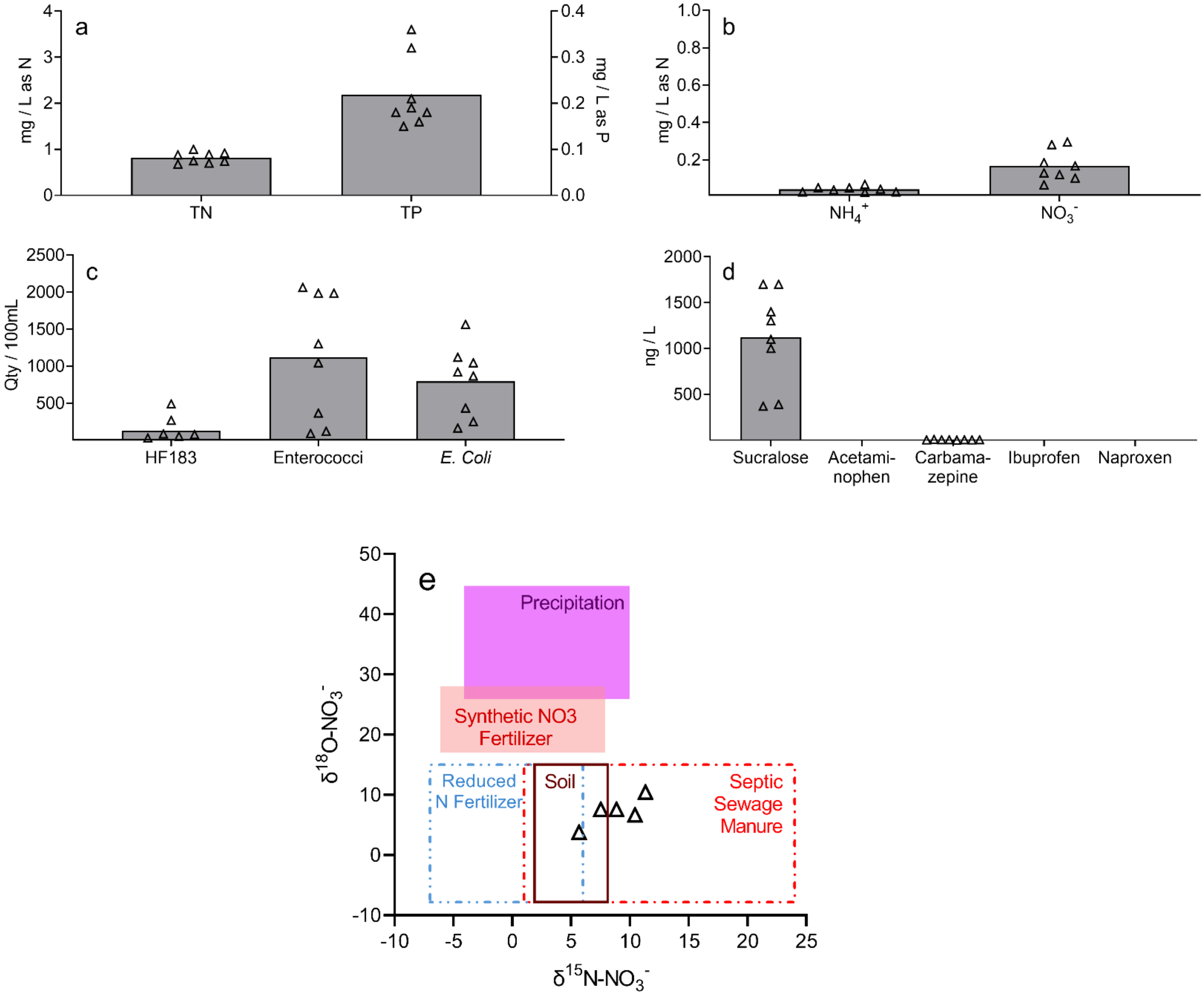

Figure S24. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Mobile Manor site. Bars show mean values and triangles show individual detections.

North Town & River

The North Town & River site was located in a residential finger canal within the Caloosahatchee River Estuary that is serviced by septic systems, but the surrounding area is primarily serviced by municipal sewer (Figure S25). Reclaimed water is applied on a golf course to the north of the study site. Total nitrogen and total phosphorus were found in moderate concentrations (Figure S26a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S26b) and soluble reactive phosphorus comprised 68% of total phosphorus on average. Bacteria concentrations were low and HF183 was not detected in any samples (Figure S26c). Sucralose was consistently found in high concentrations and carbamazepine was detected in two of eight samples (Figure S26d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of soil nitrogen (Figure S26e).

The data collected at North Town & River provide inconclusive evidence of human waste contamination from septic systems. Although carbamazepine was detected sporadically and sucralose was routinely found in high concentrations, these detections are similar to those observed at the Reference Site. Likewise, fecal indicator bacteria concentrations are also similar to those observed at the Reference Site. Surface water contaminated by septic system effluent would be expected to contain high ammonium and frequent detections of HF183, whereas neither was observed at this site. Therefore, the presence of human waste indicators in surface water at this site may be explained by background concentrations originating upstream in the Caloosahatchee River Estuary from other sources, such as wastewater treatment plant discharge. Septic systems at this site may also contribute as a contaminant source, but this is not apparent in the data. Moderate $\delta^{15}N\text{-}NO_3^-$ values in combination with low concentrations of other human waste indicators suggests that the primary nitrate source may be soil or sediment, although human waste may still contribute. Dilution may also play an important role, as this site is tidally influenced.

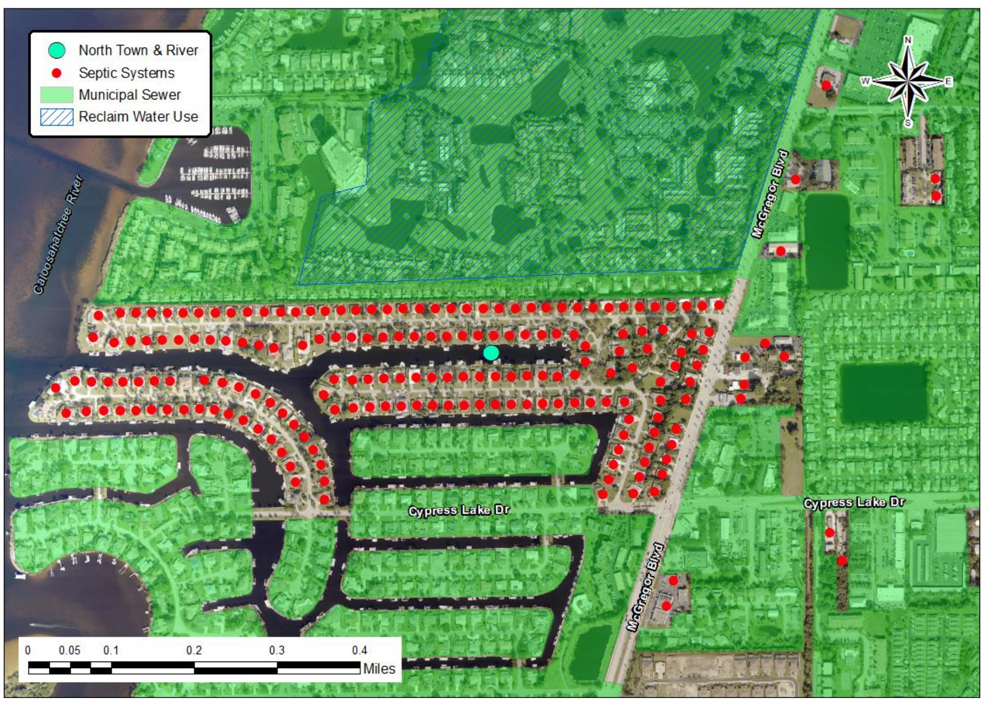

Figure S25. The North Town & River site located in a residential finger canal serviced by septic systems within the Caloosahatchee River Estuary.

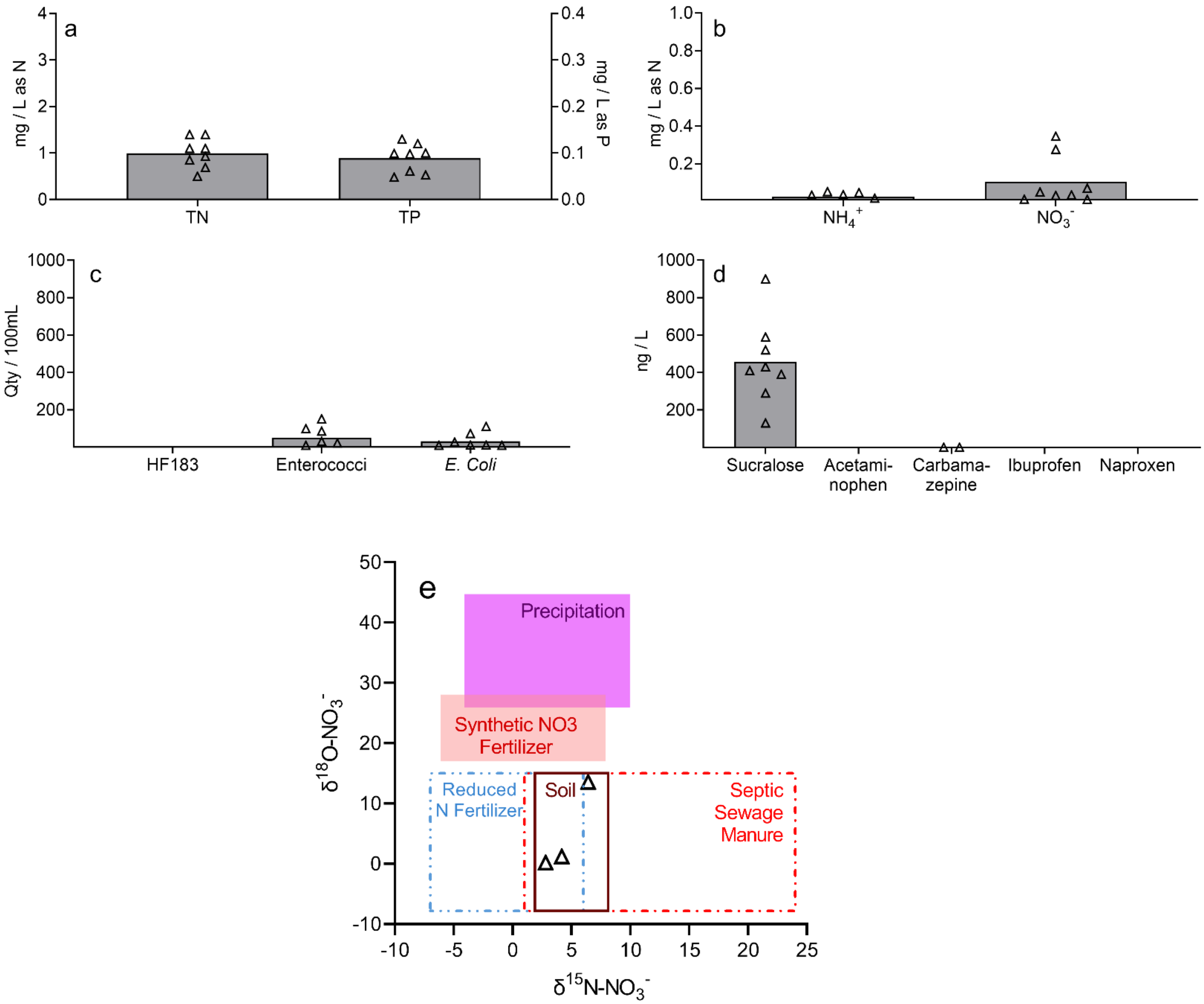

Figure S26. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the North Town & River site. Bars show mean values and triangles show individual detections.

