Spatial and seasonal variability of sedimentary features and nitrogen benthic metabolism in a tropical coastal area (Taganga Bay, Colombia Caribbean) impacted by a sewage outfall

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Abstract The effects of anthropogenic pressures in coastal areas are extensively studied in temperate but not in tropical zones, where their impact might be amplified by high water temperatures and upwelling phenomena. Sedimentary features and benthic metabolism were studied during the non upwelling (NUPW) and upwelling (UPW) seasons in Taganga Bay (Colombia). The bay is impacted by a submarine outfall of virtually untreated, organic and nutrient-rich wastewater. Samplings were performed in November

2017 (NUPW) and in January–February 2018 (UPW) at 4 stations located in the proximity and 100, 750 and 1800 m far from the outfall, respectively, at depths between 22 and 28 m. Aerobic respiration, denitrification, dissimilative nitrate reduction to ammonium (DNRA) and nutrient fluxes were measured. The influence of the outfall was detectable 750 and 1800 m away from the point pollution source, where $\delta^{13}C$ data suggested that ~ 40 and $\sim 20\%$ of organic inputs were terrigenous, respectively. In the proximity of the outfall benthic oxygen demand peaked and the presence of *Beggiatoa* mats suggested reoxidation of sulphides, that were abundant in pore water. Under sulfidic conditions, DNRA was the major driver of nitrate demand, whereas at stations far from the outfall, denitrification dominated nitrate consumption.

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Organic matter and nitrate inputs to the bay during the UPW season enhanced the effects of the outfall by increasing aerobic respiration and DNRA. Higher N availability during the UPW season reversed fluxes of molecular nitrogen and turned the sediments of 3 out of 4 sites from net sinks to net N_2 sources. Results from this study suggest that the analysis of sediments allows tracing the impact of the outfall and that such impact is enhanced during the upwelling season. In tropical areas, marine outfalls and upwelling may act in synergy and contribute to ecosystem deterioration due to high temperatures, increase of microbial respiration, sulphide toxicity and benthic biodiversity loss.

Introduction

Organic matter mineralization in coastal sediments provides an important ecosystem service such as benthic-pelagic coupling; nutrient regeneration from sediments may support 15–32% of nitrogen (N) and 17–100% of phosphorus (P) demand by phytoplankton (Aller 1988; Bonaglia et al. 2014; Carstensen et al. 2014; Mermillod-Blondin et al. 2004; Boynton et al. 2018). Sediments may also act as nutrient traps, however the supply of organic matter to the benthic compartment and the availability of oxygen are considered primary factors in controlling the direction and magnitude of solute fluxes (Banta et al. 1995; Hopkinson et al. 2001; Smith et al. 2012). Excess inputs of organic matter to sediments may uncouple microbial respiration and oxygen availability leading to anoxia, buildup of free sulphides (S^{2-} + HS^- + H_2S), simplification of benthic macrofauna communities and loss of biogeochemical services as denitrification or P-retention (Rosenberg and Loo 1988; Grall and Chauvaud 2002; Howarth et al. 2011). Oxygen shortage produces profound changes in aquatic communities, affecting energy transfer across trophic levels and biogeochemical cycles (Diaz 2001; Testa and Kemp 2011). Under hypoxic/anoxic conditions, the accumulation of toxic, reduced anaerobic metabolites within sediments and/or water depresses the

activity of microbes, plants and benthic fauna (Testa and Kemp 2011). In turn, this creates a positive feedback, as bioturbation and bioirrigation by infauna has a key role in organic matter degradation and solute transport (e.g. O_2, NO_3^-) in sediments (Aller 1994; Bonaglia et al. 2014; Carstensen et al. 2014). Strictly anoxic and chemically reduced sediments favor P mobility and its availability in water column and reduce P burial via precipitation or co-precipitation with iron (Fe) and manganese (Mn) oxides and hydroxides and organic matter (Testa and Kemp 2011; Zilius et al. 2014). Under sulphidic conditions, the activity of denitrifers is inhibited, whereas that of nitrate ammonifiers is stimulated, and the role of sediments switches form net N sink to net N source (Carstensen et al. 2014). Such switch is increasingly reported and has deep implications for benthic N cycling (An and Gardner 2002; De Brabandere et al. 2015; Hall et al. 2017).

Superimposed to or interacting with the effects of organic matter enrichment are the seasonal variations of physical variables in water column such as temperature, vertical stratification and circulation patterns. These factors, that include upwelling phenomena, are important as they control the vertical distribution and the supply of dissolved oxygen, nutrients and oligoelements in the water mass (Testa and Kemp 2011). There is a general scarcity of studies targeting benthic metabolism measurements and the potential impact of land-use alteration, nutrient and organic enrichment on sediments biogeochemistry in low latitude tropical coastal zones (Downing et al. 1999; Boynton et al. 2018). Downing et al. (1999) suggested that alterations in the N-cycle will probably have a greater impact on tropical systems than those observed in temperate areas, due to higher water temperatures and heterotrophic activity, and to lower oxygen solubility. Upwelling phenomena represent another potentially important, understudied factor regulating these dynamics, as they may alter locally the temperature of the water mass and its physicochemical composition (e.g. chlorophyll-*a*, suspended solids, oxygen, macro- and micronutrient content). Upwelling may either smooth the effects of organic enrichment or enhance them, as it represents in many areas a natural eutrophication phenomenon.

In this study, the combined effect of anthropogenic organic input associated to untreated sewage outfall and of natural upwelling on benthic biogeochemistry

was analyzed in Taganga Bay, a tropical system located in Santa Marta Coastal Area (SMCA), Caribbean Colombia. There is a strong interest in understanding the effects of multiple stressors, including wastewater discharge, and seasonal upwelling on the functioning of SMCA, and in particular on the health of coral meadows, as the area is considered a hotspot of biodiversity in the Colombian Caribbean (Bayraktarov et al. 2013, 2014; Bayraktarov and Wild 2014). Despite the importance of preserving ecosystem services in the area, no biogeochemical studies targeting the impact of wastewater outfall, alone or in combination with the seasonal upwelling phenomena were carried out to date. In particular, the sediment compartment was never studied in Taganga Bay, despite its memory for past and ongoing pollution and its importance in regulating dissolved oxygen and nutrient content in the water column.

