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Unexpected nitrogen sources in a tropical urban estuary

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Abstract

Tropical urban estuaries are severely understudied. Little is known about the basic biogeochemical cycles and dominant ecosystem processes in these waterbodies, which are often low-lying and heavily modified. The San Juan Bay Estuary (SJBE) in San Juan, Puerto Rico is an example of such a system. Over the past 80 years, a portion of the estuary has filled in, changing the hydrodynamics and negatively affecting water quality. Here we sought to document these changes using ecological and biogeochemical measurements of surface sediments and bivalves. Measurements of sediment physical characteristics, organic matter content, and stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) illustrated the effects of the closure of the Caño Martín Peña (CMP) on the hydrology and water quality of the enclosed and semienclosed parts of the estuary. The nitrogen stable isotope ($\delta^{15}N$) values were lowest in the CMP, the stretch of the SJBE that is characterized by waters with low dissolved oxygen and high fecal coliform concentrations. Despite this, the results of this study indicate that nitrogen (N) contributions from N-fixing, sulfate-reducing microbes may meet or even exceed contributions from urban runoff and sewage. While the importance of sulfate reducers in contributing N to mangrove ecosystems is well documented, this is the first indication that such processes could be dominant in an intensely urban system. It also underscores just how little we know about tropical coastal ecosystems in densely populated areas throughout the globe.

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nitrogen; urban; tropical; estuary; stable isotope; Puerto Rico

1. Introduction

> "Tropical research…must be rooted in a more inclusive set of social values. Conservation must become part of the larger agenda of sustainable and equitable development, with the development needs of the local communities receiving the same consideration as preservation goals."
>
> -Bawa et al. (2004)

Coastal areas that are less than 10 meters above sea level encompass 2% of the world's land area but are home to 13% of the world's urban population (McGranahan et al., 2007). Small island countries and territories, particularly those in tropical areas, have a disproportionate share of their citizens living in both urban and low-lying areas. For example, 88% of the population of the Bahamas lives in low-elevation coastal zones. More than 90% of the populations of the Maldives, Marshall Islands, Tuvalu, Cayman Islands, and Turks and Caicos Islands are in these low-elevation areas (McGranahan et al., 2007). The least developed countries have twice as many people living in low-elevation coastal areas when compared to the most developed. Even within cities, the residents living in lower elevation areas are more socially vulnerable (Cutter and Finch, 2008; McGranahan et al., 2007). Overall, there is an intersection among low lying coastal zones, urban areas, low socioeconomic status, and tropical regions. This results in large swaths of people who are vulnerable to the effects of increasing storm surges and tropical storms associated with climate change (e.g., Mendelsohn et al., 2012; Sajjad et al., 2018; Woodruff et al., 2013; World Bank Group, 2016). Between 1995 and 2014, lower-income countries experienced 89% of storm-related fatalities but just 26% of storms (World Bank Group, 2019). These communities often have lower quality infrastructure and are more vulnerable to hazards. Overall, there is a lack of resilience among the urban poor worldwide (World Bank Group, 2016). The field of urban ecology has grown rapidly in the past 20 years (Barot et al., 2019), but most of this research has taken place in temperate areas. Much less is known about tropical, urban dynamics. Even less is known about tropical, urban *estuarine* ecosystems.

The San Juan Bay Estuary (SJBE) in Puerto Rico, a territory of the United States, is an example of an urban, low-lying, tropical estuary. The estuary is a (formerly) interconnected series of lagoons and canals which link San Juan Bay to the west with Piñones Lagoon to the east. There is a strong urban gradient within the system itself, which runs through San Juan, the biggest city in Puerto Rico, and into the largest mangrove forest on the island. Piñones Lagoon in Loíza, the easternmost portion of the SJBE, is fully encompassed by mangroves. The western SJBE has been modified by extensive shoreline urbanization, as well as some dredging and filling (US ACE, 2016). The Caño Martín Peña (CMP), which used to connect San Juan Bay with San José Lagoon, has essentially filled in, both intentionally and unintentionally, with sediment and debris. While the western half of the CMP was dredged in the mid^{-1}980s, the eastern half has not and it regularly floods adjacent urban areas (US

ACE, 2016). The channel, which was once 1.5–4.3 m deep and 60 m wide, can now be walked across via a makeshift bridge consisting of wooden pallets at its narrowest locations (Fig. 1; US ACE, 2016). As a result, San José Lagoon, which has no direct exchange with the ocean, has seen its residence time increase from less than 4 days to more than 17 days (Pérez-Villalona et al., 2015; US ACE, 2016).

The infilling of the CMP has had a profound influence on the hydrodynamics of the estuary over a short period of time. The tidal flux in the eastern half of the CMP ranged from 4–8 $m^3 s^{-1}$ in January and February 1974 (Ellis and Gómez-Gómez, 1976; Gómez-Gómez et al., 1983). Twenty years later, in the summer of 1995, the range was given as 2–2.5 $m^3 s^{-1}$ (Bunch et al., 2000). Since then, sections of the channel have aggraded completely, and some reaches appear ponded and stagnant. In addition, several highly populated areas adjacent to the CMP are not connected to a centralized sanitary system, and thus discharge their wastewater directly into the channel (US ACE, 2016). This has resulted in impaired water quality conditions in the CMP, as well as having possible effects on other portions of the SJBE, particularly in San José Lagoon. Concerns about water quality in this area have been voiced since the 1970s, when the CMP was reported as being clogged with debris (Gómez-Gómez et al., 1983). The local communities alongside the CMP report frequent flooding of schools, homes, and streets and the bleached-out markings of flood lines are clearly visible on neighborhood walls (e.g., Fig. 1). Water samples from the CMP indicate that it is heavily contaminated by sewage, with fecal coliform concentrations sometimes exceeding 2×10^6 cfu 100 ml^{-1} (Puerto Rico health standards are 200 cfu 100 ml^{-1}) (US ACE, 2016). As a result, community action groups have been working collectively to have the CMP dredged, with the intent of reducing the flooding and improving overall water quality (http://cano3punto7.org/nuevo/index.html).

Here we use available data from the San Juan Bay Estuary Program and our own ecological and biogeochemical measurements to assess how the changes in the hydrodynamics have affected nutrient cycling in this tropical urban estuary. We sought to identify the areas most influenced by the CMP's closure and to begin to identify how recent hydrodynamic shifts may be influencing the water quality of the poorly flushed portion of the San Juan Bay Estuary that lies east of the CMP. We hypothesized that urban impacts would be greater in the CMP than in the rest of the estuary and that this would be reflected in higher organic matter content and nitrogen stable isotope values.

2. Materials and Methods

2.1 Sample Collection

Surface subtidal sediments were collected in June 2015 via a Petite Ponar Sampler and the top 5 cm of sediment was subsampled into plastic bags and frozen until analysis (Fig. 2). Mussel and oyster samples were also collected, when available, from mangrove prop roots, dock pilings, and other structures. Whole shellfish samples, consisting of at least five individuals, were transferred to bags and frozen until their tissues could be extracted and processed.

Mangrove soil cores (two from each site) were collected in March 2016 in the western CMP (N18°25’59.0808”, W66°3’30.9348”), eastern CMP (N18°25’42.2184”, W66°2’21.9408”), western San José Lagoon (N18°25’47.676”, W66°2’3.498”), Torrecilla Lagoon (N18°25’42.2184”, W66°2’21.9408”), and Piñones Lagoon (N18°25’58.6848”, W65°57’42.516”) with a Russian peat sampler. The surface sediments (0–3 cm) were used for stable isotope, nutrient, and carbon analyses in this study. In June 2017, a truncated 5-mL syringe (5 cm by 1 cm diameter) was used to extract surface sediments for the microbial biomass and truncated 50 ml syringes were used for collecting surface sediments to a depth of 3 cm for the denitrification enzyme activity (DEA) assays. Five replicates were randomly collected at each site for the DEA and microbial biomass measurements.