Orange River

The Orange River site was located in a residential neighborhood adjacent to the Orange River, a tributary for the Caloosahatchee River Estuary (Figure S27). Homes in this area are primarily serviced by septic systems, but some municipal sewer infrastructure exists nearby. Total nitrogen was found in moderate concentrations whereas total phosphorus concentrations were often high (Figure S28a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S28b) and soluble reactive phosphorus comprised 70% of total phosphorus on average. Bacteria concentrations were predominantly high and HF183 was detected in six of eight samples (Figure S28c). Sucralose was consistently found in moderate to high concentrations and carbamazepine was detected in three samples (Figure S28d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of human waste and soil nitrogen (Figure S28e).

Human waste contamination at Orange River is evidenced by moderate to high detections of sucralose and fecal indicator bacteria, as well as by frequent detections of carbamazepine and HF183. Nearby septic systems are likely a source of these contaminants, but other upstream sources may also contribute. High total phosphorus may reflect phosphorus-saturated soils in the surrounding area, as well as natural background phosphorus. Moderate $\delta^{15}N\text{-}NO_3^-$ values suggest that nitrate sources may include soil, sediment, or human waste. However, given the prevalence of other human waste indicators, it is likely a significant source of nitrate at this location.

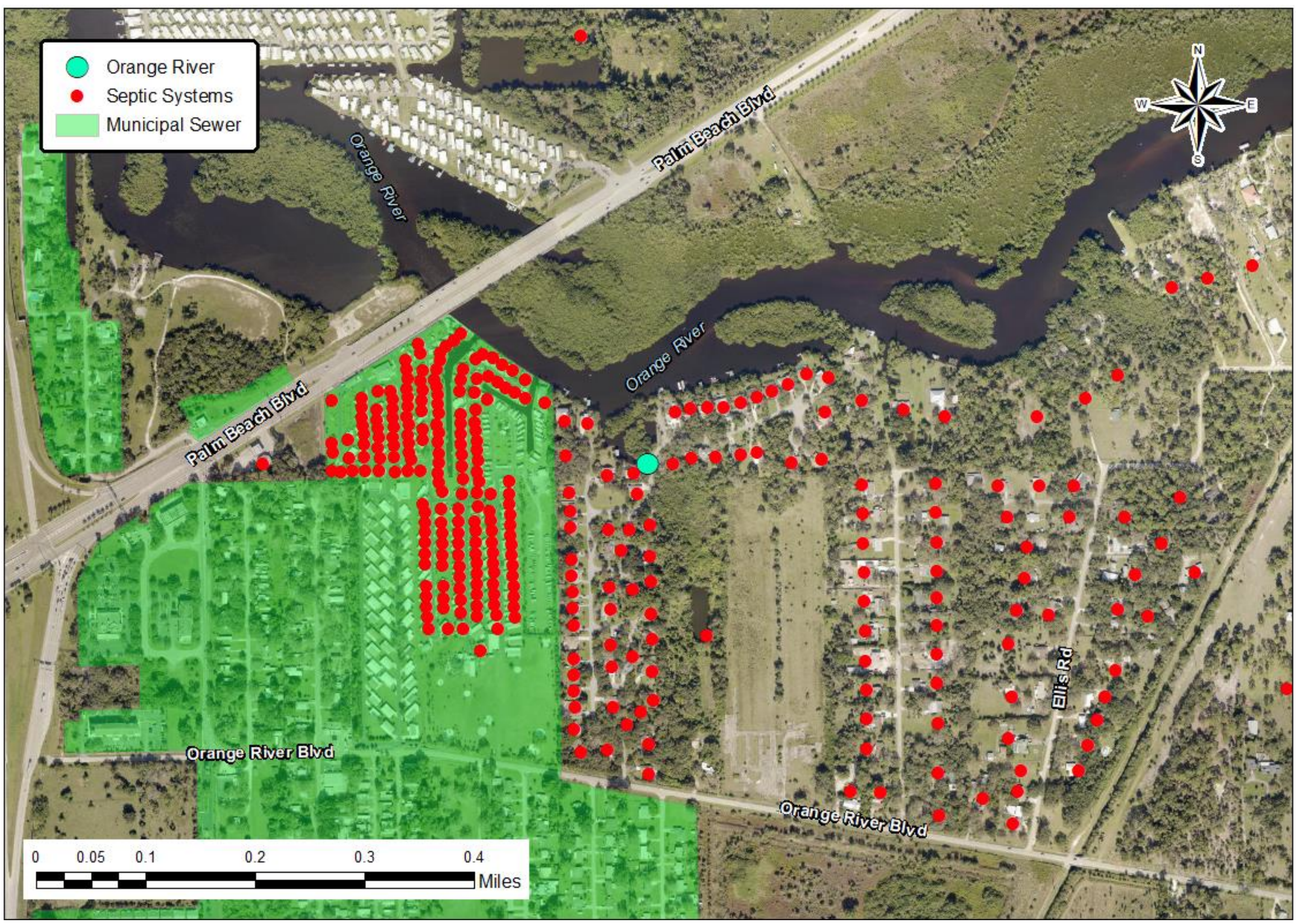

Figure S27. The Orange River site in a residential neighborhood adjacent to the Orange River, a tributary for the Caloosahatchee River Estuary.

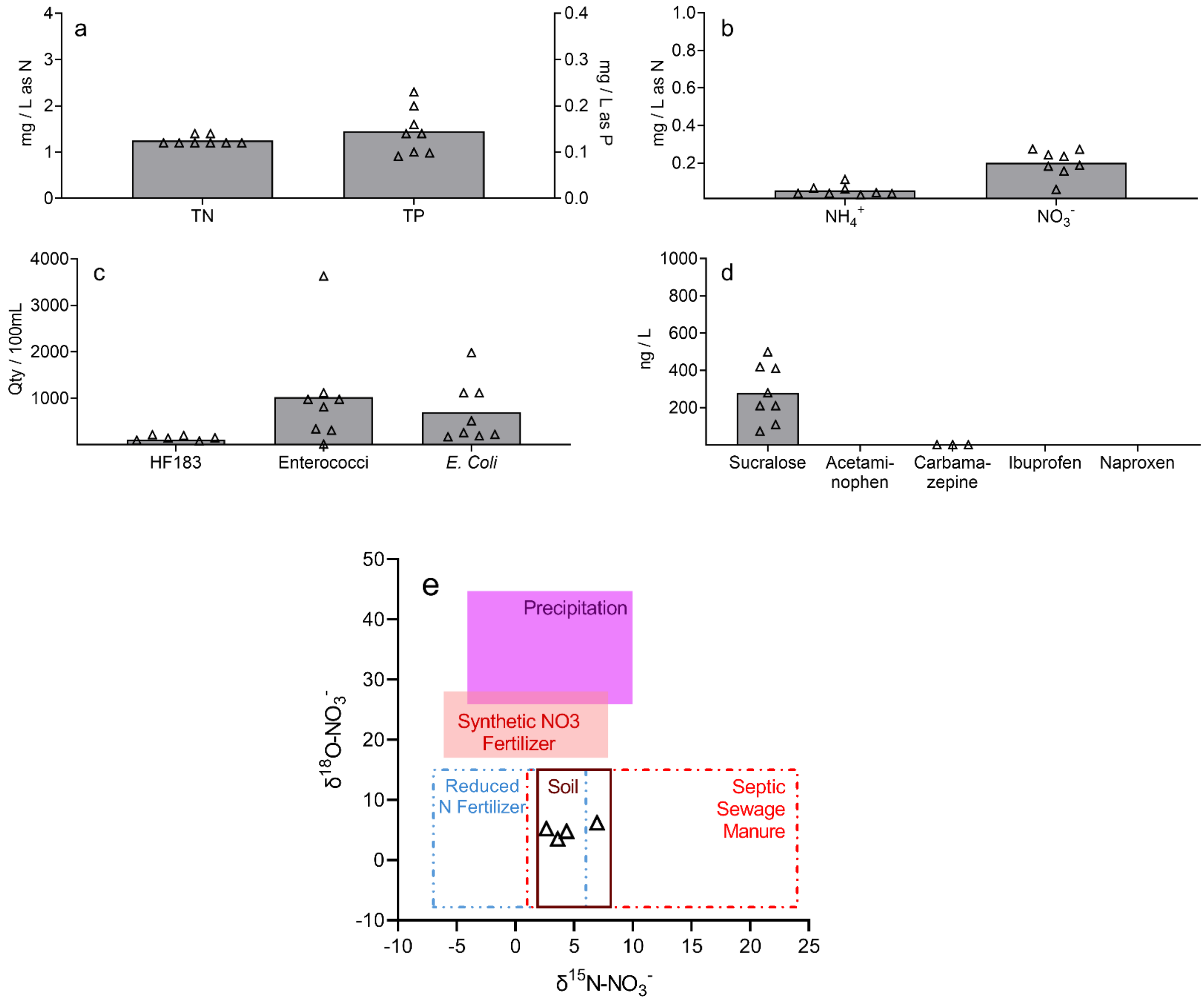

Figure S28. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Orange River site. Bars show mean values and triangles show individual detections.

Overlook Drive

The Overlook Drive site was located in a drainage ditch in a residential neighborhood serviced by both septic systems and municipal sewer (Figure S29). Reclaimed water is applied on a golf course directly upstream of the site. Total nitrogen and total phosphorus concentrations were high (Figure S30a), dissolved inorganic nitrogen was primarily in the form of ammonium (Figure S30b), and soluble reactive phosphorus comprised 28% of total phosphorus on average. Enterococci concentrations were high, frequently exceeding the upper limits of detection, whereas *E. coli* concentrations were more moderate. Additionally, HF183 was detected in six of eight samples (Figure S30c). Sucralose was consistently found in very high concentrations and carbamazepine was detected in all eight samples (Figure S30d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of fertilizer nitrogen (Figure S30e).

Many of the parameters assessed at Overlook Drive indicate significant human waste contamination. High ammonium concentrations suggest rapid transport of nutrients to surface water. Assuming proper functioning of municipal sewer infrastructure, the most likely sources of these contaminants are septic system effluent and/or reclaimed water application. Low $\delta^{15}N\text{-}NO_3^-$ values suggest that the primary nitrate source is from fertilizer, rather than human waste.