Sediment properties (organic matter quantity, C, N and P pools and $\delta^{13}C$ and $\delta^{15}N$) and benthic metabolism (aerobic respiration, denitrification, nitrate ammonification and nutrient recycling) were analyzed at increasing distances from a marine outfall and along the non upwelling and upwelling seasons in SMCA. Carbon and N stable isotopes were used to determine the organic source in sediments (marine and terrestrial) and the extent of the organic pollution. The study had a threefold aim: to trace the spatial impact of the outfall on the sediments, to quantify its effects on benthic respiration and nutrient recycling and to analyze whether such effects are enhanced by the natural upwelling. We hypothesized a large stimulation of heterotrophic activity due to the organic inputs from the outfall and a suppression of biogeochemical sediment services as N removal via denitrification, sulphide precipitation and nutrient retention. We also hypothesized that organic inputs from marine upwelling act in synergy with those from the outfall, enhancing the risk of sediment anoxia and nutrient regeneration.

Materials and methods

Study area

Taganga Bay has an open conformation, subject to oceanic and continental influences, and is impacted by multiple pressures including a submarine wastewater outfall, the harbor activity, the Manzanares and Gaira river discharge and by seasonal surface runoff (Escobar 1988; Ramos-Ortega et al. 2008; Mancera-Pineda et al. 2013) (Fig. 1). River discharge and surface runoff are low or null in the dry seasons (December–April and July–August) and peak in the wet seasons (May–June and September–November) (Arévalo-Martínez and Franco-Herrera 2008).

The Santa Marta sewage outfall (SMSO, Fig. 1) discharges on average 1 m^3 s^{-1} of untreated wastewater derived from $\sim$ 500,000 inhabitants (García 2013). The city nearly doubles its resident population during high tourist season, peaking in January and resulting in increased wastewater discharge (Díaz-Rocca and Causado-Rodríguez 2007). The outlet is located between Santa Marta and Taganga bays (11.26 Lat and $-$ 74.22 Lon) (García 2013). The sewage outfall consists of 1 m diameter tubing and extends 428 m along the coastline (García et al. 2012). Sewage only receives a preliminary treatment for large solids removal (Díaz-Rocca and Causado-Rodríguez 2007). During 2006, the loadings of total N, P and total suspended solids (TSS) released to the bay from the outfall were estimated in $\sim$ 1100 T N yr^{-1}, $\sim$ 500 T P yr^{-1} and $\sim$ 12,300 T TSS yr^{-1}, respectively (García 2013). The maximum extension of the area affected by discharge from SMSO was estimated at 600 m from the outfall from water column fecal coliforms concentration, whereas sediments were not analyzed (García 2013).

Taganga Bay is highly influenced by the Southern Caribbean upwelling system (Rueda-Roa and Muller-Karger 2013). It is subjected to strong seasonality caused by the Caribbean Low—level Jet of Northeast (NE) Trade Winds and the oscillations of the Intertropical Convergence Zone (Andrade and Barton 2005). Major (December–April) and minor (July–August) upwelling events occur during the dry season while minor (May–June) and major (September–November) no-upwelling periods occur during the rainy season (Fajardo 1979; Andrade and Barton 2005; Arévalo-Martínez and Franco-Herrera 2008). Seasonal upwelling leads to changes in physicochemical variables such as temperature decrease (from 30 to 21 °C), salinity increase (from 33 to 38), oxygen subsaturation conditions ($< 91\%$) (Ramírez 1981; Salzwedel and Müller 1983; Bayraktarov et al. 2014), and increase of nitrate and chlorophyll-a
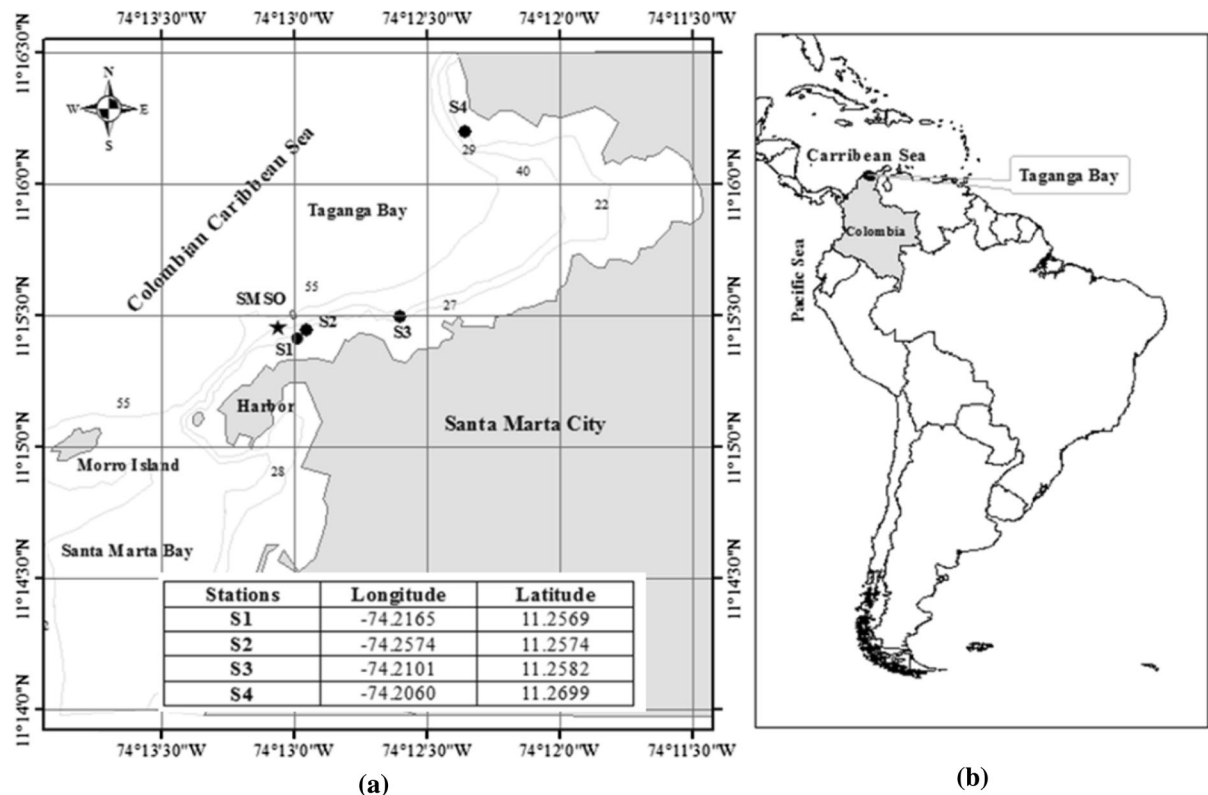
Fig. 1 Location of the Santa Marta sewage outfall (SMSO) and of the sediment sampling stations in Taganga Bay (**a**) and of the study area in Caribbean Colombia (**b**). Sampling stations coordinates are reported in decimal degrees