2.2 Sample processing

Subtidal sediment samples were thawed in the lab and a subsample was retained for grain size analysis. Another subsample was wet sieved through a 2 mm sieve and dried in a 60°C oven for at least 24 hours. One portion of the resultant material was used to determine carbonate and organic matter content via loss on ignition (LOI, described below), the other was ground to a fine powder using a mortar and pestle. The dried, ground material was used for stable isotope analysis. Shellfish samples were thawed, and tissues were extracted and rinsed with deionized water. Given the small size of the animals, three or more individuals were pooled to create one homogenized sample. Tissues were also dried in a 60°C oven and ground via mortar and pestle.

2.3 Sample Analysis

2.3.1 Subtidal sediment characteristics—To determine organic matter and carbonate content, dried subsamples were broken up with a mortar and pestle, and subsamples were weighed into crucibles (~5 g) for LOI (Heiri et al., 2001). Dried samples were then heated to 550°C for four hours, cooled, and reweighed. Percent organic matter (%OM) was calculated as 100*(1-(weight after 550°C/initial weight)). The same subsamples were then heated to 950°C for two hours, cooled and reweighed to determine carbonate content, where % carbonate was calculated as 100*(1-(weight after 950°C/initial weight)).

Additional subsamples were gently rinsed through a 2 mm sieve using deionized water. Particles <2 mm were treated with H_2O_2 to remove organic material (Gray et al., 2010) and then two replicates were analyzed with three runs each on a Malvern Hydro 2000S / Mastersizer 2000 system recording particle size distributions from 0.023–2000 μm with 84 bins. Average particle size distributions were computed from all runs for each sample, except for three individual runs with anomalous results. In each of these three cases, the remaining five runs associated with a given sample had consistent particle size distributions and the flagged run differed widely from the other replicates. Average particle size distributions were then post-processed with Gradistat.v8 software (Blott and Pye, 2001), including bin aggregation to texture classes, and statistical description. Sediment textures were defined per Friedman and Sanders (1978). Particle size (diameter, d) values were converted to phi (φ) units using the formula $\varphi = -\log_2 d$, and the full logarithmic method of moments was used to calculate mean particle size and sorting as follows:

$$Mean_{\varphi} = \frac{\sum f m_{\varphi}}{100} \tag{1}$$

$$Sorting_{\varphi} = \sqrt{\frac{\sum f\left(m_{\varphi} - Mean_{\varphi}\right)^{2}}{100}} \tag{2}$$

where f is the frequency in percent and m is the midpoint of each size class interval. Graphical analysis of $Mean_{\varphi}$ vs. $Sorting_{\varphi}$ space was conducted to discriminate between high energy 'storm/channel' and low energy 'settling' depositional domains defined by Tanner (1995) and Lario et al. (2002).

2.3.2 Carbon, nitrogen, and sulfur stable isotope analyses and content—To remove carbonates, carbon (C) sediment samples were fumigated prior to analysis with 12 M HCl following the method of Harris et al. (2001). Tissue samples were not fumigated. The C and N isotope compositions were determined using an Elementar Vario Micro elemental analyzer connected to a continuous flow Isoprime 100 isotope ratio mass spectrometer (IRMS) (Elementar Americas, Mt. Laurel, NJ). Replicate analyses of isotopic standard reference materials USGS 40 ($\delta^{13}C$ = −26.39 ‰; $\delta^{15}N$ = −4.52 ‰) and USGS 41 ($\delta^{13}C$ = 37.63 ‰; $\delta^{15}N$ = 47.57 ‰) were used to normalize isotopic values of working standards (blue mussel homogenate) to the air ($\delta^{15}N$) and Vienna Pee Dee Belemnite ($\delta^{13}C$) scales (Paul et al., 2007). Isotope values are expressed in δ notation following the formula δX (‰) = [(Rsample / Rstandard) − 1] × 10^3, where X is ^{13}C or ^{15}N and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotopic ratio, respectively. Working standards were analyzed after every 24 samples to monitor instrument performance and check data normalization. The precision of the laboratory standards was better than ± 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$. The %C and %N were calculated by comparing the peak area of the unknown sample to a standard curve of peak area versus the C or N content of a known standard.

Both sulfur stable isotopes ($\delta^{34}S$) and content (%S) were measured in sediments at the Center for Stable Isotope Biogeochemistry at the University of California at Berkeley where analyses followed the SO2 EA-combustion-IRMS method and the precision of the $\delta^{34}S$ values was better than ± 0.2 ‰.

2.3.3 Phosphorous analysis—Total phosphorous (P) concentrations were determined in the subtidal sediments via a method presented in Strickland and Parsons (1972), with modifications by Aspila et al. (1976). Briefly, 0.2–0.3 g of homogenized, sieved sediment was combined with 12 ml of 1M HCl, shaken for 16 hours, and then settled. Then 1 ml of the liquid was diluted with Milli-Q water (1:10), combined with 1 ml of a working color reagent, and left in a dark room for 20 minutes prior to analysis on a spectrophotometer at 885 nm. Measurements were calibrated against a blank (Milli-Q) and six standards (0, 50, 100, 200, 400, 800 µM). Inorganic P was measured in the same manner as total P, except that the sediment samples were heated to 550°C for 1 hour prior to analysis. Concentrations (in µM) were converted to grams of P and divided by the initial sample weights to calculate %P.

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Organic P was calculated as the difference between total and inorganic P. Total P was used, in conjunction with total sediment N to calculate molar N:P ratios.

2.3.4 Mangrove DEA and microbial biomass—

The DEA was measured using the acetylene-based anaerobic assay in which conditions were made nonlimiting by the addition of excess nitrate at a rate of 1.4 mg KNO_3 g^{-1} sediment wet weight and at a rate of 1 mg glucose g^{-1} sediment (Groffman, 1987; Groffman et al., 1996; Smith and Tiedje, 1979). Chloramphenicol was added to the soil samples to inhibit the growth of the bacteria at a rate of 0.25mg g^{-1} sediment. The combined nitrate-glucose-chloramphenicol media was prepared in deionized water, and 18 ml were added to each 8.6 grams of soil to create a slurry and placed in a 100-ml dark serum bottle. Serum bottles were made anaerobic by a series of evacuations and flushes with nitrogen gas, and then 10 ml of acetylene was added to the headspace. Samples were collected from the headspace at 30 and 90 minutes, stored in evacuated glass tubes submerged in deionized water, and analyzed for N_2O with electron capture gas chromatography.

Microbial biomass from mangrove soils (C/N) was estimated using the chloroform fumigation-extraction method (Beck et al., 1987; Brookes et al., 1985) using five grams of wet sediment, a fumigation time of five days, and extraction using K_2SO_4. The filtrate was analyzed using Kjeldahl digestion (for microbial N) and via a TOC analyzer (for microbial C). Total microbial biomass was estimated as proportional to the chloroform-labile pool by dividing by correction factors (k_N=0.79; k_C = 0.45). Five replicates were analyzed from each site.

2.4 Statistics and data management

Differences among sites for nitrogen and carbon microbial biomass in mangrove sediments were assessed using a one-way analysis of variance (ANOVA). Both normality (Shapiro-Wilk) and equal variance tests (Brown-Forsythe) were passed and pairwise multiple comparisons were done via the Holm-Sidak method. Overall significance level was <0.05. The DEA data failed the Shapiro-Wilk normality test, so a Kruskal-Wallis one-way ANOVA on ranks was performed (H=18.402 with four degrees of freedom). A Tukey test was used for all pairwise multiple comparisons. Statistics were performed in Sigma Plot 14.0.