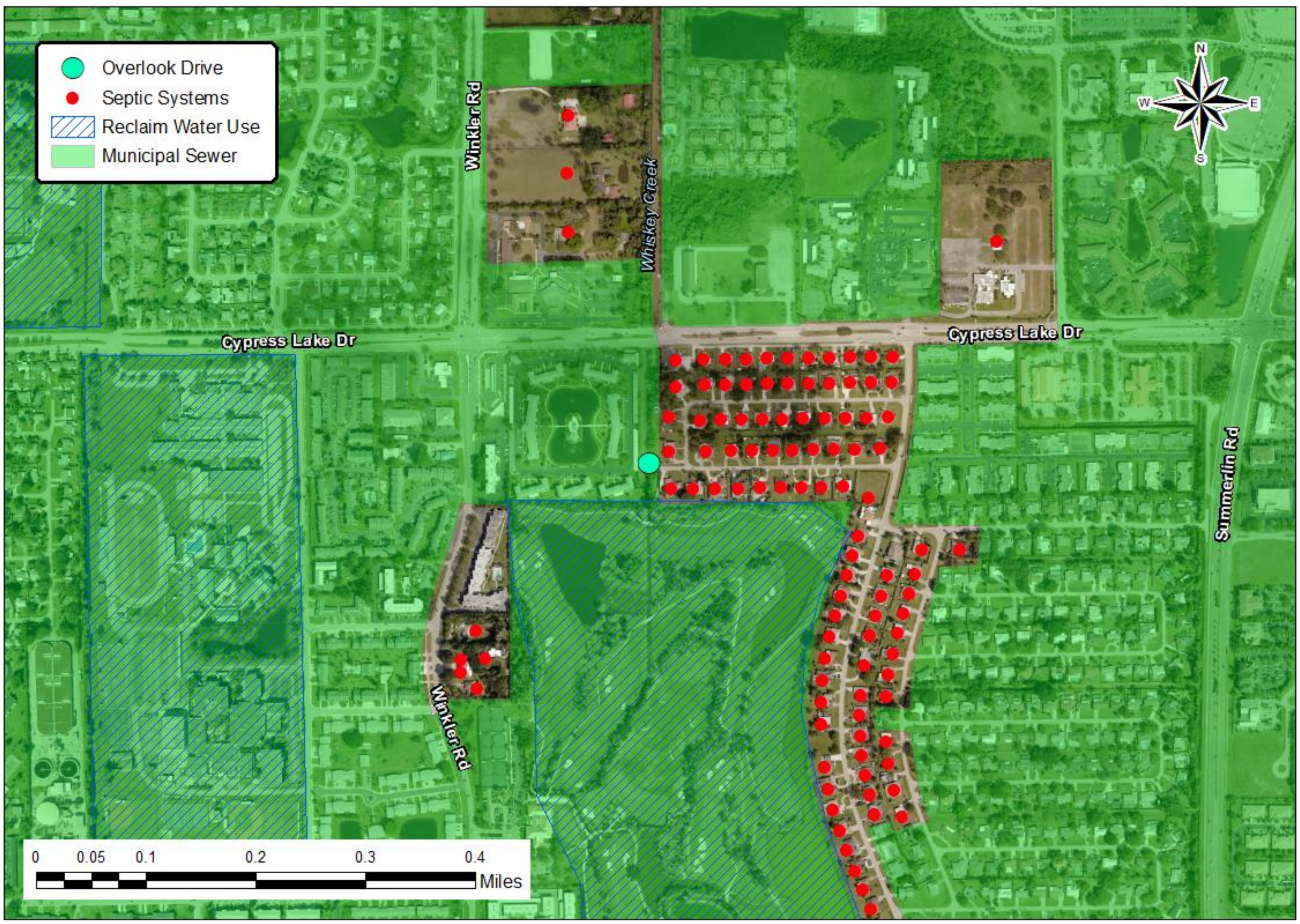

Figure S29. The Overlook Drive site located in a drainage ditch in a residential neighborhood serviced by both septic systems and municipal sewer.

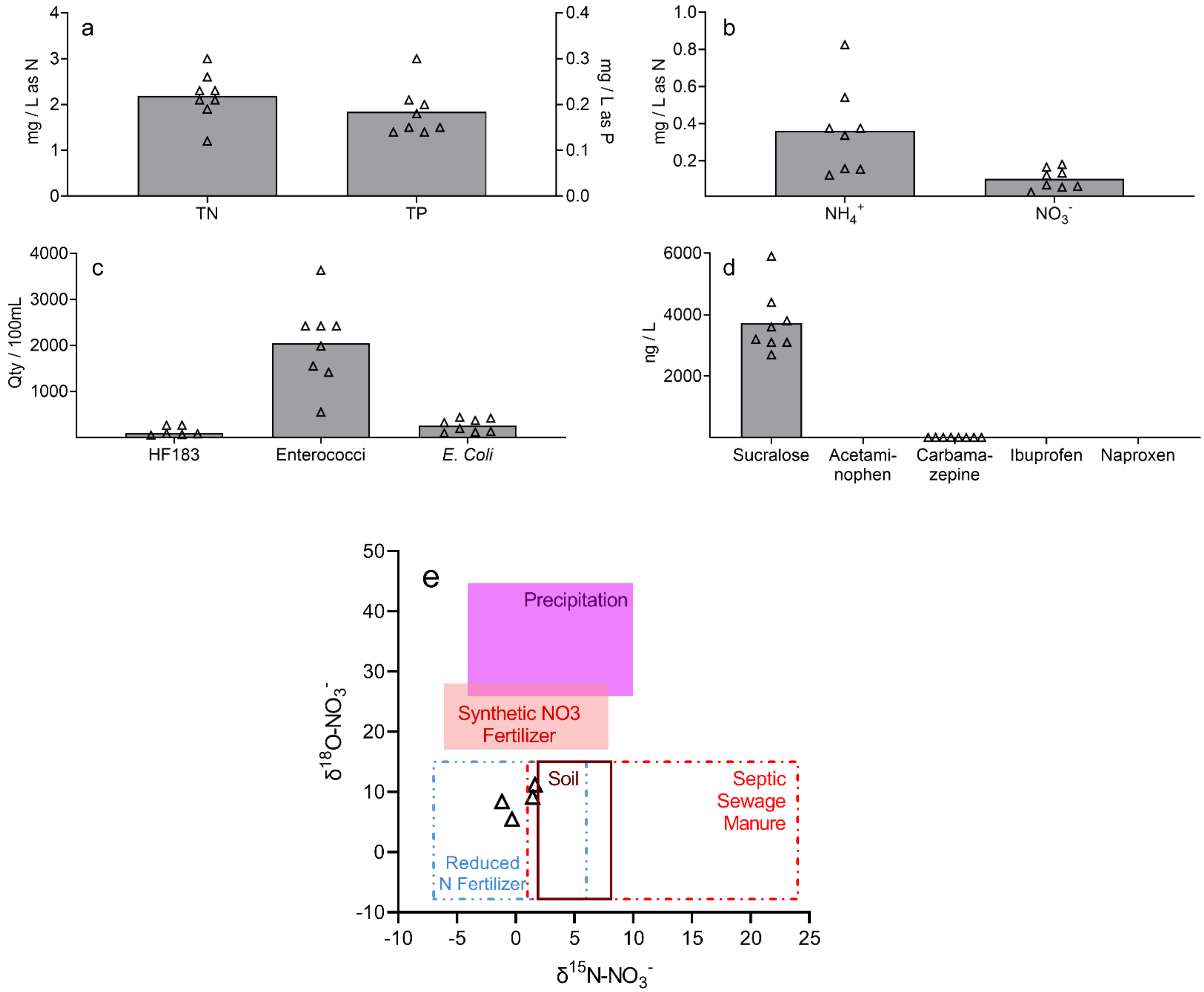

Figure S30. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Overlook Drive site. Bars show mean values and triangles show individual detections.

Page Park

The Page Park site was located in a drainage ditch in an urban area serviced by both septic systems and municipal sewer. The surrounding upstream area is mixed use, including residential homes, commercial businesses, a community golf course, and a private school (Figure S31). There are no reclaimed water influences at this site. Total nitrogen and total phosphorus were found in low concentrations (Figure S32a). Dissolved inorganic nitrogen was evenly comprised of ammonium and nitrate (Figure S32b) and soluble reactive phosphorus comprised 29% of total phosphorus on average. Bacteria concentrations were frequently high and HF183 was detected in three of eight samples (Figure S32c). Sucralose was consistently found in high concentrations and carbamazepine was detected in all eight samples (Figure S32d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of human waste nitrogen (Figure S32e).

The presence of human waste at Page Park is evidenced by the high concentrations of sucralose and fecal indicator bacteria, as well by the frequent detection of carbamazepine and HF183. However, this human waste influence does not appear to contribute high nutrient loads to surface water. These may be removed by microbial activity or plant uptake in soil before reaching the surface water. High $\delta^{15}N\text{-}NO_3^-$ values provide further evidence of human waste contamination. Assuming proper functioning of municipal sewer infrastructure, septic systems are the most likely local source of this human waste contamination.

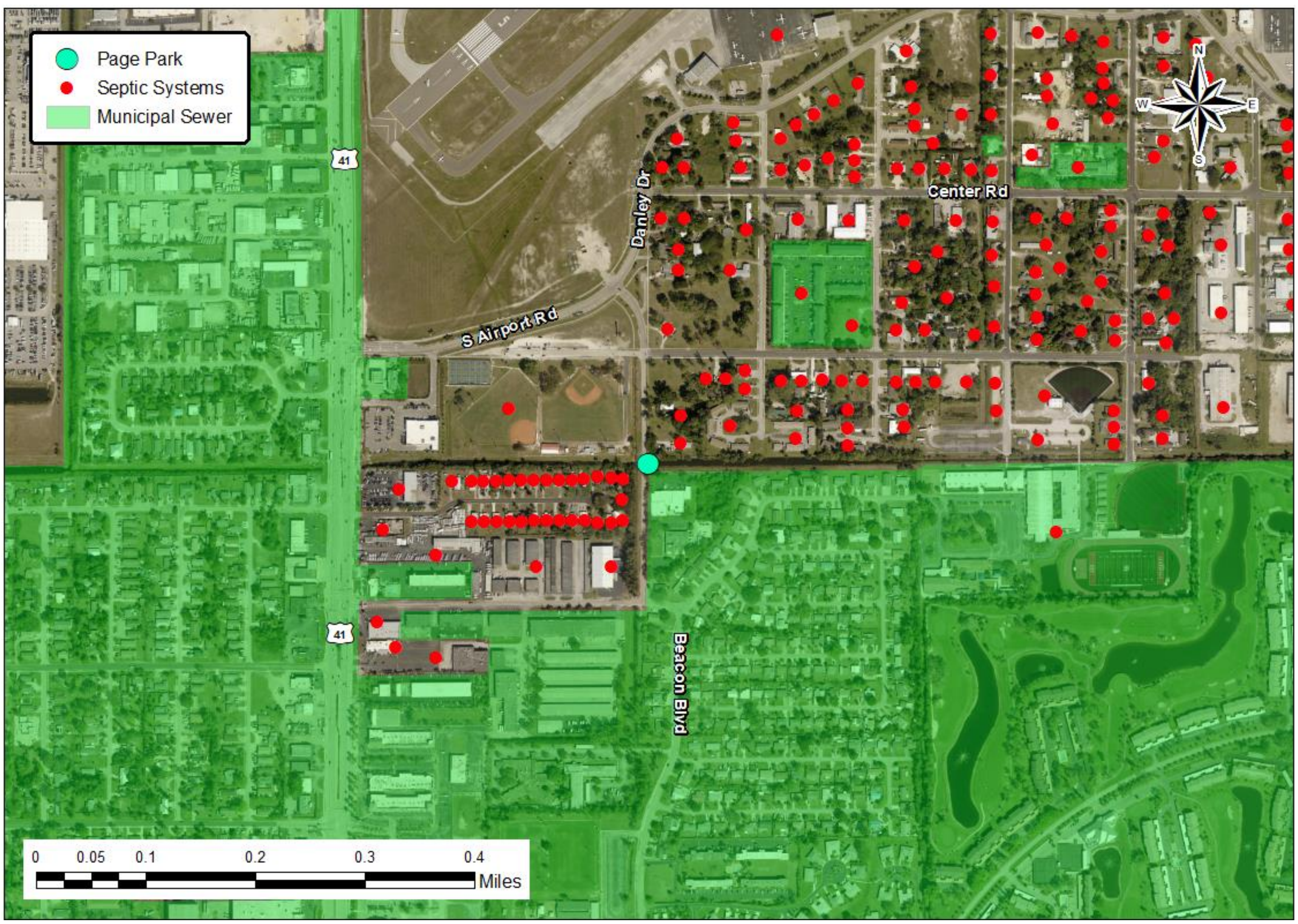

Figure S31. The Page Park site located in a drainage ditch in an urban area serviced by both septic systems and municipal sewer.