concentration, turning the system from oligotrophic to mesotrophic (Arévalo-Martínez and Franco-Herrera 2008; García-Hoyos et al. 2010; Paramo et al. 2011).

Water and sediment sampling was carried out at four stations (S1 to S4, located in the proximity and at 100, 750 and 1800 m from the outfall, respectively) (Fig. 1). Specifically, S1 was located near the outfall diffusers whereas S4 was close to Punta Venado, in a sandy area with low coral covering (Martínez and Acosta 2005; Vega-Sequeda et al. 2008). On every sampling occasion the physico-chemical features of the water overlying sediments (temperature, salinity and dissolved oxygen) were analyzed with a YSI 560 multiple probe.

Sediment cores collection

Sediments were sampled with minimum disturbance by scuba divers using transparent plexiglass liners (internal diameter = 4 cm, length = 20 cm), in order to have approximately equal heights (8–9 cm) of

sediment and water column. Sampling was conducted during major non upwelling rainy season—NUPW—(S1: 27 November, S2: 28 November, S3: 30 November and S4: 2 December 2017) and during major upwelling dry season—UPW—(S1: 26 January, S2: 28 January, S3: 31 January and S4: 4 February 2018). Twenty sediment cores were collected at each station. The cores were intentionally scattered along a 22–28 m depth profile, due to the variable topography of the bottom and in order to have overlapping depths among sites. Once collected, the cores were bottom and top capped and brought to the boat where the top lid was removed and the cores were submersed in a tank containing *in-situ* water cooled with ice packs (Fig. S1, Supplementary Material). Different tanks were used for the different stations. Bottom water (∼ 40 L per site) was collected through four large PVC pipes equipped with valves at both ends. Pipes were submersed and the valves were closed near the bottom by divers. Cores were stored vertically in the dark, and immediately transferred to the laboratory

where they were equipped with magnetic stirrers (more details provided in next paragraphs). During the overnight preincubation the cores were maintained submersed with the top open and the stirring on; the water in the tank was maintained at in situ temperature and at 100% oxygen saturation.

Net flux measurement at the sediment–water interface

The incubations were carried out at the chemistry laboratory of the Jorge Tadeo Lozano University, campus Santa Marta, in a temperature-controlled room maintained at 29 and 23 °C during the NUPW and UPW season, respectively. The day after sampling, dark incubations began by sealing each core with gas-tight lids. During the preincubation and incubation periods the temperature of the incubation tank varied by less than 0.3 °C. The water inside the cores was gently stirred by Teflon- coated magnetic bars without suspending the sediment (Fig. S1, supplementary material). The magnetic bars were driven by an external magnet rotated by a motor at 40 rpm. Five cores per station were used to measure the fluxes of dissolved gas (O_2:Ar, N_2:Ar and CH_4), metals (Fe^{2+} and Mn^{2+}) and inorganic nutrients (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-} and SiO_2) (Table S1, Supplementary Material)). Solutes concentrations were measured at the beginning and at the end of the incubation, assuming that their uptake or release rate during the incubation was linear. At time zero, four water samples (ca. 60 ml) were collected from the incubation tank before cores were capped. Sediment incubation time was maximum 6 h to keep oxygen within 20–30% of the initial value. Incubation time was set with pilot incubations carried out before starting the experiments. At the end of the incubation, lids were removed and water samples were collected from each core. Subsamples of 12 ml were transferred into exetainers, poisoned with 200 µl of $ZnCl_2$ solution (7 M) and later analyzed for O_2:Ar and N_2:Ar ratios and for CH_4 concentrations with a Membrane Inlet Mass Spectrometer (MIMS) equipped with a furnace with a copper reduction column (Bay instruments). Subsamples of 15 ml were transferred to glass vials, acidified with 50 µl of concentrated HNO_3 and analyzed for Fe^{2+} and Mn^{2+} with a Varian 240FS AA. Subsamples of 20 ml were filtered (Whatman, GF/F), refrigerated and stored at − 20 °C for later

nutrient analysis. Nutrients were measured with a continuous-flow analyzer (San^{++}, Skalar) according to Grasshoff et al. ([1983]). Detection limits were 0.1 µM for NH_4^+ and NO_3^-, 0.05 µM for NO_2^- and PO_4^{3-} and 0.3 µM for SiO_2. The Dissolved Inorganic Nitrogen (DIN) was calculated as $DIN = NH_4^+ + NO_2^- + NO_3^-$. Dissolved oxygen (DO) was also measured in the incubation tank and in the cores with a calibrated oxygen sensor (Orion Star Water[TM] A326). Solute fluxes across the sediment–water interface were calculated according to Eq. ([1]) (Dalsgaard et al. [2000]):

$$F = \frac{(C_f - C_o) \cdot V}{A \cdot t} \tag{1}$$

where F is flux of measured solutes (µmol m^{-2} h^{-1}), C_0 is concentration at time zero (µmol l^{-1}), C_f is concentration at the end of incubation (µmol l^{-1}), V is the volume of water in the core (L), A is area of sediment surface in the core (m^2) and t is the incubation time (h). Fluxes directed from the sediment to water column were considered as positive.