3. Results

Data available from the San Juan Bay Estuary Program (SJBEP; https://web.estuario.org) illustrates the range of water quality across the SJBE (Fig. 3). While the system ranged from fully marine (San Juan Bay) to polyhaline (CMP, La Torrecilla, and Piñones), the San José Lagoon and Suárez Canal were mesohaline (salinity between 5 and 18). Between 2015 and 2017 fecal coliform concentrations were just under the 200 cfu 100 ml^{-1} limits in San Juan Bay, with only one exception (710 cfu 100 ml^{-1} on 1 Aug 2016). San Juan Bay dissolved oxygen (DO) concentrations were generally just above the 4.8 mg l^{-1} concentration threshold that the U.S. Environmental Protection Agency has defined as the concentration below which unacceptable chronic effects to aquatic life occur (at least for the mid-Atlantic; US EPA 2000). All fecal coliform measurements exceeded the health standard of 200 cfu 10 ml^{-1} with a range of 3,700 to 72,000 cfu 100 ml^{-1}) and almost all DO concentrations were below 4.8 mg l^{-1} (range 0.0–6.6 mg l^{-1}) in the CMP. SJBEP stations in San José Lagoon, Suárez Canal, and La Torrecilla Lagoon were intermediary between these two extremes, with fecal coliform concentrations ranging from 3 to 18,000 cfu 100 ml^{-1} and DO concentrations between 0.0 and 12.9 mg l^{-1}.

3.1 Sediment characteristics

The subtidal biogeochemistry from the eastern CMP had the highest organic matter (mean was 31%, ranged from 10 to 50%) (Fig. 4). The lowest %OM was measured in San Juan Bay and %OM in the surface sediment was generally ~20% throughout most of the inner SJBE, east of the CMP.

The texture of surficial sediment deposits in the San Juan Bay Estuary system ranged from mud to muddy sand; and were predominantly sandy mud (Fig. 4). The CMP east samples contained the most sand (mean of 43%, ranged from 5 to 58%) in the system; however muddy sands were also found at two sites in San Juan Bay, at a site near the fisherman's cooperative and boat launch in San José Lagoon, in Suárez Canal at the mouth of a small creek, and at the site closest to the mouth of La Torrecilla Lagoon. Most muds were bimodal with strong clay and fine-medium silt peaks, while most sandy muds were unimodal with coarse silt to fine sand peaks. Muddy sands ranged from uni- to bimodal with major peaks from fine to coarse sand (Fig. 5). The particle size distributions of poorly sorted, coarser sediments identified as channel/storm deposits tended to be bimodal. Channel/storm deposits were more prevalent than settling deposits in all sub-systems, particularly the CMP, with the exception of La Torrecilla (Fig. 5).

The $\delta^{15}N$ isotope values in surface sediments ranged from 1 to 9‰, with the lowest values in the CMP and the highest in San José Lagoon(Fig. 4). While living shellfish samples were unavailable throughout the eastern CMP and most of San José Lagoon and Suárez Canal, they generally followed the same spatial trends, with lower values in the western CMP than measured throughout the rest of the system.

The highest nitrogen content (%N), >1%, was measured in the CMP east sediments (mean %N was 1.0±0.6). These high N concentrations also drove high N:P. While San Juan Bay (N:P=5±3, N=0.12±0.04%), the western CMP (N:P=13±10, N=0.46±0.10%), and portions of La Torrecilla (overall N:P=7±7, N=0.34±0.10%) were N-limited (N:P below 16:1), the eastern CMP was strongly P-limited with ratios exceeding 30:1.

The $\delta^{13}C$ values ranged from −30‰ to −3.5‰ throughout the system but were less than −20‰ across most of the estuary. Values in the San Juan Bay were exceptionally high relative to the rest of the estuary. The $\delta^{13}C$ values at the six westernmost stations, closest to the mouth of the estuary, ranged from −10.7 to −3.5‰. There was also a wide range of $\delta^{34}S$ values where the highest $\delta^{34}S$ values were measured in the CMP, reaching up to 8‰, and the lowest were measured in La Torrecilla, with a low value of −27‰.

3.2 Mangrove biogeochemistry

The $\delta^{15}N$ values of surface sediment from locations adjacent to mangrove forests were similar to those measured in the subtidal sediments (Fig. 6). The lowest values were observed in the CMP and the highest in San José Lagoon. The CMP east mangrove site had high denitrification potential (DEA), where rates were significantly higher than those measured in the mangroves adjacent to CMP west and Piñones Lagoon. For both the N and C concentrations in microbial biomass, CMP east and San José were significantly higher than concentrations measured at the other mangrove sites. Overall, the Piñones site had the lowest, or among the lowest, values for DEA, N fluxes, and microbial biomass.

4. Discussion

4.1 Hydrodynamic changes & sediment effects

Here we focus on the hydrodynamic changes to the San Juan Bay Estuary that occurred between the mid^{-1}930s and late 1990s. The differences between aerial photos taken of the eastern Caño Martín Peña in 1936 and again in 1948 are clear. The earlier photos show a 60120 m wide channel filled with mangroves and, by 1948, the deforestation and encroachment of homes is unmistakable (US ACE, 2016). The earliest measurements of flow in this area were made in 1974: 26 years later. Flow between San Juan Bay and San José Lagoon in the eastern CMP was 4–8 m^{-3} s^{-1} in the fall and winter of 1974 (Ellis and Gómez-Gómez, 1976; Gómez-Gómez et al., 1983). But even at that time there were distinct hydrodynamic differences between the eastern and western CMP. For example, in January 1974, flows in the eastern CMP ranged from 4 to 7 m^3 s^{-1} while flows at the western CMP were 2 to 5-fold higher, ranging from 25 to 17 m^3 s^{-1} (Ellis and Gómez-Gómez, 1976). By 1995, flow through CMP east had been reduced by more than half, ranging from 2–2.5 m^3 s^{-1} (Bunch et al., 2000). The first report of the channel being “closed” was in 2000 (US ACE, 2004). While CMP east was infilling, the inlet at La Torrecilla Lagoon was widened and deepened in 1962 (Ellis, 1976), increasing flushing in La Torrecilla.

Currently, the tidal ranges at La Torrecilla Lagoon and San Juan Bay are both about 60 cm (Webb and Gómez-Gómez, 1998). The tidal range in San José Lagoon is much less, 5–10 cm, and tidal fluctuations in Piñones Lagoon are similarly small (Bunch et al., 2000). In these latter two systems, about half of the variation in water levels can be explained by variations in precipitation. In a recent study, the hydrodynamics of Piñones could not be modeled using a tidal constituent (Branoff, 2019). In contrast, in San Juan Bay and CMP west, with their greater tidal ranges, precipitation explained less than 1% of the water level variations. Flushing and water quality are correlated in the SJBE; San José and Piñones have poorer water quality than La Torrecilla and San Juan Bay. While surrounded by mangrove forest, Piñones has been characterized as being ~2°C warmer than other coastal estuaries in Puerto Rico and having significantly higher total Kjeldahl N, total organic C, turbidity, and biological oxygen demand (Branoff, 2019). While the lagoon has few direct sewage inputs, possibly from a small number of residences near the lagoon (<10) and a village to the northwest (Finca Piñones), fecal coliform concentrations in Piñones Lagoon frequently exceed Puerto Rico’s health standards (Fig. 3; www.estuario.org). San José Lagoon does receive large amounts of urban runoff, particularly via untreated wastewater that is discharged into creeks which drain into the lagoon, and the Baldorioty de Castro flood control pump station which discharges urban stormwater as well as runoff from the Caño Martín Peña (Pérez-Villalona et al., 2015).

The SJBE in general, but San José Lagoon and the CMP in particular, have had water quality issues detailed since the first ecological reports emerged in the early 1970s (e.g., US EPA, 1971; US AED, 1978). Phytoplankton concentrations in San José in January 1971 averaged 17,400 cells ml^{-1} with more than half of the cells being flagellates. In the same study, San Juan Bay, which has open exchange with the Atlantic Ocean, averaged 640 cells ml^{-1} (US EPA, 1971). At that time, the northeast shores of San José were matted with algae and the lagoon water was described as completely green. A series of 1969 surveys of DO levels in the SJBE found concentrations consistently below 3 mg l^{-1} in the CMP (US AED, 1978). Water quality is widely considered to have improved in portions of the SJBE, largely due to the sewering of most of the city of San Juan in 1985, but the SJBE estuary east of San Juan Bay in general, and San José Lagoon and CMP east in particular, remain impaired (Fig. 3; www.estuario.org).