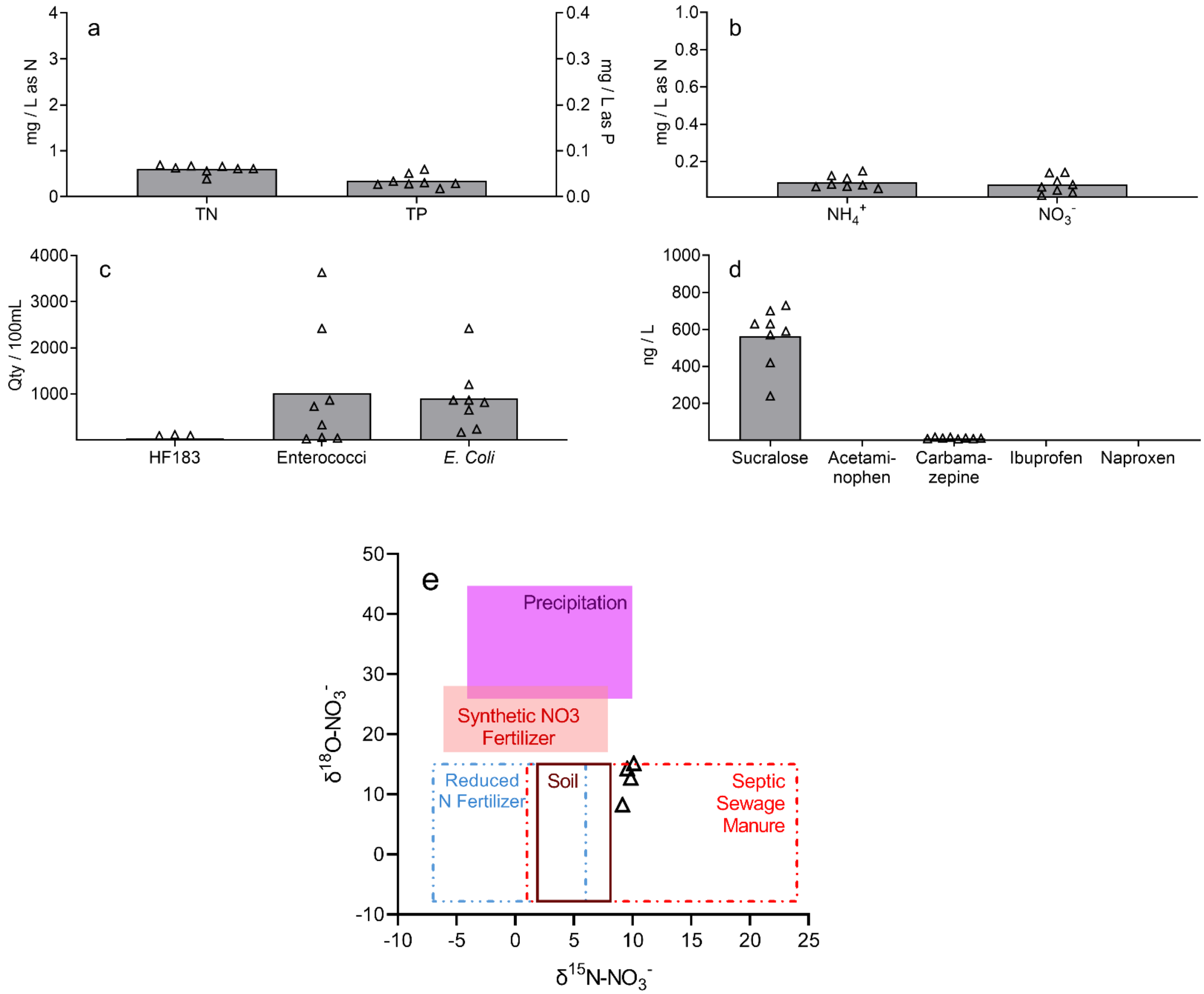

Figure S32. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Page Park site. Bars show mean values and triangles show individual detections.

Pine Island

The Pine Island and Pine Island 02 sites were located in tidally influenced residential finger canals with homes serviced by septic systems (Figure S33). There is no municipal sewer infrastructure or reclaimed water application nearby. Total nitrogen and total phosphorus concentrations were moderate (Figures S34a and S35a). Dissolved inorganic nitrogen was primarily in the form of ammonium (Figures S34b and S35b) and soluble reactive phosphorus comprised 40% of total phosphorus on average. Bacteria concentrations were predominantly low but very high concentrations of *E. coli* were found in two samples. HF183 was detected in six of eight total samples at Pine Island and once at Pine Island 02 (Figures S34c and S35c). Sucralose was consistently found in moderate concentrations, but no other chemical indicators were detected (Figures S34d and S35d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of fertilizer nitrogen (Figure S34e and S35e).

Human waste contamination at the two Pine Island sites is evidenced by the presence of HF183, as well as moderate concentrations of sucralose and occasionally high concentrations of *E. coli*. Although nutrients were not found in exceedingly high concentrations, the proportionately high amount of ammonium relative to nitrate suggests rapid transport of nutrients to surface water before ammonium oxidation and uptake in soil can occur. Septic systems are a likely source of human waste contaminants at these two sites; however, low $\delta^{15}N\text{-}NO_3^-$ values suggest fertilizer as the primary nitrate source, rather than human waste. Although the concentrations of many human waste indicators were lower at the Pine Island sites compared to other sites in this study, this may be due in part to shorter residence times and a greater dilution effect rather than decreased human waste loading, since these two sites have the greatest marine and tidal influence.

Figure S33. The Pine Island and Pine Island 02 sites in tidally influenced residential finger canals within the Caloosahatchee River Estuary.

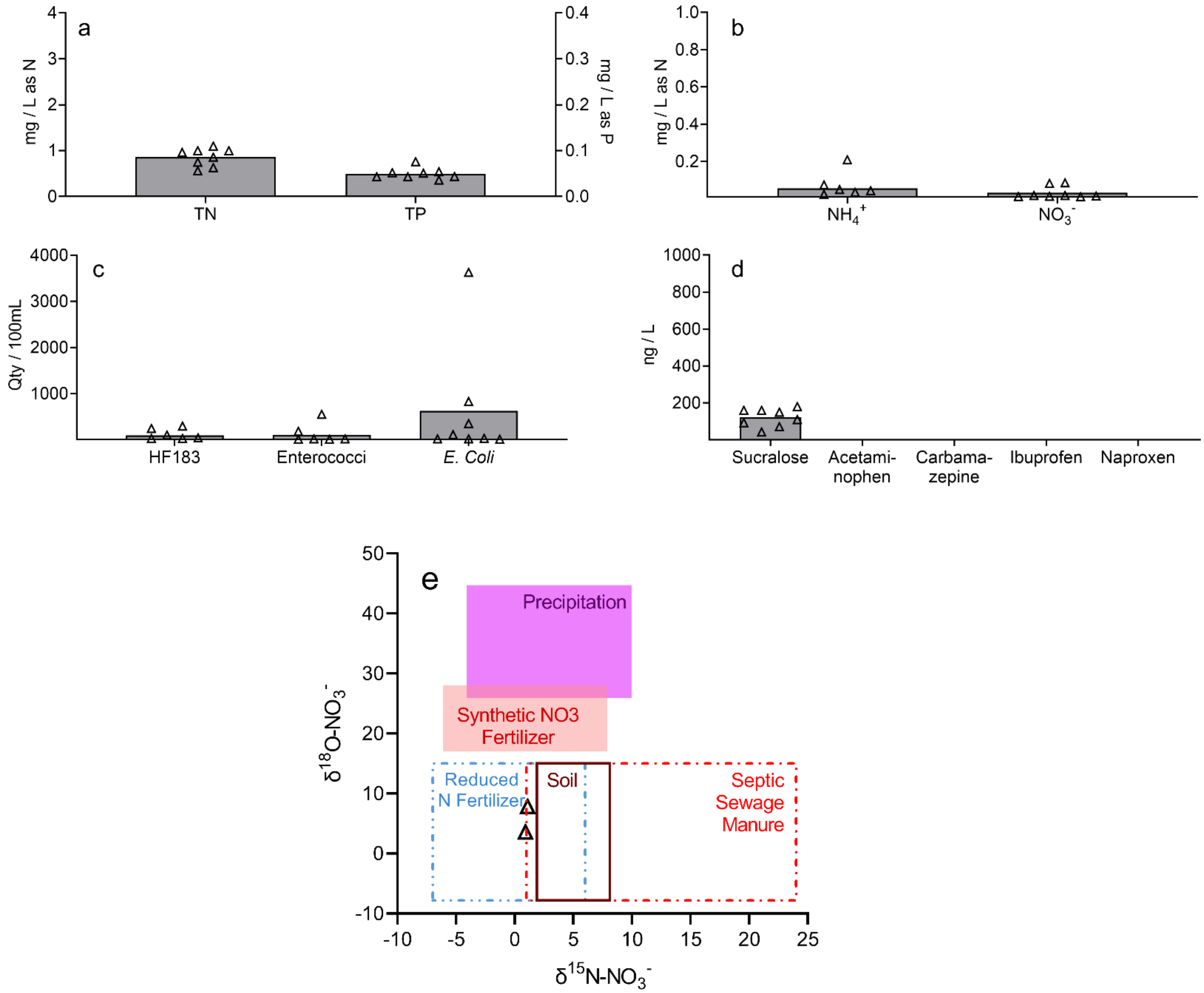

Figure S34. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Pine Island site. Bars show mean values and triangles show individual detections.

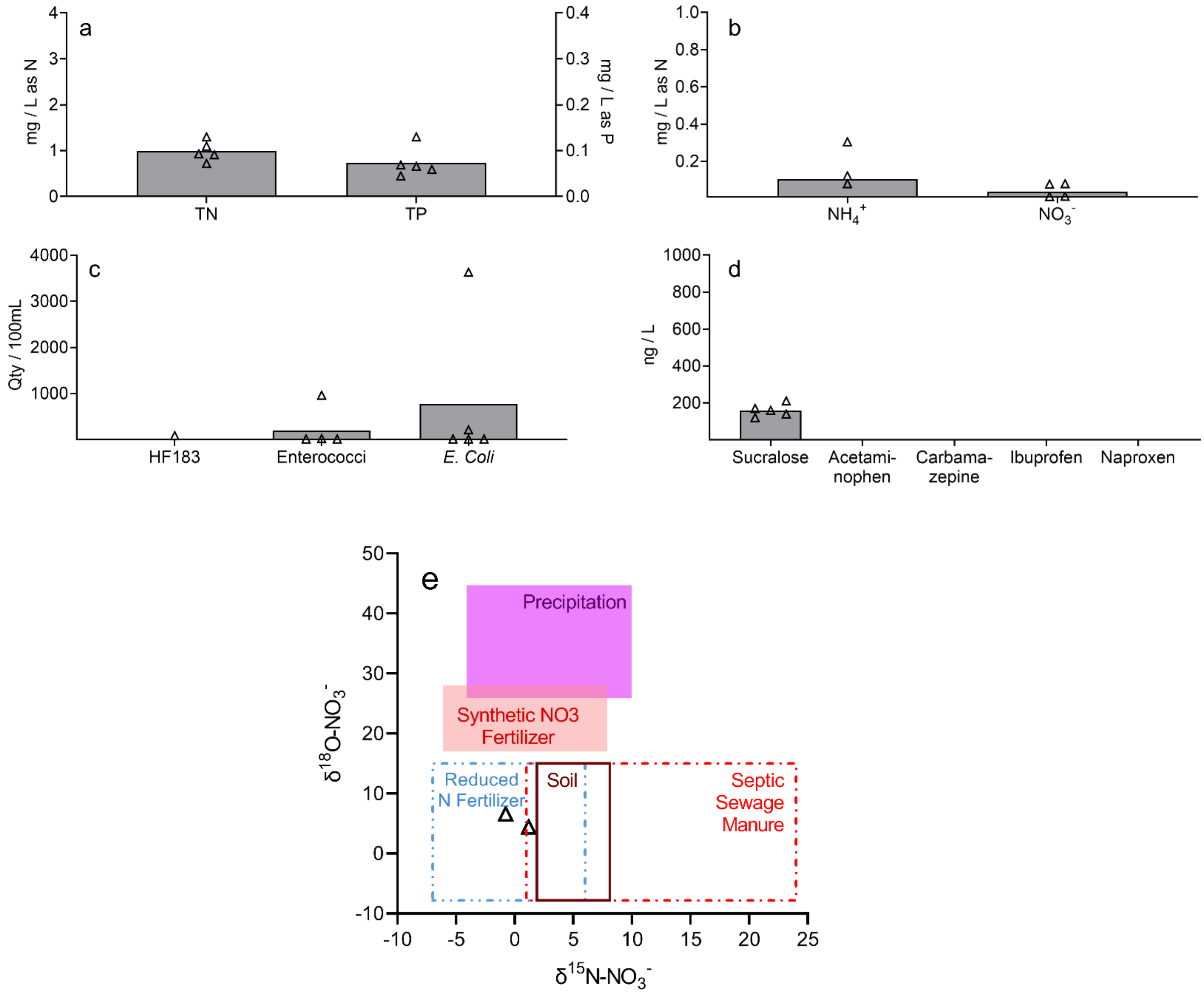

Figure S35. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate isotopes (e) measured in surface water samples collected from the Pine Island 02 site. Bars show mean values and triangles show individual detections.