Denitrification and DNRA measurements

Sequential to net fluxes, the isotope pairing technique ITP (Nielsen [1992]) was used to measure the dark rates of denitrification and nitrate ammonification (DNRA). The IPT allows partioning NO_3^- source for denitrification in the contribution of nitrate diffusing to the anoxic sediment from the water column (Dw) and denitrification of nitrate produced within the sediment due to nitrification (Dn). Besides denitrification (and DNRA) the concentration series approach we adopted allows to test for the occurrence of the anaerobic oxidation of NH_4^+ to N_2 (ANAMMOX) (Dalsgaard et al. [2005]; Trimmer et al. [2006]; Burgin and Hamilton [2007]; Koop-Jakobsen and Giblin [2009]). Different amounts of $^{15}NO_3^-$ from a 30 mM Na$^{15}NO_3$ solution (> 98% Sigma Aldrich) were added to the water column of each of the 12 replicate cores to perform a concentration series experiment (Dalsgaard et al. [2000]). Three levels of $^{15}NO_3^-$ concentration (20, 40 and 60 µM, each level with 4 replicates) were created adding increasing amounts of the stock $^{15}NO_3^-$ solution to the cores water phase (Table S1, Supplementary Material). The incubation length was similar to that for fluxes, in order to keep oxygen variations within 20–30% of the initial value. At the end of the

incubation the sediment and water were gently mixed. An aliquot of the slurry was transferred to a 12 ml Exetainer and poisoned with 200 μl of $ZnCl_2$ solution (7 M); $^{14}N^{15}N$ and $^{15}N^{15}N$ abundance in N_2 were analyzed by MIMS. As calculated $^{28}N_2$ production was independent from the level of $^{15}NO_3^-$ addition (no detectable ANAMMOX), denitrification rates were calculated according to the equations and assumptions of Nielsen ([1992]). Another aliquot of the slurry (20 ml) was transferred in 50 ml falcon tubes, treated with KCl (1 M), shaken for 1 h in the dark, centrifuged, filtered and frozen for later analysis of the $^{15}NH_4^+$ fraction in the ammonium pool. $^{15}NH_4^+$ was determined via chemical oxidation to $^{29}N_2$ and $^{30}N_2$ by alkaline hypobromite addition as described in Warembourg ([1993]). DNRA was then calculated as described in Bonaglia et al. ([2014]). All cores were finally sieved (mesh size 500 μm) to check for the occurrence of large macrofauna.

Sedimentary features

After incubations, the analysis of sedimentary features was conducted on subsamples from the upper sediment layer (0–3 cm) in four cores per site (Table S1, Supplementary Material). Such sediment horizon was decided on the basis of the homogeneous color of sediment (Fig. S1, Supplementary Material) and in order to get information about the most reactive, upper layer. Each sediment slice was homogenized and a subsample was taken using a cut-off 5 ml syringe. Water content and porosity were determined after drying at 60 °C until constant weight. Then each sample was homogenized and ground with a porcelain mortar and pestle prior analysis of total organic carbon (TOC), total nitrogen (TN), isotopic measurements ($\delta^{13}C$ and $\delta^{15}N$), total phosphorus (TP), total inorganic phosphorus (TIP) and organic material content (OM). TOC, TN, $\delta^{13}C$ and $\delta^{15}N$ were measured with a Thermo Finnigan EA/NA-1100 CHN elemental analyzer coupled with an isotope ratio mass spectrometer after removing carbonates with HCl (25% v/v) and subsequent rinsing with deionized water to neutralize and dry (30 °C). The samples were run in duplicate and the analytical precision was ± 0.2% for TOC and ± 0.01% for TN. TOC and TN percent measured on sediment was corrected by the weight change due to the procedure (Tucker and Giblin [2010]). For $\delta^{13}C$, the reference was Vienna Pee Dee Belemnite (VPDB),

and for $\delta^{15}N$, it was atmospheric nitrogen. Isotopic ratios were expressed in the usual δ-notation (part per mill, ‰). Samples replicate analyses were within ± 0.2% for $\delta^{13}C$ and ± 0.3% for $\delta^{15}N$. Organic matter content (%) was measured by loss on ignition (LOI) of 0.2 g sediment at 350 °C in a muffle furnace. TP was extracted from ashes with 37% HCl whereas TIP was extracted after treating 0.2 g of dry sediment with 1 M HCl. Both TP and TIP were determined spectrophotometrically (Aspila et al. [1976]). Total Organic phosphorus (TOP) was estimated as difference between TP and TIP. Redox Potential (Eh) was measured with a Thermo Fisher ORP sensor Orion Star™ A326, inserted within the gently homogenized sediment slices. The sensor response was checked against a standard redox solution (200 mV). As the sediment slice was exposed to air the measured Eh value can be slightly overestimated. The porewater from the same slice was extracted from the sediment via centrifugation (4000 rpm, 5 min) in glass vials filled with N_2 and analyzed for sulphide concentration by spectrophotometry (Cline [1969]).

Contribution of terrestrial organic matter to Taganga Bay sediments

The contribution of terrestrial and marine organic matter to sediment organic carbon was estimated using a two-end-member mixing model based on the equation derived by Calder and Parker ([1968]) taken from Schlunz et al. ([1999]). This approach has been widely applied in organic matter pollution studies of marine sediments (Li et al. [2016]; Zhou et al. [2006]). The terrestrial organic carbon contribution was calculated using Eq. ([2]):

$$\text{Terrestial TOC}(\%) = \frac{\left(\delta^{13}C_{\text{marine}} - \delta^{13}C_{\text{Org}}\right)}{\left(\delta^{13}C_{\text{marine}} - \delta^{13}C_{\text{terrestrial}}\right)} \times 100\% \quad (2)$$

The contribution of marine organic matter to the TOC was then estimated from Eq. ([3]):

$$\text{Marine TOC}(\%) = 100 - \text{Terrestrial OC}(\%) \quad (3)$$

where $\delta^{13}C_{\text{marine}}$ is the $\delta^{13}C$ of marine end member, $\delta^{13}C_{\text{terrestrial}}$ is the $\delta^{13}C$ of terrestrial end member and $\delta^{13}C_{\text{org}}$ is the measured value in sediment samples. A $\delta^{13}C_{\text{terrestrial}}$ value of − 27.0‰ was used as end member based on the low $\delta^{13}C$ values of sediment

samples in the study area (Li et al. 2016). For the marine end member ($\delta^{13}C_{marine}$) a value of -20.3 ‰ was used as this is a typical carbon isotopic composition for diatoms in temperate marine ecosystems (Gearing et al. 1984). Furthermore, Gearing et al. (1984) indicated that planktonic isotope ratios varied little with water temperature (0 to 20 °C). A prevalence of diatoms and dinoflagellates has been found in the Santa Marta and Taganga bays (Ramírez-Barón et al. 2010; Garcés-Ordóñez et al. 2016).