The particle size distribution of sub-tidal sediments in the SJBE system was consistent with the hydrodynamic changes, but sediment organic matter content appeared to be unrelated to particle size variations. Sandier sediments were generally found in areas of higher transport energy such as canals, lagoon and creek mouths, and the portions of San Juan Bay exposed to open marine forcings. Highly organic sediments were found across the range of sediment textures, from mud to muddy sand. Generally, in coastal and marine sediment deposits organic matter content tends to scale inversely with mineral sediment particle size (Burdige, 2006). This is usually attributed to the fact that organic material is predominantly co-depositional with the finer fraction of mineral sediment, and finer sediment deposits tend to lose carbon through mineralization more slowly (Hedges and Keil, 1995; Mayer, 1994; Wakeham et al., 2009). However, no relationship was found between organic matter content and particle size distribution in the surficial sediments of the SJBE. For example, CMP sediments were sandy with high % OM, while finer-grained sediments in San José Lagoon were lower in organic content. This indicates that local productivity and organic material supply are more important drivers of surficial sediment organic loading than depositional environment. However, this is not entirely unexpected in shallow (i.e., recently deposited) estuarine sediments, where rapid, recent accretion and large local differences in organic material supply rates that diverge from sediment transport pathways can decouple mineral sediment grainsize and organic carbon content, particularly with strong human forcings on organic carbon delivery and production (Pelletier et al., 2011).

Nitrogen concentrations were very high in the subtidal surface sediments of the eastern CMP, with concentrations reaching close to 2% (Fig. 4). These high N concentrations drove P-limitation (where N:P exceeds 16:1; N:P was 34±26). In contrast, both N:P and %N were low in San Juan Bay, which is characterized by its open exchange with the Atlantic Ocean (Fig. 2). In general, the sites east of the CMP have higher N:P and N content, probably reflecting a poorer flushing regime. It is clear from the sediment nutrient content alone that CMP east looks very different from the other sites.

4.2 Stable isotopes as indicators

4.2.1 Nitrogen—Stable isotopes of N, C, and S are biogeochemical measurements that are commonly used to identify human influences on coastal ecosystems. In general, the expectation is that human inputs will be reflected in elevated $\delta^{15}N$ values (often >10‰), the $\delta^{13}C$ values will distinguish between terrestrial and marine C sources, and $\delta^{34}S$ will differentiate between fresh and marine waters as well as indicate levels of sulfate reduction (e.g., Fry, 2006). In contrast to this paradigm, we found that the lowest $\delta^{15}N$ values in the SJBE surface sediments and shellfish were from the CMP, which is characterized by high urban runoff and high fecal coliform concentrations as well as high N content in the sediment samples (Figs. 3 and 4; US ACE, 2016). The $\delta^{15}N$ values in surface sediment averaged 2.7±0.9‰ (S.D.) across our 29 sampling locations throughout the length of the CMP. Sediments, mussels, and oysters had significantly lower $\delta^{15}N$ values in the CMP, relative to the rest of the SJBE. In contrast, the highest $\delta^{15}N$ values were measured in the adjacent San José Lagoon. A recent study of isotope values in sediment cores collected from the SJBE suggested that since the 1980s San José has been increasingly functioning as a settling basin and the higher $\delta^{15}N$ values reflected a mix of urban runoff and nutrient cycling (Oczkowski et al., 2019). The CMP likely receives as much, if not more, urban runoff as San José Lagoon, but the $\delta^{15}N$ values in those sediment cores declined over time. There are only three known processes that result in lower $\delta^{15}N$ values in coastal ecosystems. The first is preferential uptake of the lighter N isotope (^{14}N) over the heavier (^{15}N) when bioavailable N is abundant. The second process is the direct application of synthetic fertilizer, which has a value of ~0‰ (produced by converting inert N_2 gas into bioavailable forms), and the third is N-fixation, which also yields $\delta^{15}N$ values of ~0‰ (Fry, 2006). Recent 14-day experiments designed to document preferential uptake in a temperate estuarine water column measured maximum decreases in $\delta^{15}N$ values of 9‰ in plankton and 2‰ in bivalves when nutrient limitation was eliminated (Pruell et al., 2020). Despite this, $\delta^{15}N$ values of nutrient-enriched coastal systems tend to be >6‰ (e.g., Lapointe et al., 2015; Nixon et al., 2007). Given the intensely urban environment surrounding the CMP, fertilizer is not a significant source of low $\delta^{15}N$.

Nitrogen fixation, which is the conversion of N_2 gas into bioavailable forms, is a dominant process in mangrove forests, even in urban areas, and fixation in mangrove sediments and tropical estuarine waters is correlated with the supply of organic matter and carbon rather than N availability (Bhavya et al., 2016; O'Neil and Capone, 1989; Shiau et al., 2017). Nitrogen fixation is associated with sulfate reduction, where the activity of nitrogenase, the enzyme responsible for N reduction, has been linked to sulfate-reducing bacteria (Romero et al., 2015; Shiau et al., 2017). Fixation studies conducted in mangroves in Taiwan and Belize found that the addition of carbon increased soil nitrogenase activity dramatically. In a mangrove (*Kandelia obovata*) forest in Taiwan the soil nitrogenase activity increased from ambient rates of 1.74±0.58 μmol C_2H_4 m^{-2} h^{-1} to potential rates of 171±11 μmol C_2H_4 m^{-2} h^{-1}, supporting the idea that the N-fixers are generally present in mangrove soils but their rate of fixation is limited by the availability of C sources. Salinity did not affect these rates (Shiau et al., 2017). Further, these rate observations are underestimates of potential fixation as they did not account for soil diatoms and cyanobacteria nor did they measure water column fixation.

The $\delta^{15}N$ values in the surface sediments from this study and recent sediment core deposits (Oczkowski et al., 2019) from the CMP are consistent with the observations of others and suggest that N-fixation may be an important process in the CMP sediments and water column. The enriched nature of the CMP water, as evidenced by high fecal coliform concentrations, suggests that N and C loads from sewage are high. Yet the $\delta^{15}N$ values do not reflect sewage sources, which are typically >10‰ (Fry, 2006). The fact that the $\delta^{15}N$ values in CMP sediments averaged <3‰ gives some indication as to how important fixation may be in contributing bioavailable N to this system, relative to sewage. While indirect evidence, hydrogen sulfide levels in the air around the CMP exceed minimum acceptable level for chronic exposure for children, supporting the supposition that sulfate reduction rates are high in this area (US ACE, 2016).

Surface subtidal sediment and mangrove peat samples collected at five paired sites from the SJBE had similar $\delta^{15}N$ values. Further, DEA analyses of mangrove peat revealed that the CMP east had the highest denitrification potential, followed by San José and La Torrecilla, and that the highest concentrations of N in the microbial biomass of the surface peat were also measured in CMP east and San José Lagoon (Fig. 6). Microbially mediated denitrification is associated with an isotope fractionation factor on the order of 10–30‰ (Fry, 2006). While denitrification potential is high in CMP east, it does not appear to be so great as to drive the $\delta^{15}N$ values higher. Denitrification is the process that removes biologically available N from the water column, reducing it to N_2 gas; essentially the opposite of N-fixation. Thus, while the potential for denitrification in CMP east mangrove peat may be high, the $\delta^{15}N$ values suggest that this is not a dominant process. In fact, when organic carbon supply and rates of sulfate reduction are high, as they appear to be in the CMP, dissimilatory nitrate reduction to ammonium (DNRA) can outcompete denitrification in removing nitrate (Bernard et al. 2015; Domangue and Mortazavi 2018; Gardner et al. 2006). DNRA retains bio-available N in the system as ammonium, as opposed to denitrification which converts it to and releases inert N_2 gas. The high concentrations of microbial biomass N in the CMP east and San José peats indicate more N-related microbial activity than the other sites. In the case of the CMP east, this activity may be associated with fixation, but the $\delta^{15}N$ values indicate that the opposite may be true in San José Lagoon. The high values for $\delta^{15}N$, DEA, and microbial biomass data are consistent with the observations of others that nitrate removal processes dominate in this basin (e.g., Oczkowski et al., 2019; Pérez-Villalona, 2014; Pérez-Villalona et al., 2015).