San Carlos Park

The San Carlos Park site was located in a drainage ditch in a residential neighborhood (Figure S36). There is some municipal sewer infrastructure upstream of the site but the area immediately surrounding the site is almost entirely serviced by septic systems. Total nitrogen and total phosphorus were found in low to moderate concentrations (Figure S37a). Dissolved inorganic nitrogen was primarily in the form of ammonium (Figure S37b) and soluble reactive phosphorus comprised 32% of total phosphorus on average. *E. coli* concentrations were moderate, while enterococci concentrations were high, and HF183 was detected in all samples at high concentrations (Figure S37c). Sucralose was consistently found in moderate to high concentrations and carbamazepine was detected in six of eight samples (Figure S37d). δ^{15}N-NO_3^- values were in the range of fertilizer nitrogen (Figure S34e).

Human waste influence at San Carlos Park is evidenced by the presence of chemical indicators and fecal indicator bacteria, as well as by very high concentrations of HF183. Further, proportionately high concentrations of ammonium compared to nitrate suggests rapid transport of nutrients to surface water before complete oxidation of ammonium in soil can occur. Although high concentrations of total nitrogen and total phosphorus were not observed, this may possibly be explained by rapid uptake by aquatic vegetation, as water lettuce and other aquatic macrophytes were observed at this site. It is likely that high-density septic systems directly adjacent and upstream of the study site are contributing to the observed human waste contamination. However, low δ^{15}N-NO_3^- values suggest fertilizer as the primary nitrate source, rather than human waste.

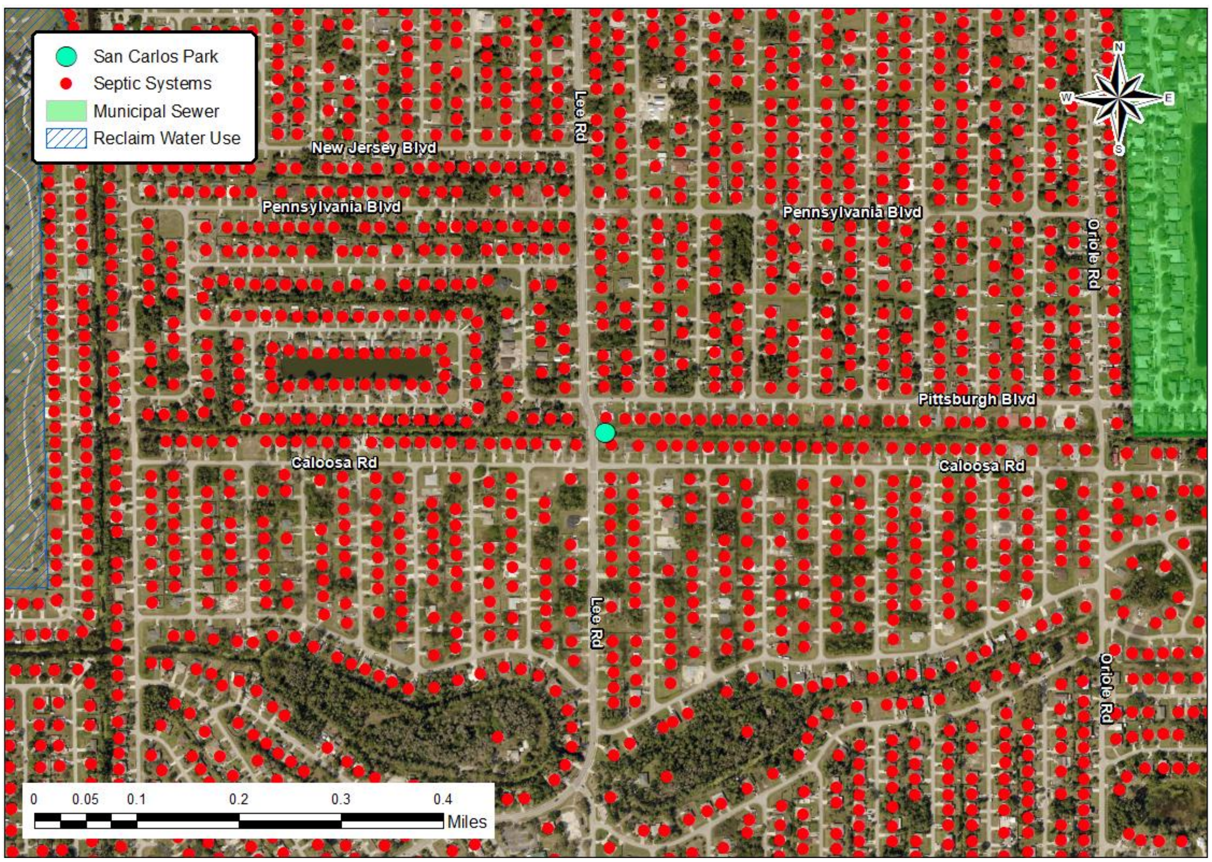

Figure S36. The San Carlos Park site located in a fast-flowing residential drainage ditch serviced by septic systems.

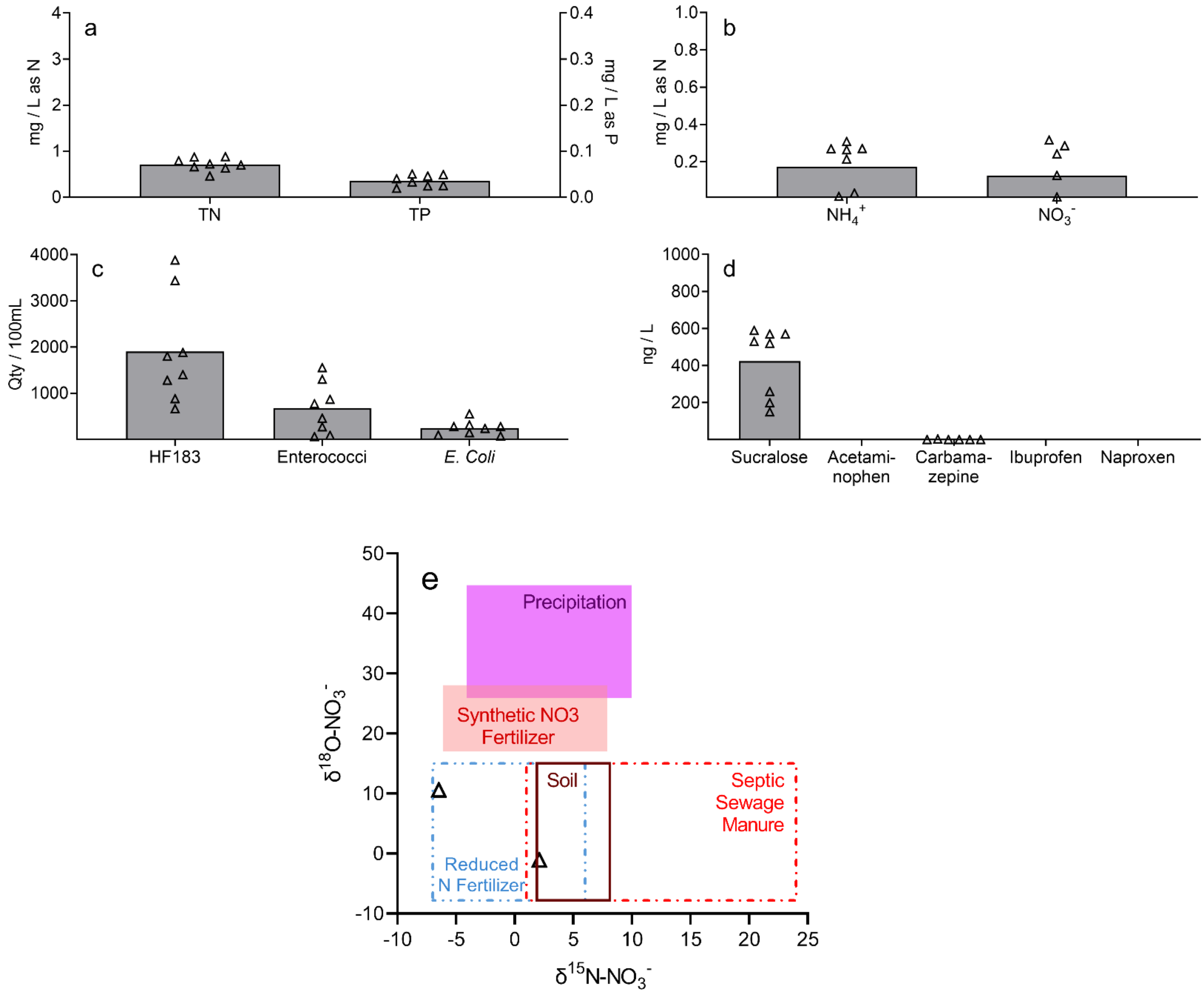

Figure S37. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the San Carlos Park site. Bars show mean values and triangles show individual detections.

Summerwood Drive

The Summerwood Drive site was located in a drainage ditch in a residential neighborhood that is serviced by both municipal sewer and septic systems (Figure S38). Total nitrogen and total phosphorus were found in high concentrations (Figure S39a). Dissolved inorganic nitrogen was primarily composed of ammonium (Figure S39b) and soluble reactive phosphorus comprised 60% of total phosphorus on average. *E. coli* concentrations were moderate, while enterococci concentrations were high. HF183 was detected in four of eight samples, including one extremely high detection in January 2021 (Figure S39c). Sucralose was consistently found in high concentrations and ibuprofen was detected in two samples (Figure S39d). $\delta^{15}N\text{-}NO_3^-$ values were not assessed due to very low nitrate concentrations.

Human waste contamination at Summerwood Drive is evidenced by high concentrations of HF183, fecal indicator bacteria, ammonium, sucralose, and ibuprofen. These human waste sources appear to contribute high nutrient loads to the surface water. Very high ammonium concentrations found in surface water samples collected from this site suggests rapid transport of human waste nutrients to surface water. Assuming proper functioning of municipal sewer infrastructure, the most likely source of these contaminants is septic system effluent.

Figure S38. The Summerwood Drive site located in a drainage ditch in a residential neighborhood serviced by municipal sewer and septic systems.