Statistical Analysis

A two-way analysis of variance (2-way ANOVA) was carried to test the significance of the factors season (NUPW versus UPW period), sampling site and of their interactive effects on sedimentary properties, stable carbon and nitrogen isotopes ($\delta^{13}C$ and $\delta^{15}N$), sedimentary fluxes and microbial N transformations. Rcmdr package of the R-Project for Statistical Computing (R version 3.5.1) was used to fit different models to the data (Fox 2005). Kolmogorov–Smirnov's test and Levene's tests were employed to assess the data normality ($p > 0.05$) and variance homogeneity ($p > 0.05$), respectively. In the case of heteroscedasticity, data were $1/x$, $\sqrt{x}$, $\ln(x)$, $\log(x^2)$ or $\log(x)$ transformed. A goodness of fit was obtained after transformations. For significant factors, post hoc pairwise comparisons were performed using the Tukey HSD test. Significance was accepted for $p < 0.05$.

Correlations between sediment properties and nutrient fluxes were analyzed using simple and multiple lineal regression models, Pearson—test analysis (significance level $p < 0.0001$) and redundancy analysis (RDA), respectively. RDA was performed in the R environment using the vegan package. Before RDA analysis, a data exploratory multivariate analysis through Principal Component Analysis (PCA) (results not shown) was made on sedimentary properties (explanatory variables) and nutrient fluxes to detect and remove redundant variables lead to multicollinearity and affect data interpretation. Then, in the RDA analysis, one replicate of nutrient fluxes was randomly deleted to have a balanced arrangement along with sedimentary properties (4 replicates). This process was repeated three times to verify the validity of RDA results. The significance of sedimentary

properties was tested against 999 permutation of ANOVA function included in vegan package ($p < 0.05$). RDA results were interpreted based on the scaling used. The angles vectors representing sedimentary properties and nutrient fluxes reflect their linear correlation (scaling 1). Angles between all vectors reflect linear correlation (scaling 2) (Legendre and Legendre 1998).

Two ways PERMANOVA was applied to analyze the multivariate variance to the raw variables of nutrient fluxes, denitrification and DNRA. The adonis function within vegan package was used to perform PERMANOVA test against 999 permutation. Post hoc pairwise comparisons were made using the function pairwise adonis in R. Significance was accepted for $p < 0.05$.

Results

Water and sedimentary features

The upwelling altered the bottom water physicochemical conditions in Taganga Bay. It resulted in a nearly 7 °C decrease of average water temperatures, from 29.16 ± 0.25 to 22.58 ± 0.21 °C, in a decrease of DO concentration and saturation rates, from 235 ± 10 μM (123% saturation) to 208 ± 15 μM (106% saturation) and in an increase of salinity, from 35.98 ± 0.19 to 37.05 ± 0.02.

Sediments from the four stations were sandy, with comparable density, porosity and water content but with marked differences in terms of OM, C, N and P content, and redox status (Table 1). There was a clear gradient of impact from the sediments closest to the outfall (S1), that were more organic, sulphidic and covered by *Beggiatoa* mats, to the outer station (S4) that had less organic loaded and more oxidized sediments. The two-way ANOVA adopted for the analysis of sediment features showed a good fit to experimental data ($R^2 > 75\%$, Table 2). The season, station and interaction (season x station) terms were statistically significant for all parameters except for TOP. The effect of station was higher for TOC and TN than that of season while both effects were strong for OM, TP and TIP (Table 2). Sediment C, N, P, and OM content were in general highly correlated across the stations (Table S2, Supplementary Material).

Table 1 Sedimentary features measured in the upper 3 cm layer of stations S1 to S4

Station	November 2017, NUPW				January–February 2018, UPW			
	S1	S2	S3	S4	S1	S2	S3	S4
Density (g cm^{-3})	1.73 ± 0.04	1.80 ± 0.11	1.89 ± 0.04	1.89 ± 0.08	1.68 ± 0.10	1.71 ± 0.05	1.96 ± 0.02	1.82 ± 0.06
Porosity	0.53 ± 0.02	0.47 ± 0.05	0.46 ± 0.01	0.43 ± 0.01	0.54 ± 0.03	0.44 ± 0.02	0.35 ± 0.03	0.35 ± 0.01
Water (%)	30.66 ± 1.92	26.10 ± 4.55	24.26 ± 0.43	22.98 ± 1.05	31.97 ± 2.00	25.63 ± 1.56	18.03 ± 1.52	19.45 ± 0.59
OM (%)	3.78 ± 0.99	2.59 ± 0.47	2.70 ± 0.35	2.27 ± 0.18	6.68 ± 0.64	3.32 ± 0.47	2.23 ± 0.13	2.11 ± 0.38
TOC (%)	0.34 ± 0.15	0.36 ± 0.22	0.18 ± 0.03	0.20 ± 0.02	1.32 ± 0.35	0.55 ± 0.25	0.16 ± 0.07	0.14 ± 0.01
$\delta^{13}C_{(V\text{-}PDB)}$ (‰)	− 24.69 ± 0.28	− 24.50 ± 0.75	− 23.62 ± 0.24	− 21.77 ± 0.42	− 26.12 ± 0.56	− 25.53 ± 0.20	− 23.07 ± 0.54	− 21.36 ± 0.23
TN (%)	0.044 ± 0.009	0.022 ± 0.004	0.026 ± 0.003	0.012 ± 0.001	0.134 ± 0.012	0.038 ± 0.002	0.029 ± 0.010	0.012 ± 0.001
$\delta^{15}N_{(AIR)}$ (‰)	3.26 ± 0.39	3.35 ± 0.40	6.23 ± 0.37	4.45 ± 0,88	4.92 ± 0.98	13.40 ± 2.75	7.77 ± 3.40	4.00 ± 2.09
TP (%)	0.063 ± 0.016	0.051 ± 0.008	0.058 ± 0.004	0.039 ± 0.005	0.123 ± 0.017	0.074 ± 0.008	0.054 ± 0.006	0.044 ± 0.002
TIP (%)	0.051 ± 0.007	0.049 ± 0.014	0.051 ± 0.004	0.035 ± 0.005	0.105 ± 0.014	0.063 ± 0.013	0.043 ± 0.008	0.033 ± 0.011
TOP (%)	0.019 ± 0.009	0.008 ± 0.009	0.007 ± 0.004	0.004 ± 0.001	0.029 ± 0.014	0.010 ± 0.008	0.012 ± 0.004	0.017 ± 0.008
TOC/TN (molar)	9 ± 3	13 ± 2	8 ± 2	20 ± 3	12 ± 4	14 ± 7	7 ± 4	13 ± 1
Eh (mV)	− 432 ± 21	− 320 ± 63	− 101 ± 48	49 ± 102	NM	NM	NM	NM
$H_2S + HS^- + S^{2-}$ (µM)	870 ± 130	170 ± 80	0	0	NM	NM	NM	NM