4.2.2 Sulfur and carbon—Seawater sulfate, which is the primary source of sulfur to the estuary, has a $\delta^{34}S$ value of 21‰ (Bottcher et al., 2004) but most of the $\delta^{34}S$ values measured in the surface sediments of the SJBE were negative. Negative values are common in low-oxygen bottom waters in coastal systems and are associated with high rates of sulfate reduction. There is a 20–40‰ kinetic fractionation associated with the reduction of sulfate to sulfide, which leaves the resultant sulfide more negative than the sulfate (Brownlow, 1996; Canfield, 2001). Values from some of the stations in San Juan Bay, La Torrecilla, and Piñones were <−15‰, consistent with sulfate reduction. In contrast, $\delta^{34}S$ values were positive in the CMP, where we hypothesized that N-fixing sulfate reducers were predominant. These high $\delta^{34}S$ values may be reflecting the poorly flushed nature of the CMP rather than the magnitude of sulfate reduction. When water is ponded, the sulfate supply is limited and the sulfate reducers are forced to take up more of the heavier ^{34}S, which results in higher $\delta^{34}S$ values (e.g., Emery and Robinson, 1993). In general, $\delta^{34}S$

values were lowest at some of the most well flushed sites, San Juan Bay and La Torrecilla, suggesting that the $\delta^{34}S$ values may be correlated with residence time in this highly altered tropical urban estuary.

In contrast to the other geochemical parameters that reflected the influences of urbanization and biogeochemical processing, the $\delta^{13}C$ values were fairly homogenous throughout most of the system (≈−26‰) and similar to literature values for mangroves (<−29‰; Fry, 2006). Sediments from San Juan Bay were very different; values were much higher, reaching a maximum of −3.5‰ at our westernmost site. Values this high are unusual in coastal systems. For example, $\delta^{13}C$ values in marine plankton typically range from −19 to −24‰ (Fry, 2006). However, values on the order of +4 to +5‰ have been measured in the shallow water carbonate sediments from the Great Bahamas Bank, to the northwest of Puerto Rico, and seagrasses in that area range from −4.9 to −9.7‰ (Burdige et al., 2010; Hu and Burdige, 2007). The high $\delta^{13}C$ values from the Great Bahamas Bank have been attributed to the processes of carbonate dissolution and reprecipitation. While the amount of carbonate in the San Juan Bay samples was not particularly high (Fig. 4), and we did remove carbonates prior to analysis via acid fumigation, the open exchange with the Atlantic and nearby shallow shelf waters and coral reefs may be influencing the carbon in these surface sediments. Regardless of the cause, the contrast between the high $\delta^{13}C$ values in the San Juan Bay and the rest of the system underscore how disconnected the San Juan Bay Estuary system has become.

4.3 Synthesis

There is no question that the closure of the Caño Martín Peña greatly affected the San Juan Bay Estuary system. Because so many people live in and among the CMP and the urbanization and changes to the San Juan Bay Estuary hydrodynamics have occurred in the last 100 years, local communities have observed these changes firsthand. We know that the areas around the channel flood frequently with sewage-rich waters and that residents are concerned about not just the CMP but also the water quality of the adjacent San José Lagoon. While there is some gray literature, primarily in the form of government reports, there is a lack of peer-reviewed scientific literature to document these changes. Thus, we know what has occurred, and roughly when, but supporting documentation has generally not undergone peer-review. Tropical urban coastal ecosystems are extremely understudied, providing little context for our results. The very low $\delta^{15}N$ values in the CMP were surprising, especially given the high N and organic matter content of the sediments, contradicting our initial hypothesis. Fecal coliform concentrations in these waters can exceed 2,000,000 cfu 100 ml^{-1} (health standards are 200 cfu 100 ml^{-1}; US ACE, 2016) and the communities around CMP east experience higher frequencies of gastrointestinal illness and asthma when compared to the rest of Puerto Rico (Sheffield et al., 2014). We know that the CMP east water has very low dissolved oxygen levels (e.g., US ACE, 2016), and yet the $\delta^{34}S$ values were high. Sulfate reduction in low-oxygen coastal waters is characterized by low $\delta^{34}S$ values (e.g., Brownlow, 1996; Canfield, 2001; Yamanaka et al., 2003), like those seen in La Torrecilla and Piñones lagoons (Figure 7). Further, the water in the CMP (east and west) generally presents as cloudy and the hydrogen sulfide levels in the air around the channel are unhealthy (US ACE, 2016). Both factors further indicate high rates of sulfate reduction.

The hypothesis that fits all the geochemical observations is that the sediments and water column of the Caño Martín Peña are hotspots for microbes that fix nitrogen during sulfate reduction. The only probable explanation for low $\delta^{15}N$ values in this area is N-fixation and it is well-documented that S-reducers in mangrove ecosystems also fix N (Romero et al., 2015; Shiau et al., 2017). We suggest that the $\delta^{34}S$ values are so high because the flushing in this part of the estuary is poor, forcing the microbial communities to reduce the heavier ^{34}S that they would otherwise preferentially avoid (e.g., Emery & Robinson, 1993). Because N-fixation is driven by the supply of organic matter to the S-reducers in mangrove systems such as this, and not the amount of N already available, the N:P ratios in the CMP are consistent with this scenario as they indicate strong P-limitation (Figure 7).

If the San Juan Bay, with its open exchange with the tropical southwest Atlantic Ocean, reflects a mix of terrestrial and offshore sources (as suggested by the $\delta^{13}C$ values), and the CMP is a zone of S-reduction and N-fixation, then the adjacent San José Lagoon is different from either of these subsystems. The high $\delta^{15}N$ values that we measured are consistent with the observations of Pérez-Villalona (2015) that San José is a receiving basin for urban runoff, including discharge from a large pump station that concentrates stormwater mixed with failed septic waste, and an area where N is processed either through DNRA or denitrification. The lagoon has no direct exchange with the sea and its residence time has increased from <4 days to 17+ days (Pérez-Villalona et al., 2015; US ACE, 2016). Further, the bottom water of the lagoon is anoxic and fish kills and nuisance algal blooms are periodically reported (CCMP, 2000; US ACE, 2016). In contrast, the urban influences on the Suárez Canal and La Torrecilla and Piñones lagoons are less clear. They appear to be better flushed and, as they are surrounded by mangrove forests, certainly receive less urban influence than the San Juan Bay and CMP. But some of the sediments from Piñones were enriched in N with strong P-limitation, and fecal coliforms still often exceeded acceptable limits. The source of the N enrichment, whether allochthonous or autochtonous, is unknown.

Overall, we found it surprising how little is known about tropical, urban estuaries given both their prevalence and their vulnerability. A recent synthesis of fundamental processes and trends related to urban mangrove ecology and ecosystem services found that, while flushing is the primary driver of mangrove physiology, faunal assemblages associated with mangroves were affected by urban pollution (Branoff 2019). If our revised hypotheses are correct, then the most disturbed portion of this estuary, where raw sewage is actively pouring in and only episodically flushing, is also a hotspot for N-fixation, adding an unprecedented (and unaccounted for) amount of bioavailable N to an ecosystem with well-documented water quality problems. Aside from episodic fish kills in San José Lagoon, the impacts of this on higher trophic levels are unknown. The findings and observations presented in this paper should be a call to arms to the ecological community, particularly for those concerned with environmental justice, to study nutrient cycling in these urban, low-lying coastal ecosystems.