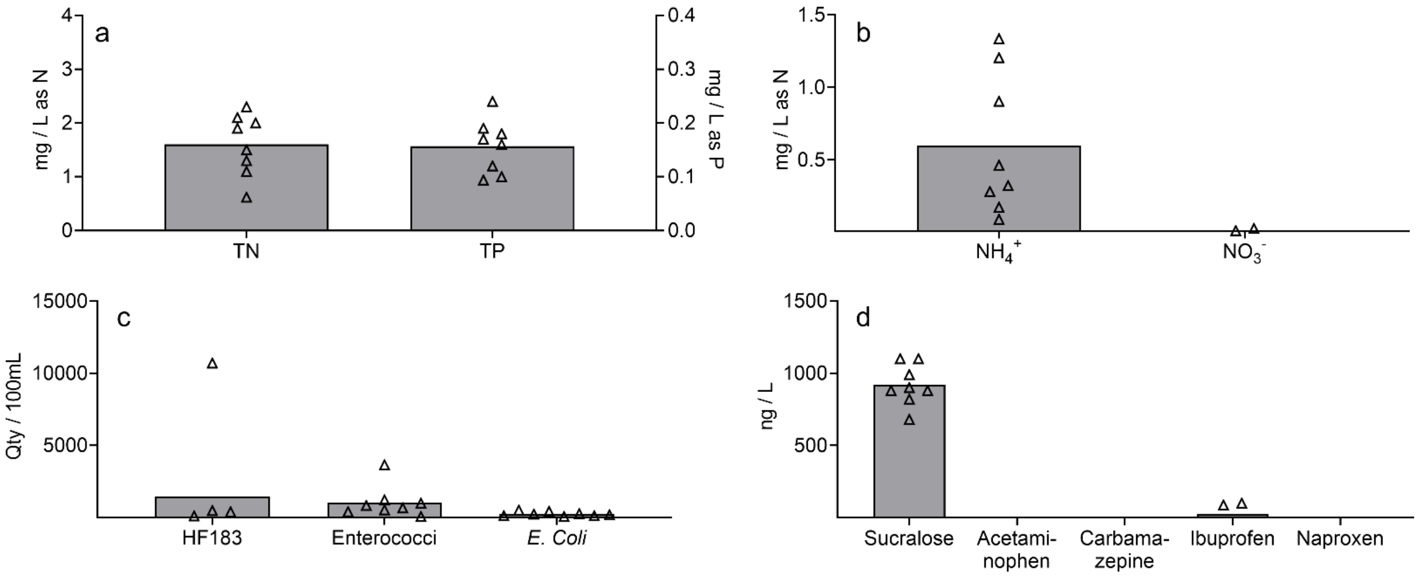

Figure S39. Total nutrients (a), nitrogen speciation (b), bacteria (c), and chemical tracers (d) measured in surface water samples collected from the Summerwood Drive site. Bars show mean values and triangles show individual detections.

Tidewater Island

The Tidewater Island sites were located in tidally influenced residential finger canals serviced by septic systems. These sites are found within the Mullock Creek watershed which drains into Estero Bay (Figure S40). There are no direct municipal wastewater or reclaimed water influences at this site. Total nitrogen and total phosphorus were found in low to moderate concentrations (Figures S41a and S42a). Dissolved inorganic nitrogen was evenly comprised of ammonium and nitrate (Figures S41b and S42b) and soluble reactive phosphorus comprised 30% of total phosphorus on average. Bacteria concentrations were moderate at Tidewater Island 01, whereas *E. coli* concentrations at Tidewater Island 02 were high and enterococci were in the moderate range. HF183 was detected in three of eight samples at Tidewater Island 01 and in two of eight samples at Tidewater Island 02 (Figures S41c and S42c). One sample taken from Tidewater Island 02 in September 2020 contained very high concentrations of HF183. Sucralose was consistently found in high concentrations at both sites and carbamazepine was also regularly detected at both sites (Figures S41d and S42d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of soil, human waste, and fertilizer nitrogen (Figure S41e and S42e).

The presence of human waste at Tidewater Island is evidenced by the presence of fecal indicator bacteria, HF183, and sucralose in high concentrations, as well as by the regular detection of carbamazepine. Nutrient loading does not appear to be high at Tidewater Island. Septic systems adjacent to finger canals are the likely a source of human waste at Tidewater Island, although additional upstream sources may also exist. Moderate $\delta^{15}N\text{-}NO_3^-$ values suggest soil, sediment, or fertilizers as additional nitrate sources. These sites are tidally influenced, so short residence times and dilution may play a role in removing human waste contaminants.

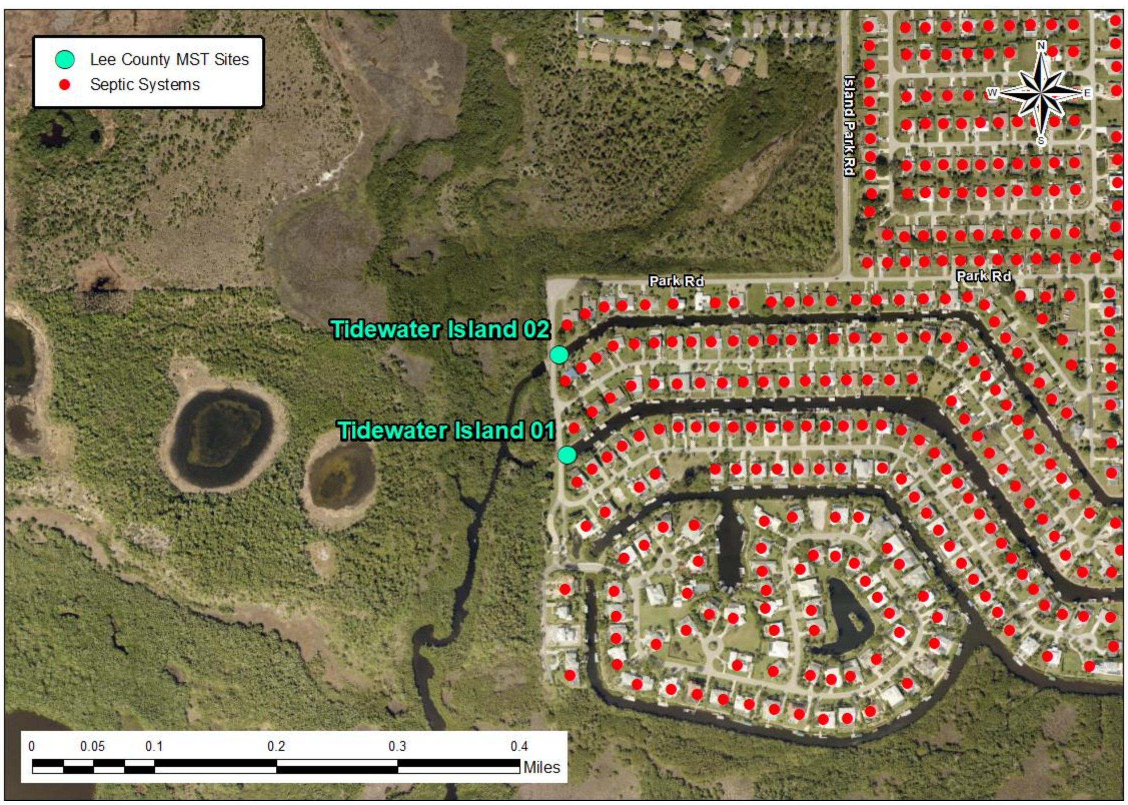

Figure S40. The Tidewater Island sites located in tidally influenced residential finger canals serviced by septic systems.

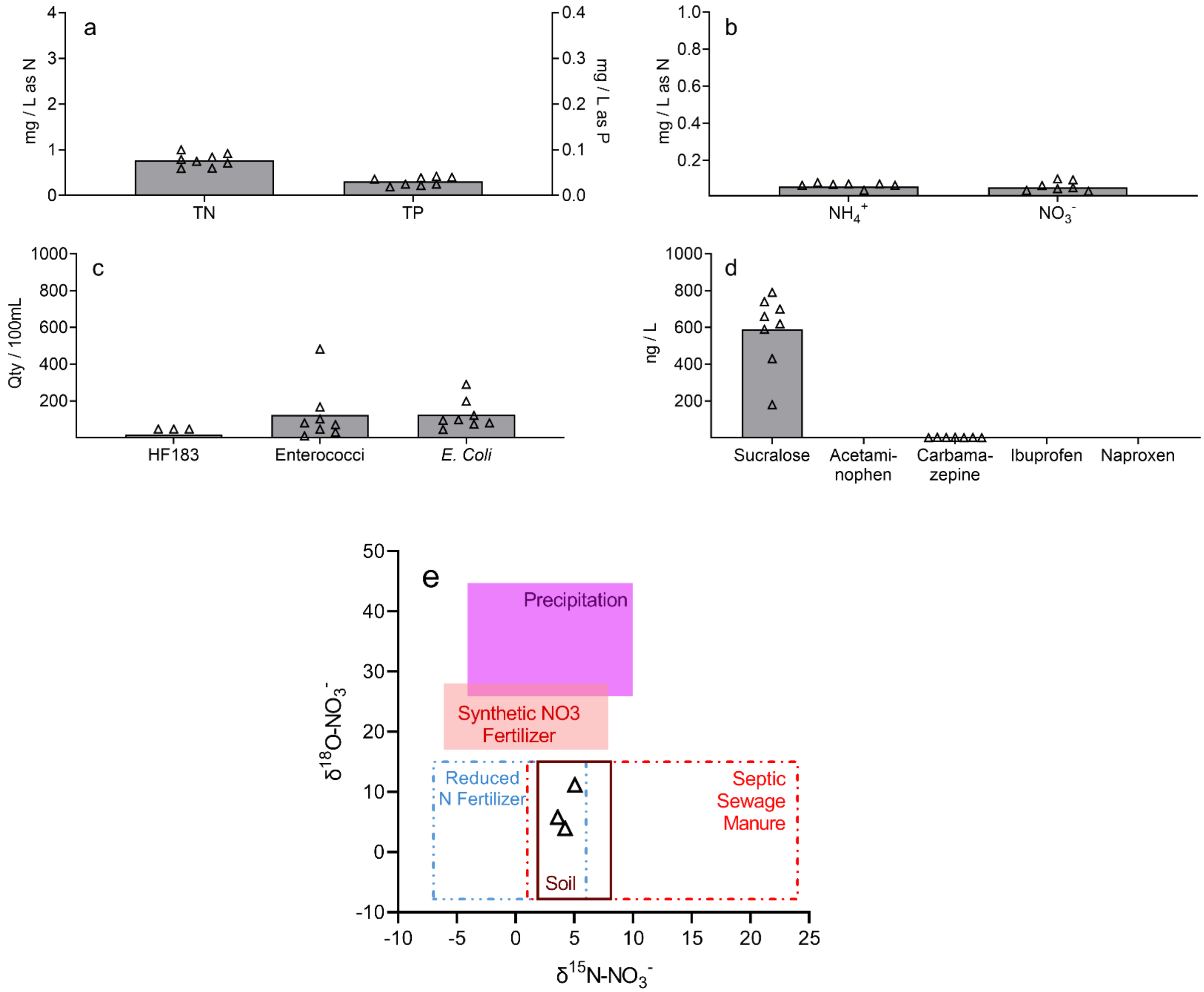

Figure S41. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Tidewater Island 01 site. Bars show mean values and triangles show individual detections.