Sampling stations are located along a gradient of impact, with S1 closest to the sewage outfall. Sediments were collected in November 2017, NUPW and in January–February 2018, UPW in Taganga Bay-Colombian Caribbean. NM = not measured

Table 2 Results of two-way ANOVA to test the effects of the factors sampling season and stations on sedimentary features of Taganga Bay

Dependent variable	Independent variable	F	P	η^2	Adjusted R^2	Tukey HSD test
OM	Season	60.66	**< 0.0001**	0.48	0.98	
	Station	30.06	**< 0.0001**	0.35		S1–S2, S1–S3, S1–S4, S2–S4
	Interaction	6.33	**0.002**	0.07		
TOC	Season	10.25	**0.0006**	0.16	0.93	
	Station	22.40	**< 0.0001**	0.52		S1–S3, S1–S4, S2–S3, S2–S4
	Interaction	5.70	**< 0.05**	0.13		
TN	Season	35.62	**< 0.0001**	0.12	0.98	NUPW–UPW
	Station	165.81	**< 0.0001**	0.82		S1–S2, S1–S3, S1–S4, S2–S4, S3–S4
	Interaction	4.35	**< 0.05**	0.02		
TP	Season	106.38	**< 0.0001**	0.62	0.98	NUPW–UPW
	Station	29.26	**< 0.0001**	0.26		S1–S2, S1–S3, S1–S4, S2–S4, S3–S4
	Interaction	5.39	**< 0.05**	0.05		
TIP	Season	27.78	**< 0.0001**	0.44	0.94	
	Station	11.94	**< 0.0001**	0.28		S1–S4, S2–S4, S3–S4
	Interaction	3.36	**< 0.05**	0.08		
TOP	Season	52.56	**< 0.0001**	0.78	0.76	
$\delta^{13}C$	Season	13,089.30	**< 0.0001**	0.98	0.98	NUPW–UPW
	Station	124.90	**< 0.0001**	0.01		S1–S3, S1–S4, S2–S3, S2–S4, S3–S4
	Interaction	10.18	**< 0.0001**	0.001		
$\delta^{15}N$	Season	88.46	**< 0.0001**	0.71	0.94	NUPW–UPW
	Station	6.97	**0.001**	0.08		S1–S3, S3–S4
	Interaction	9.51	**0.0002**	0.09		

Bold values indicate factors or their interactions were significant when P < 0.05

OM, TOC, TN, TP, TIP, $\delta^{15}N$ data were 1/x transformed while TOP data was $\sqrt{x}$. $\delta^{13}C$ data were not transformed. Significant differences in the two-way ANOVA and in the Tukey HSD tests were set at P < 0.05

$\eta^2 = 0.01$ represents a small effect, $\eta^2 = 0.10$ a medium effect, $\eta^2 = 0.25$ a large effect (Keith, 2015)

Organic carbon and nitrogen isotopic composition in the Taganga Bay sediments

The sedimentary C and N isotopic composition displayed significant spatial and seasonal variations (Fig. 2). The most and last depleted average $\delta^{13}C$ values were measured in S1 and in S4, respectively, both during the UPW season (Table 1). The seasonal effect was considerably stronger than the spatial variation and the interaction (Table 2). The pairwise comparisons between S1 and S2 did not show a clear distinction, but differences between these stations and stations S3 and S4 were significant. Most and least depleted values of $\delta^{13}C$ were negatively correlated with TOC, OM, TN, TP, and TIP but not with TOP, or $\delta^{15}N$ (Table S2, Supplementary Material).

Values of $\delta^{15}N$ also displayed high spatial and temporal variability with the more depleted values measured in S1 and S2 during the NUPW season (Table 1). Average $\delta^{15}N$ values increased from the NUPW to UPW season, in particular at S2, and the effect of season was greater than the effect of station and of the interaction. Pairwise comparisons suggested significant differences between $\delta^{15}N$ values measured at S1–S3 and S3–S4 (Table 2). Outcomes of pairwise and Pearson's coefficient indicate that spatial distribution of $\delta^{15}N$ did not correspond to that of TN. Furthermore, there were no significant correlations with other sedimentary properties.