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Key Points

- We lack a basic understanding of the biogeochemical dynamics of tropical, urban coastal regions
- Sediment geochemistry from San Juan Bay Estuary's most urbanized region suggests that nitrogen fixation is an important nitrogen source
- Nitrogen fixation by sulfate reducing microbes is well-documented in mangroves, but never in such an intensely urban system

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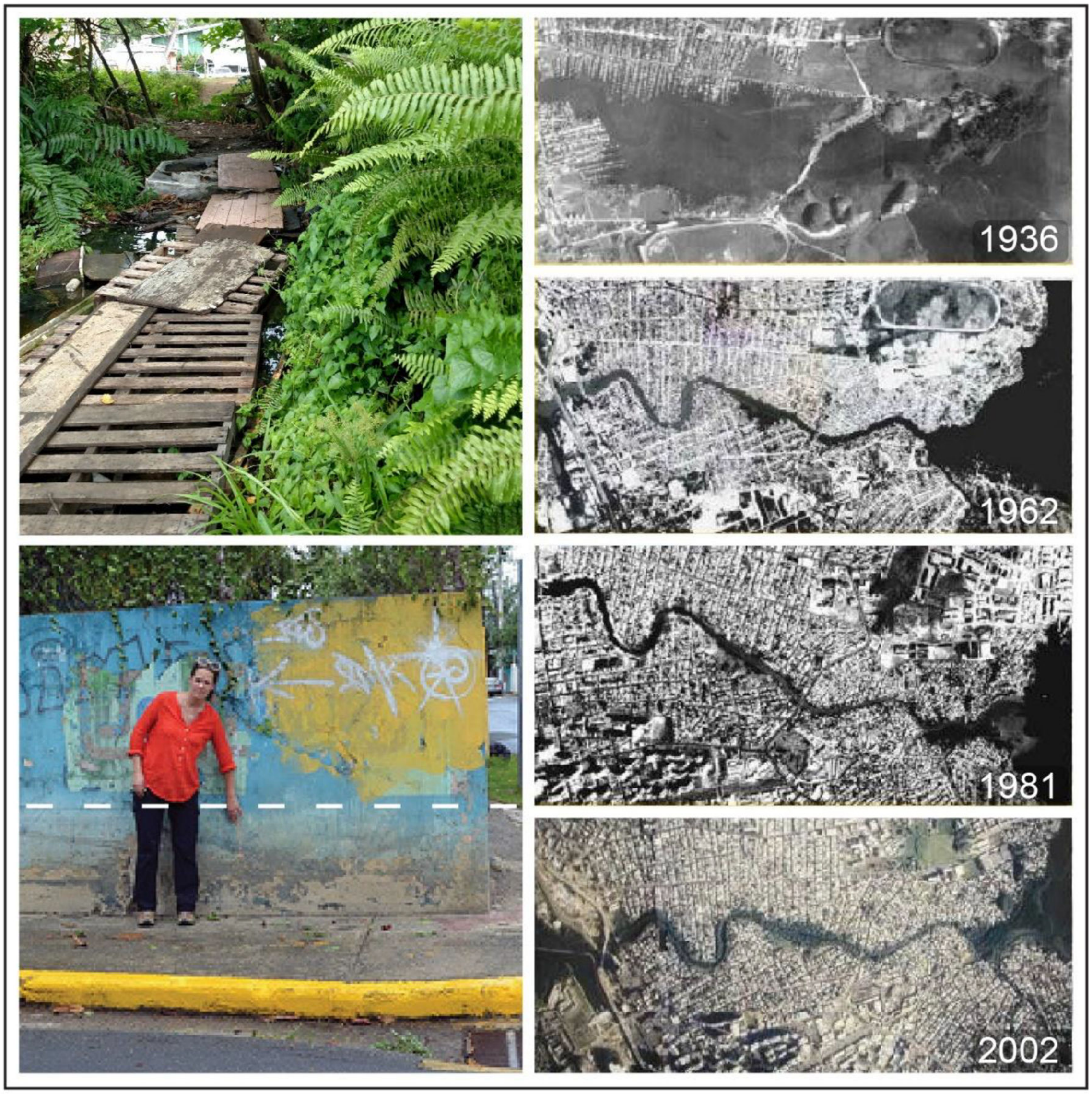

Fig. 1.

Top left: picture of the narrowest point of the Caño Martín Peña (CMP), where the waterway can be crossed on wooden debris. Lower left: Wall showing the bleached-out markings associated with local flooding. A white dashed line has been added to the approximate high flood mark. The aerial photos on the right show the evolution of the CMP east from 1936 to 2002. Aerial photos courtesy of ENLACE (http://cano3punto7.org/nuevo/index.html).

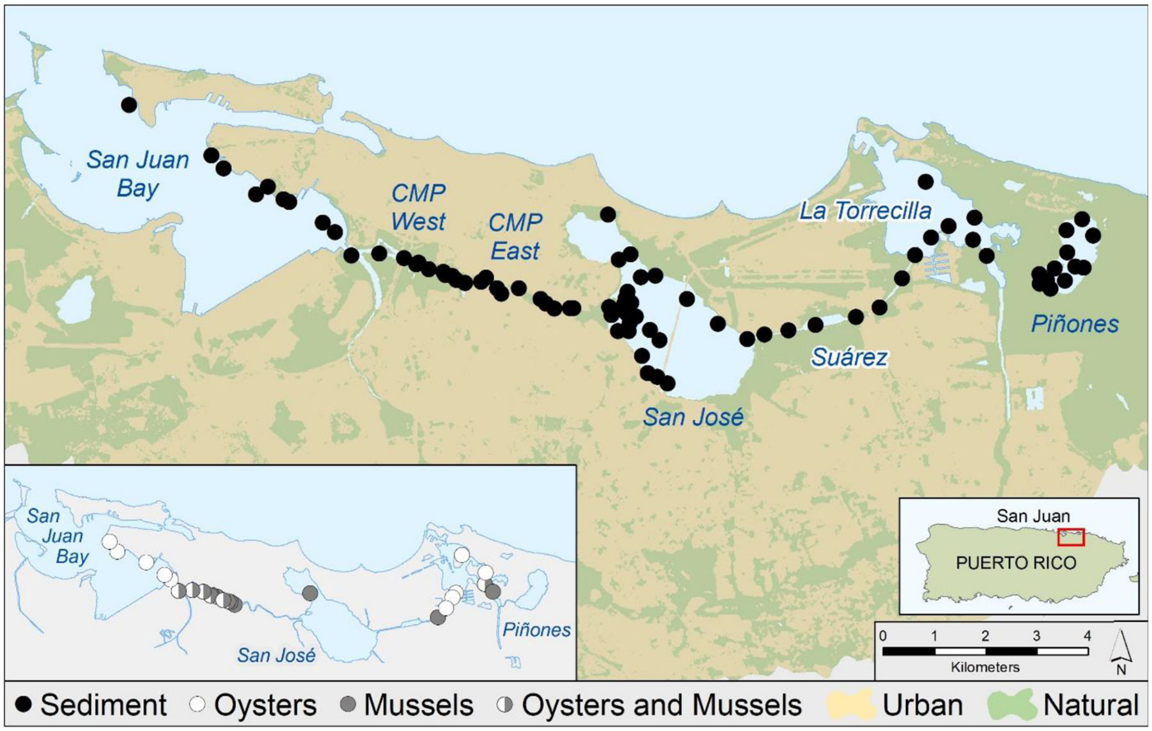

Fig. 2.

Map of the San Juan Bay Estuary (SJBE) located on the northeast coast of Puerto Rico. Closed black circles represent surface sediment sampling locations. Locations of oyster and mussel sample collections are shown in the inset map. Urban land-use associated with the city of San Juan is shown in gold and forested, mangrove, and wetland landcover is shown in green.