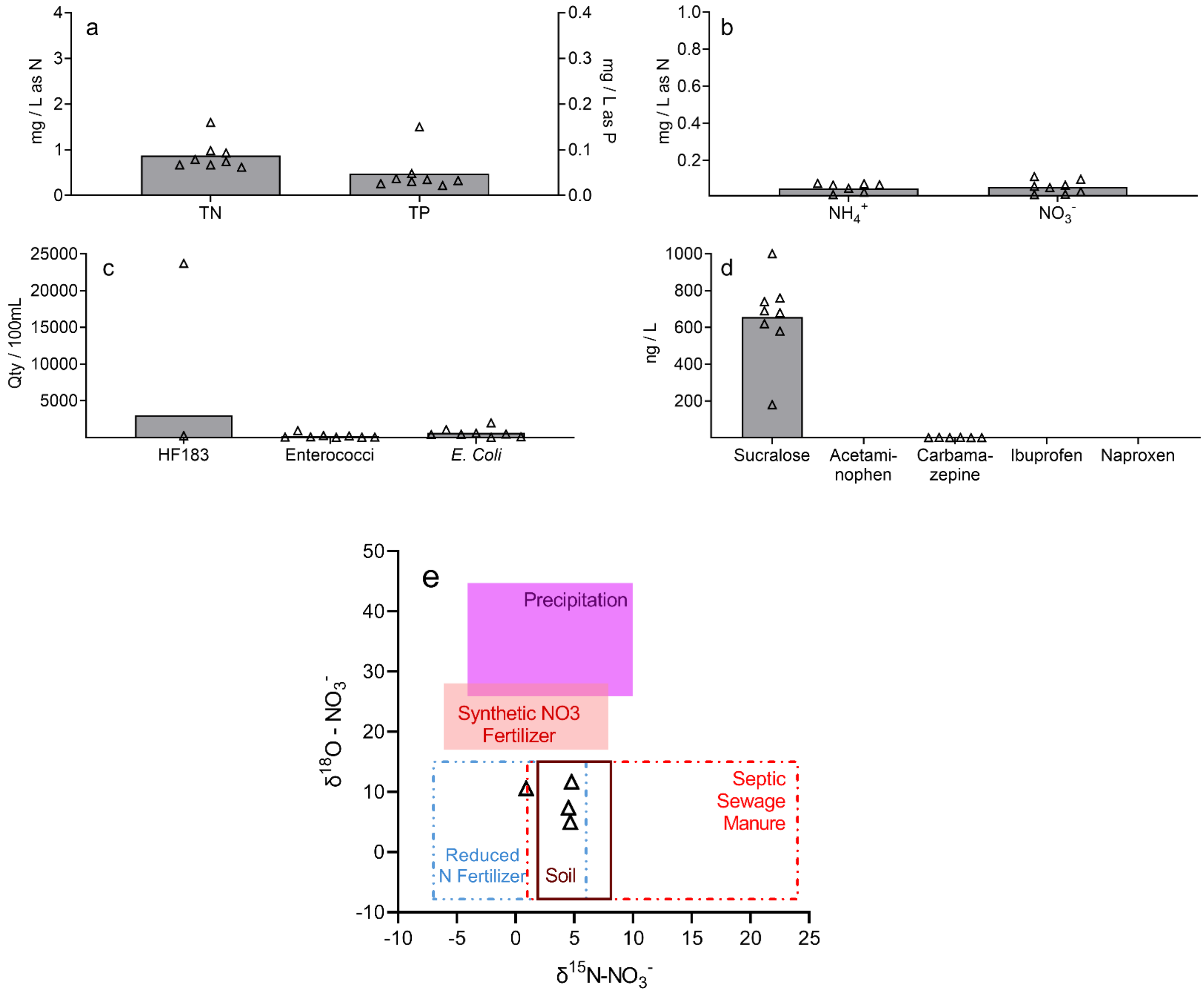

Figure S42. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Tidewater Island 02 site. Bars show mean values and triangles show individual detections.

Waterway Estates 01

The Waterway Estates 01 site was located in a tidally influenced finger canal in North Fort Myers serviced by septic systems (Figure S43). Total nitrogen was found in moderate concentrations, whereas concentrations of total phosphorus ranged from moderate to high (Figure S44a). Dissolved inorganic nitrogen was evenly comprised of ammonium and nitrate (Figure S44b) and soluble reactive phosphorus comprised 70% of total phosphorus on average. Bacteria concentrations ranged from low to high and HF183 was detected in four out of eight total samples (Figure S44c). Sucralose was detected in all samples at moderate to high concentrations and carbamazepine was detected in five samples (Figure S44d). $\delta^{15}N\text{-}NO_3^-$ values were in the range of soil, human waste, and fertilizer nitrogen (Figure S44e).

The presence of human waste contamination at Waterway Estates 01 is evidenced by high concentrations of HF183, fecal indicator bacteria, and sucralose, as well as by the detection of carbamazepine in a majority of samples. Nutrient pollution is evidenced by moderate and high concentrations of nitrogen and phosphorus. Nearby septic systems are likely a contributing source for these contaminants, although upstream sources may also exist which contribute to background contaminant levels in the Caloosahatchee River Estuary. Elevated ammonium and phosphate may indicate rapid transport of contaminants to surface water before ammonium oxidation and phosphorus adsorption in soils can be completed. Moderate $\delta^{15}N\text{-}NO_3^-$ values suggest soil or sediment may also contribute as a nitrate source.

Figure S43. The Waterway Estates 01 site located in a tidally influenced residential finger canal serviced by septic systems.

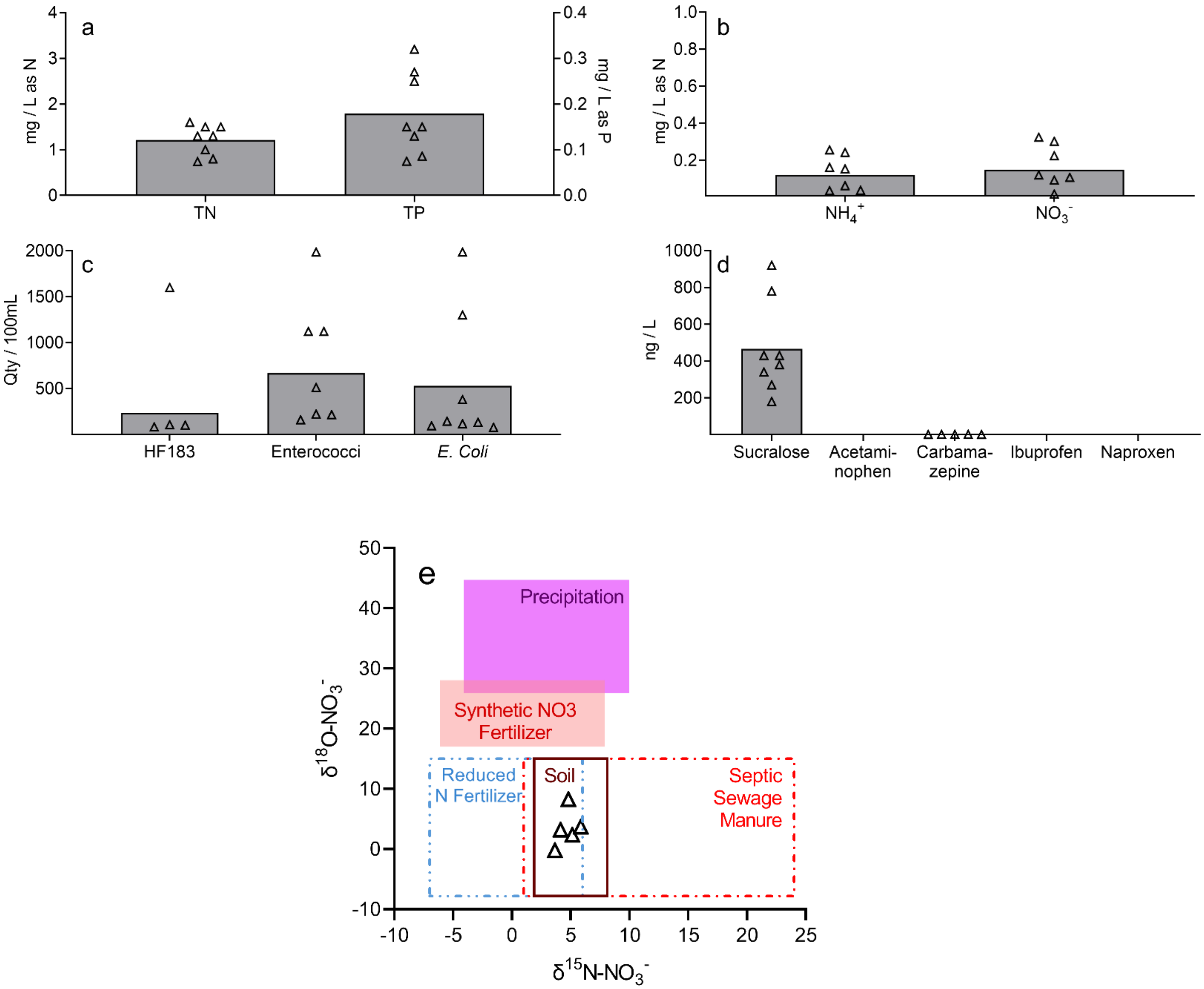

Figure S44. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Waterway Estates 01 site. Bars show mean values and triangles show individual detections.

Waterway Estates 02

The Waterway Estates 02 site is located in a tidally influenced residential finger canal serviced by septic systems (Figure S45). The surrounding area is serviced by municipal sewer. Total nitrogen was mostly found in moderate concentrations, whereas total phosphorus concentrations ranged from moderate to high (Figure S46a). Dissolved inorganic nitrogen was primarily in the form of nitrate (Figure S46b) and soluble reactive phosphorus comprised 64% of total phosphorus on average. Bacteria concentrations were predominantly high for enterococci and ranged from moderate to high for *E. coli*. HF183 was detected in four out of eight total samples at low concentrations (Figure S46c). Sucralose was consistently found in moderate to high concentrations, carbamazepine was detected in one sample, and ibuprofen was detected in one sample (Figure S46d). $\delta^{15}N\text{-}NO_3^-$ values were primarily in the range of soil and human waste (Figure S46e).

Moderate to high nutrient concentrations with elevated soluble reactive phosphorous at Waterway Estates 02 may originate from nearby human waste sources, but moderate $\delta^{15}N\text{-}NO_3^-$ values suggest that soil or sediment may also contribute as a nutrient source. High concentrations of fecal indicator bacteria and sucralose in addition to detections of HF183, carbamazepine, and ibuprofen provide some evidence of human waste contamination. Septic systems are likely a source of human waste at Waterway Estates 02, but background levels in the Caloosahatchee River Estuary may also contribute. Dilution may also play an important role, as this site is tidally influenced.

Figure S45. The Waterway Estates 02 site located in a tidally influenced residential finger canal serviced by septic systems.