The molar ratio TOC:TN calculated at the different sites and over the two sampling seasons varied by an order of magnitude, from 3 to 24 (Table 1). In general,
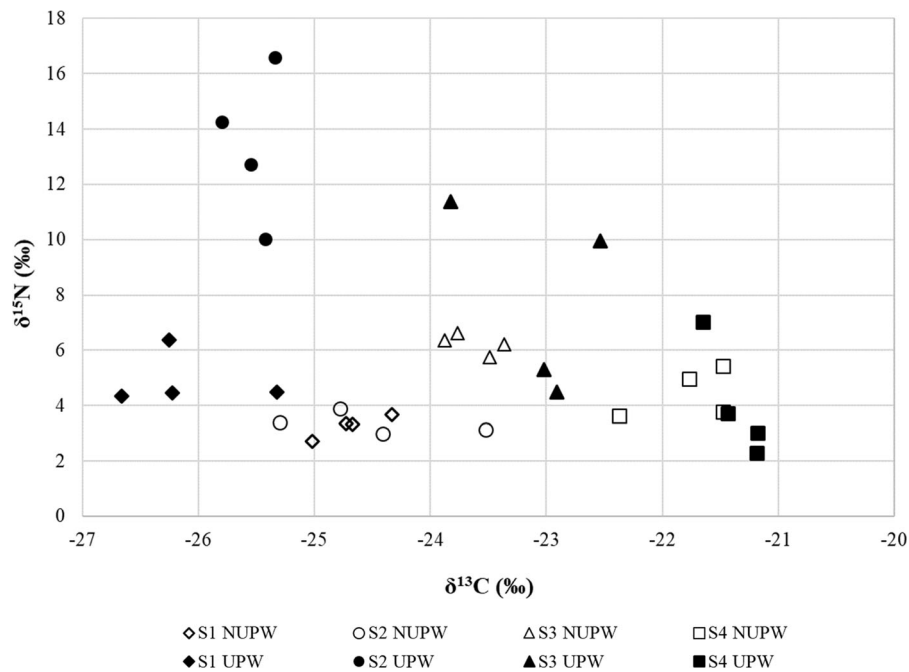
Fig. 2 Signatures of $\delta^{13}C_{(V\text{-}PDB)}$ and $\delta^{15}N_{(AIR)}$ in surface sediments (0–3 cm) of 4 stations (S1 to S4) located along increasing distances from a sewage outfall. Station S1 is the closest to the outfall. Measurements were performed in November 2017 and in January–February 2018, during non-upwelling (NUPW) and upwelling season (UPW), respectively
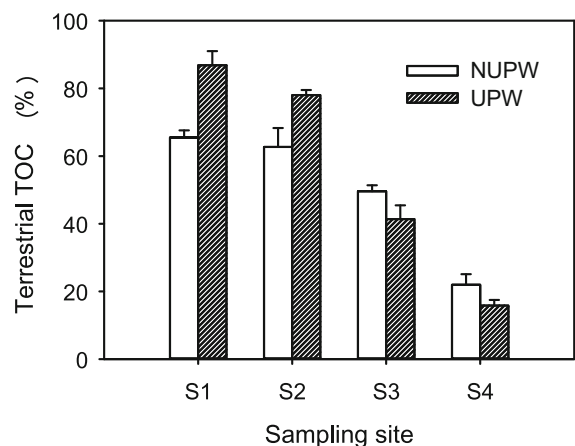
TOC:TN molar ratios were lower at S1 and S3 in both seasons; the highest ratio was found at S4 during the NUPW season.

The relative contribution of terrestrial organic carbon ranged from 13.0 to 94.9%, with the highest average contribution calculated for S1 during the UPW season (86.8 ± 8.4%) and declining from S1 to S4 (Fig. 3). At S3, 750 m far from the outfall, the average terrestrial contribution of organic C nearly halved as compared to S1 (45.5 ± 7.3%, n = 8) while at S4, 1.8 km far from the outfall, it was minor (18.9 ± 5.7%, n = 8) and marine sources prevailed. There was an increase of terrestrial TOC average values from the NUPW to UPW season for S1 and S2, whereas the opposite occurred at S3 and S4.

Aerobic and anaerobic metabolism

Oxygen uptake rates displayed clear spatial and seasonal trends, with decreasing respiration along the transect S1–S4 and with higher rates measured at S1 and S2 during the UPW as compared to the NUPW season (Table 3 and Fig. 4). The effects of season and sampling station were highly significant but there was

Fig. 3 Relative contribution of terrestrial versus marine organic carbon in surface sediments (0–3 cm depth) calculated for 4 stations along increasing distance from a sewage outfall in Taganga Bay—Colombian Caribbean. Measurements were carried out during the non-upwelling (NUPW) and upwelling (UPW) season. Averages ± standard deviations (n = 4) are reported

an interaction between the two factors (Table 3). Rates of oxygen consumption ranged from -1018.4 to -4519.0 μmol O_2 m^{-2} h^{-1} (NUPW season) and from -1269.6 to -6311.0 μmol O_2 m^{-2} h^{-1}

Table 3 Results of the two-way ANOVA on the effects of the factors sampling season, sampling stations and of their interaction on benthic fluxes, denitrification and nitrate ammonification rates

Dependent variable	Independent variable	F	P	η^2	Adjusted R^2	Tukey HSD test
O_2 flux	Season	986.19	**< 0.0001**	0.73	0.99	NUPW–UPW
	Station	210.12	**< 0.0001**	0.23		S1–S2, S1–S3, S1–S4, S2–S3, S2–S4, S3–S4
	Interaction	25.36	**< 0.0001**	0.03		
NO_3^- flux	Season	561.88	**< 0.0001**	0.78	0.98	NUPW–UPW
	Station	91.11	**< 0.0001**	0.19		S1–S2, S1–S3, S1–S4, S2–S3, S2–S4
	Interaction	5.15	**0.005**	0.01		
NH_4^+ flux	Season	9.44	**0.0006**	0.11	0.81	
	Station	9.31	**0.0001**	0.16		S1–S4, S2–S4
	Interaction	33.14	**< 0.0001**	0.56		
DIN	Season	104.30	**< 0.0001**	0.38	0.89	NUPW–UPW
	Station	92.45	**< 0.0001**	0.51		S1–S2, S1–S3, S1–S4, S2–S3, S2–S4, S3–S4
	Interaction	8.64	**0.0002**	0.05		
DIP flux	Season	161.73	**< 0.0001**	0.89	0.89	NUPW–UPW
DSi flux	Season	19.96	**< 0.0001**	0.25	0.97	
	Station	12.95	**< 0.0001**	0.25		S1–S2, S2–S3, S2–S4
	Interaction	15.41	**< 0.001**	0.29		
N_2 flux	Season	13.79	**< 0.001**	0.37	0.53	NUPW–UPW
	Interaction	2.49	**< 0.05**	0.2		
Dw	Season	220.71	**< 0.0001**	0.78	0.94	NUPW–UPW
	Station	10.93	**< 0.0001**	0.06		S1–S2, S1–S3, S2–S4, S3–S4
Dn	Season	162.22	**< 0.0001**	0.61	0.90	NUPW–UPW
	Station	38.78	**< 0.0001**	0.22		S1–S3, S1–S4, S2–S3, S2–S4, S3–S4
Dt	Season	168.86	**< 0.0001**	0.62	0.90	NUPW–UPW
	Station	39.03	**< 0.0001**	0.21		S1–S3, S1–S4, S2–S3, S3–S4
DNRA	Season	655.21	**< 0.0001**	0.66	**0.98**	NUPW–UPW
	Station	217.09	**< 0.0001**	0.33		S1–S3, S1–S4, S2–S3, S2–S4, S3–S4
	Interaction	3.30	**< 0.05**	0.005		