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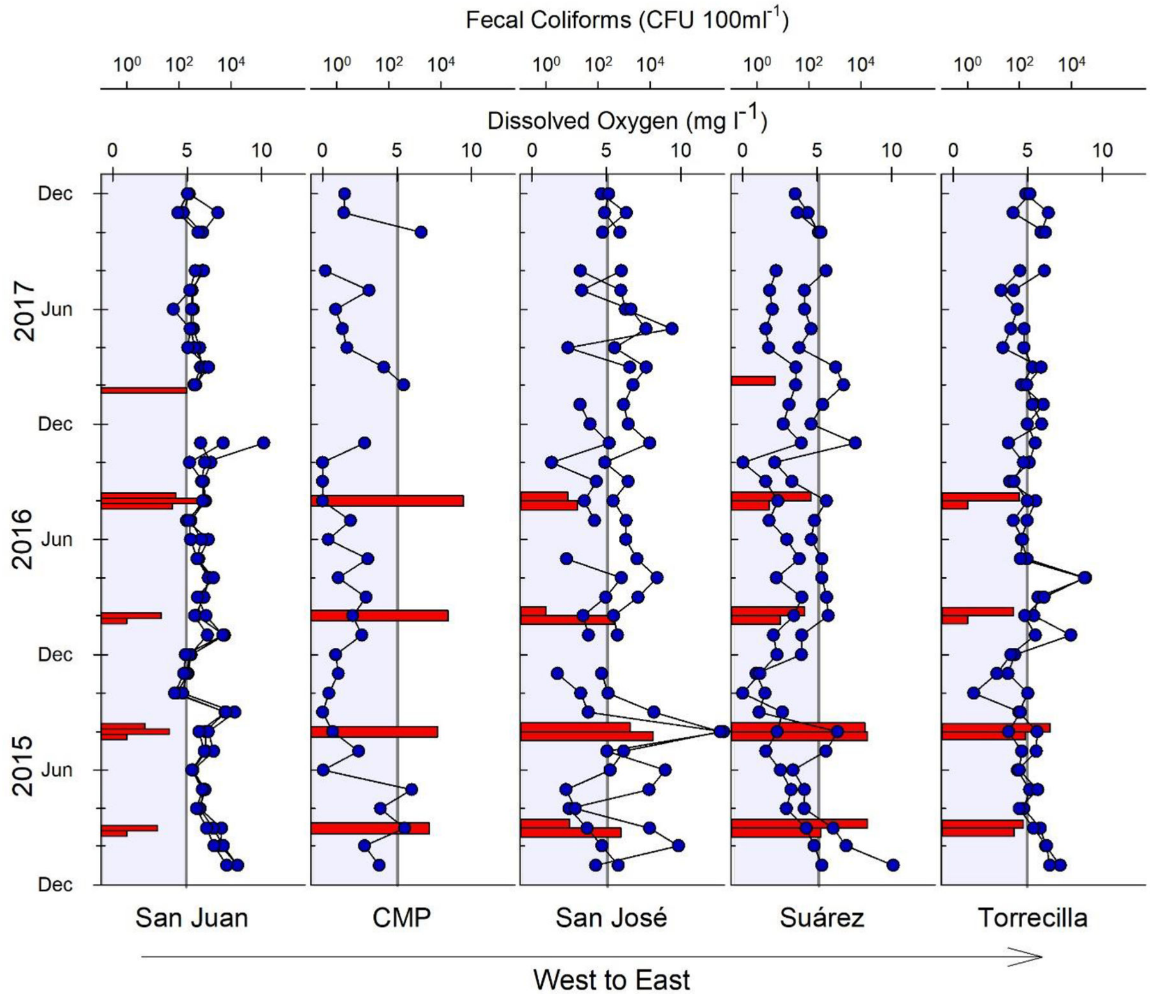

Fig. 3.

Fecal coliform concentrations (red bars) and dissolved oxygen concentrations (blue circles) for the San Juan Bay Estuary. Data were from a monitoring program conducted by the San Juan Bay Estuary Program (www.estuario.org). Multiple bars or circles represent individual sampling locations. Bars that exceed the gray line are exceeding Puerto Rico's acceptable health standards for fecal coliform concentrations (>200 cfu 100 ml^{-1}; US ACE, 2016). Circles that fall below the gray line are <5 mg l^{-1}, which is the concentration belowwhich the ecosystem experiences negative effects, at least for temperate regions (US EPA, 2000).

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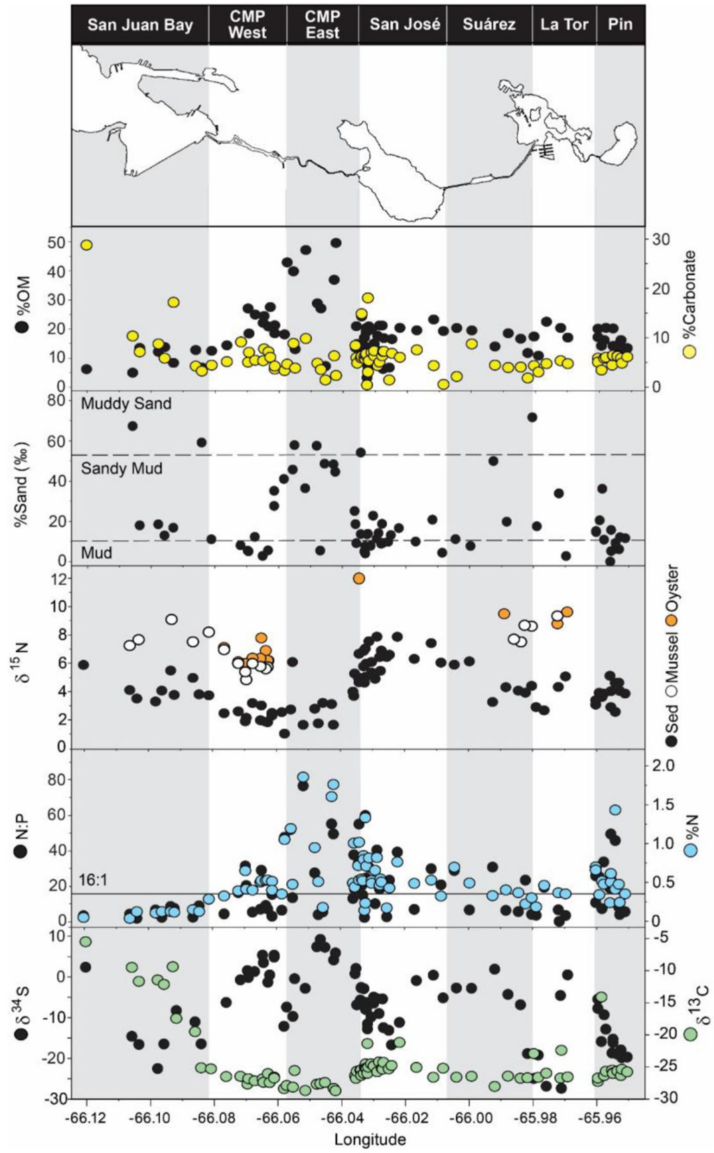

Fig. 4.

From top to bottom: the organic matter (OM) and carbonate content of surface sediments, the sand content where muddy sand, sandy mud, and mud were delineated, nitrogen stable isotope (δ^{15}N) results for surface sediments and mussel and oyster tissues, the N:P and %N of surface sediments, and the sulfur (δ^{34}S) and carbon (δ^{13}C) values in sediments from the San Juan Bay Estuary. Data are plotted against longitude. An outline of the estuary is given at the top of the figure and shading is used to indicate the sections of the estuary that are described in the text. Note the 16:1 line is specified in the N:P figure. Values <16:1 suggest P-limitation.