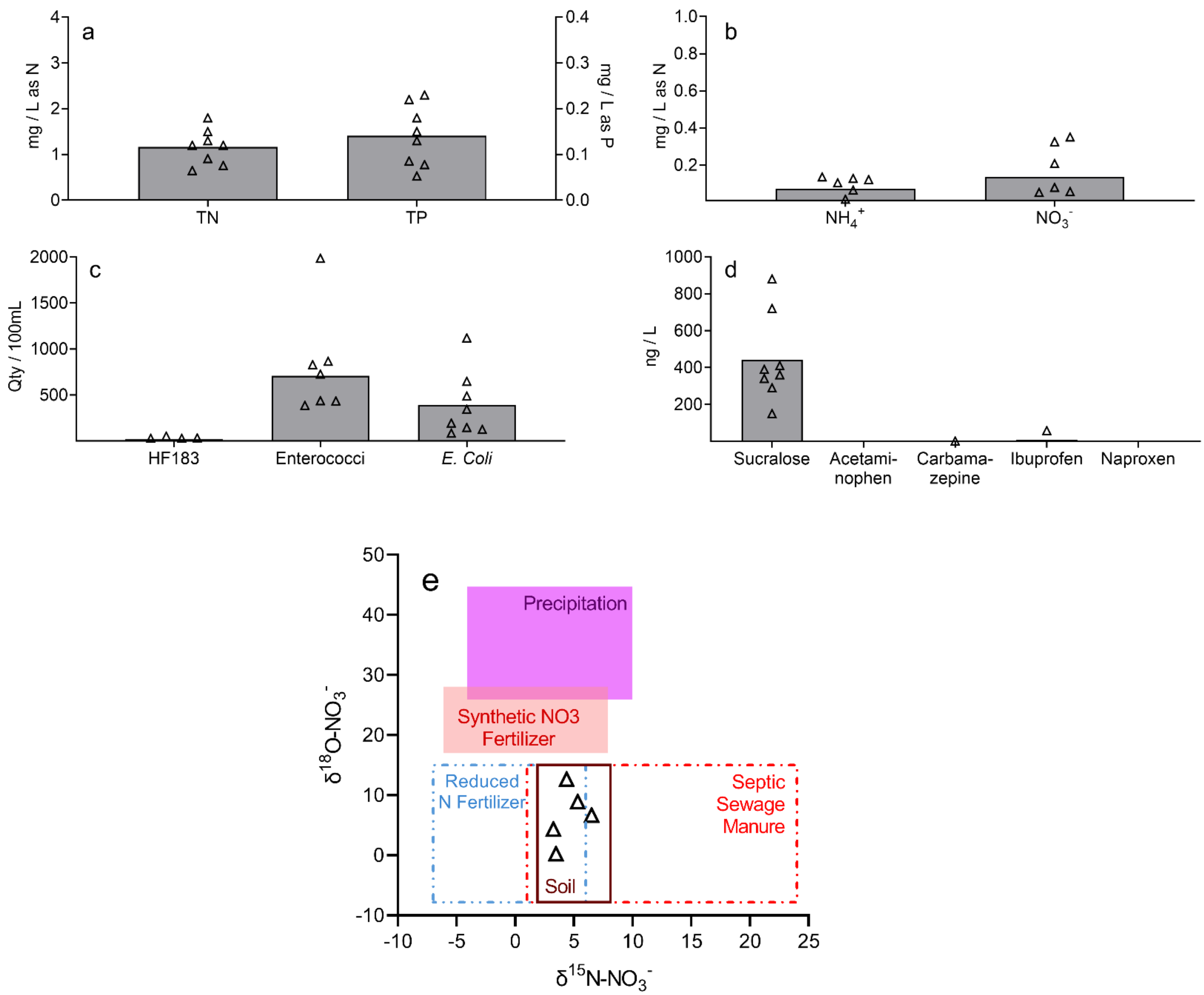

Figure S46. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Waterway Estates 02 site. Bars show mean values and triangles show individual detections.

Yacht Club

The Yacht Club site is located in a residential finger canal serviced by septic systems within the Caloosahatchee River Estuary (Figure S47). There are no direct reclaimed water or municipal sewer influences at this site. Total nitrogen and total phosphorus were found in moderate concentrations (Figure S48a). Dissolved inorganic nitrogen was primarily in the form of ammonium (Figure S48b) and soluble reactive phosphorus comprised 63% of total phosphorus on average. Bacteria concentrations were low and HF183 was detected in two of eight samples at low concentrations (Figure S48c). Sucralose was consistently found in moderate concentrations and carbamazepine was detected in one sample (Figure S48d). $\delta^{15}N$-NO_3^- values were primarily in the range of soil nitrogen (Figure S48e).

Nutrient concentrations at this site are very similar to those found at the Reference site, and therefore may reflect background concentrations within the Caloosahatchee River Estuary rather than loading from human waste sources. In fact, all parameters exhibited similarity to the Reference site, except for sucralose, which was found in lower concentrations at Yacht Club than at the Reference site. Further, moderate $\delta^{15}N$-NO_3^- values suggest soil or sediment may contribute as a significant nitrate source. $\delta^{15}N$-NO_3^- values also fall in the human waste range which may indicate periodic nitrogen loading from septic systems.

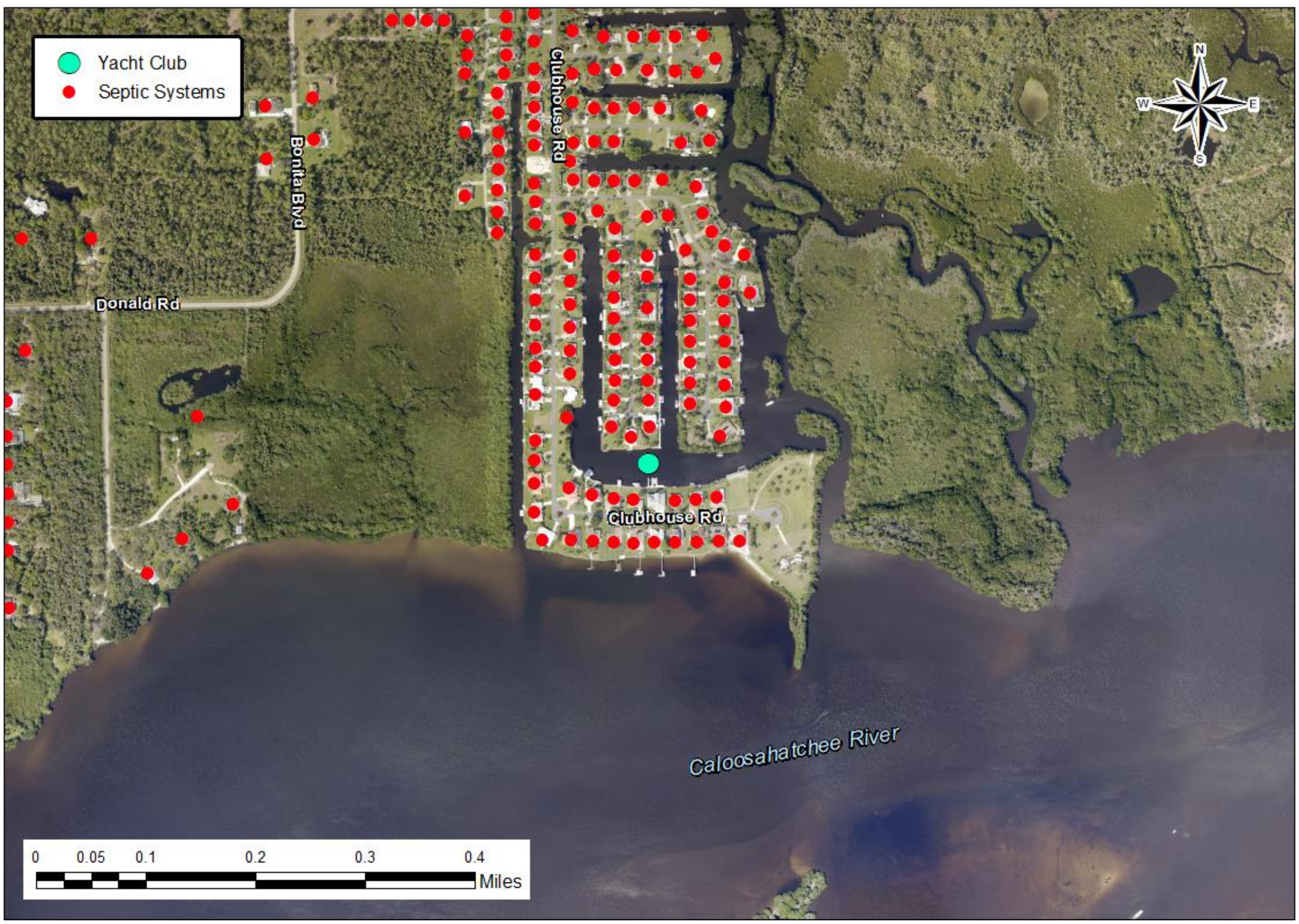

Figure S47. The Yacht Club site located in a finger canal serviced by septic systems within the Caloosahatchee River Estuary.

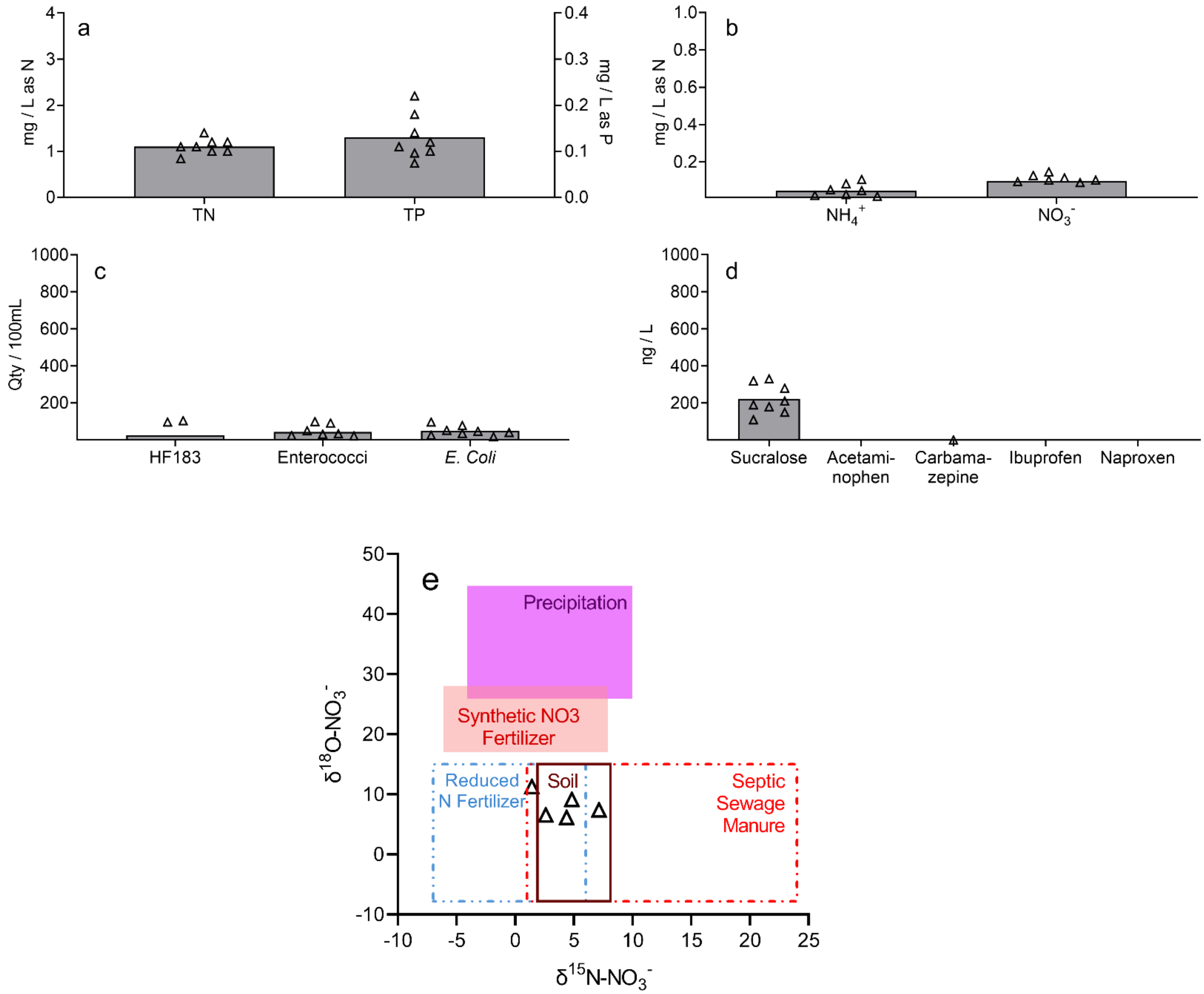

Figure S48. Total nutrients (a), nitrogen speciation (b), bacteria (c), chemical tracers (d), and nitrate stable isotopes (e) measured in surface water samples collected from the Yacht Club site. Bars show mean values and triangles show individual detections.

HARBOR BRANCH

FLORIDA ATLANTIC UNIVERSITY