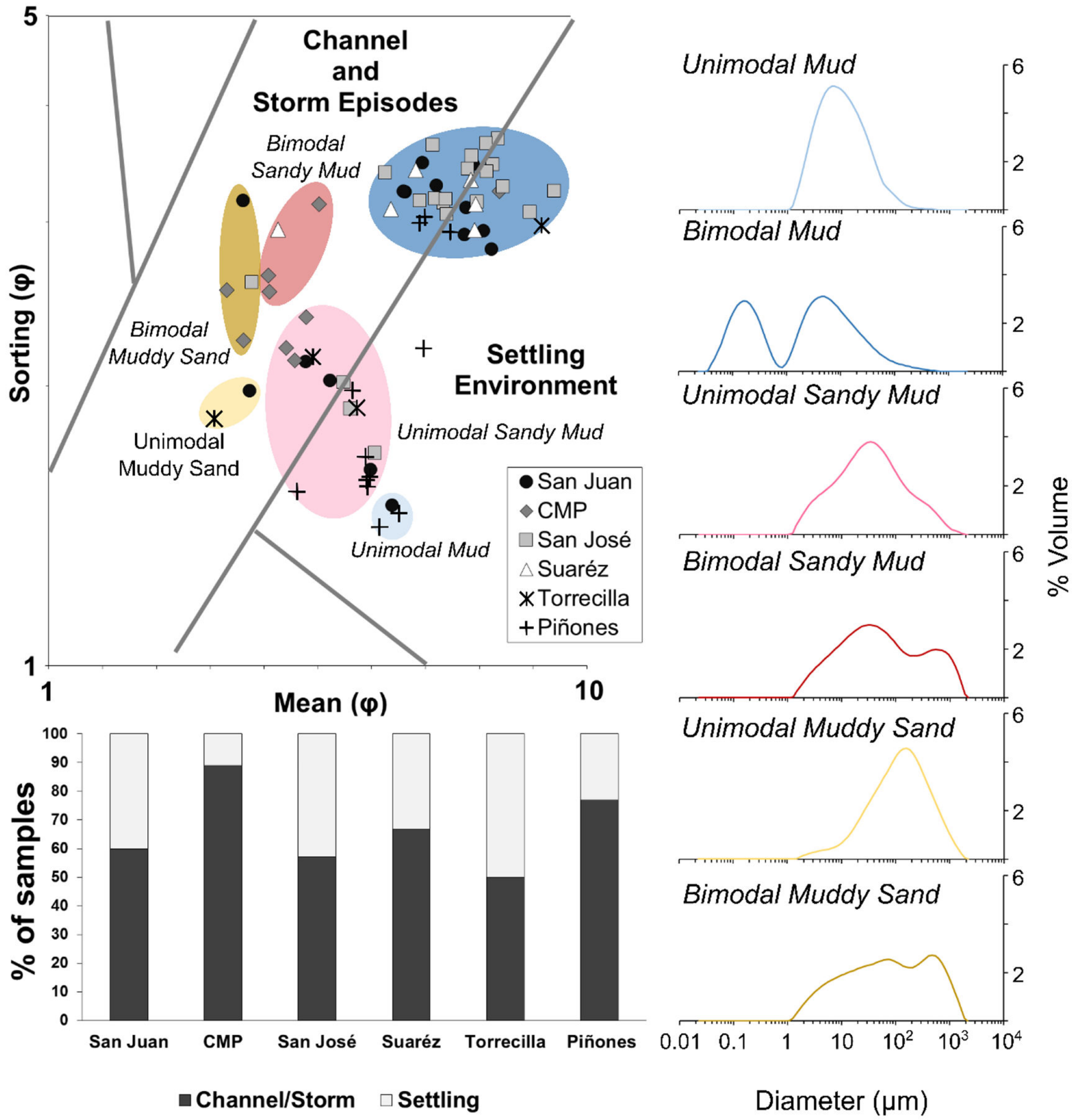

Fig. 5.

Average particle size distributions for the SJBE sediments. Delineations into channel and storm episodes and settling environments were determined using Gradistat.v8 software (Blott and Pye, 2001). In the top image, sediment textures (plotted as phi units) are plotted using overall sediment sorting mean grain size. Frequency distributions of the two settling environments (channel/storm versus settling) are given in the bottom graph.

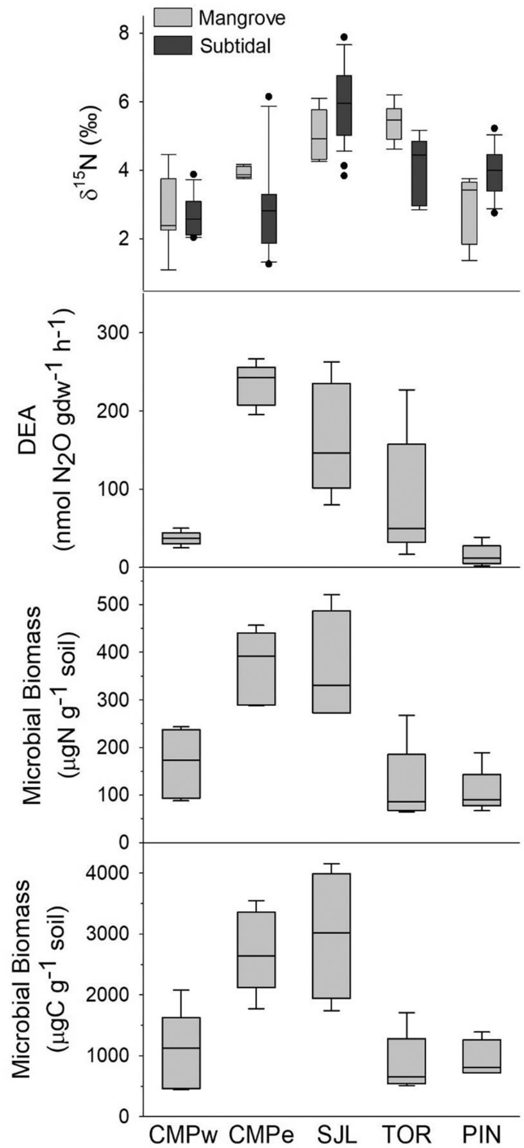

Fig. 6.

Boxplots of nitrogen stable isotope values ($\delta^{15}N$) for mangrove and subtidal sediment, mangrove soil denitrification enzyme activity (DEA), and mangrove soil nitrogen and carbon microbial biomass are given for Caño Martín Peña west (CMPw), Caño Martín Peña east (CMPe), San José Lagoon (SJL), La Torrecilla Lagoon (TOR), and Piñones Lagoon (PIN) (Fig. 1). The boxplots show the median, quartiles (top and bottom of box) and 90th percentiles (whiskers). Outliers are shown as closed circles.

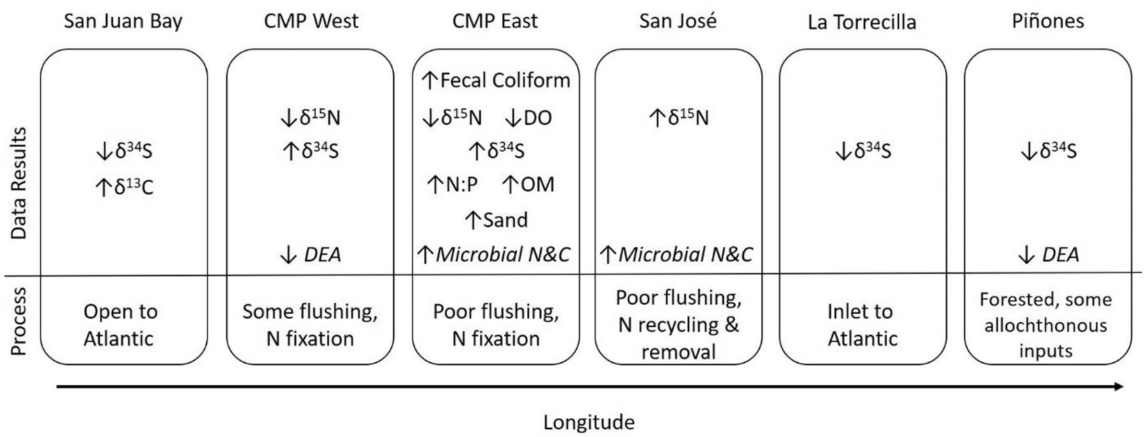

Fig. 7.

Conceptual diagram highlighting results and the suggested process that is driving them. Results and processes are broken down by subsections of the SJBE (Fig. 1) and organized geographically, from west to east. This diagram is intended to represent a summary of the information presented in this study. Italics indicate results and processes measured in adjacent mangrove peats. Note that Suárez Canal is not included because no driving processes nor distinguishing results were identified.