Bold values indicate factors or their interactions were significant when $P < 0.05$

See the text for more details. Squared root reflected transformation was applying to O_2 while ln(x) reflected transformation was used to NO_3^-, NH_4^+, SiO_2, N_2 and DIN data were not transformed. DIP data was log(x^2) transformed while Dw and DNRA data were log(x). Dn and Dt data were 1/x transformed. Significant differences in the two-way ANOVA and Tukey HSD tests were set at $P < 0.05$

$\eta^2 = 0.01$ represents a small effect, $\eta^2 = 0.10$ a medium effect, $\eta^2 = 0.25$ a large effect (Keith 2015)

(UPW season). In both investigated seasons the average O_2 uptake rate measured at S1 was nearly 3.6 times higher than that at S4. During the UPW season oxygen demand increased significantly (S1 and S2, by 24 and 62%, respectively) or tended to increase (S3 and S4, by 4 and 11%, respectively) as compared to the NUPW season. Variation coefficients were generally low (< 15%) at all stations and sampling seasons. Sediment sieving at the end of the incubations revealed the occasional presence of a few, small oligochaetes.
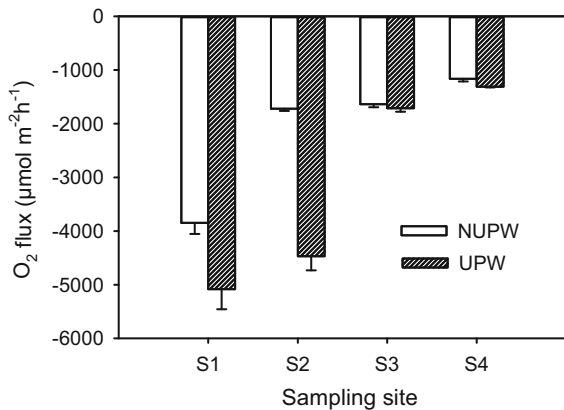
Fig. 4 Dark oxygen fluxes measured at the four stations during the non upwelling (NUPW) and upwelling (UPW) season. Averages (n = 5) and standard deviations are reported

Fluxes of ferrous iron, manganous manganese and methane were below the limit of detection of our methods (< 1 μmol Fe^{2+} or Mn^{2+} m^{-2} h^{-1} and < 0.1 μmol CH_4 m^{-2} h^{-1}) and are not reported.

Net N_2 fluxes and rates of denitrification and nitrate ammonification

The net fluxes of N_2 were characterized by small scale variability, as suggested by high variation coefficients, and by marked seasonal differences, depending upon the interaction with the factor site (Fig. 5 and Table 3). Rates were both positive, suggesting the dominance of denitrification over N_2 fixation, and negative,
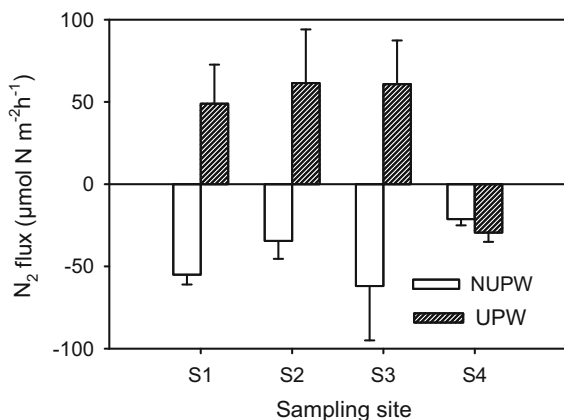
Fig. 5 Dark net N_2 fluxes measured as N_2:Ar ratios at the 4 stations during the non upwelling (NUPW) and upwelling (UPW) season. Averages (n = 5) and standard deviations are reported

suggesting the dominance of N_2 fixation. In the transition from the NUPW to the UPW season negative N_2 fluxes measured in S1, S2 and S3 became positive whereas at station S4 net fluxes were negative in both seasons. Net N_2 fluxes ranged from -109.6 to 67.6 μmol N m^{-2} h^{-1} during the NUPW season and from 50.0 to 186.3 μmol N m^{-2} h^{-1} during the UPW season.

Calculated rates of $^{28}N_2$ production from the IPT concentration series were always independent from the level of $^{15}NO_3^-$ added to the core water phase, suggesting that ANAMMOX was not contributing to N_2 production at the sampling stations. Rates of total denitrification were very low at three out of four stations ($2 < x < 4$ μmol N m^{-2} h^{-1}) with S3 as only exception ($8 < x < 11$ μmol N m^{-2} h^{-1}). At all sampling sites denitrification was mostly sustained (90%) by production of nitrate within sediments by nitrification (Fig. 6). Total denitrification (Dtot) showed high significant spatial and temporal variations (Table 3). Lowest average values of Dtot were found at S1 during the UPW season while highest values were observed at S3 during the NUPW season. Dtot decreased at S1 (by $\sim 35\%$) and S3 (by $\sim 25\%$) whereas rates remained virtually unchanged at S2 and S4 between the NUPW and UPW season. During the same time lag, average rates of Dw increased in all stations. At S1, S2 and S3 Dw increased by a factor of ~ 2.4 whereas at S4 rates increased by a factor of ~ 3.

Rates of DNRA were consistently higher during the UPW season, except at S4, and during both seasons DNRA rates decreased from S1 to S4 (Table 3 and Fig. 7). More in detail, S1 and S2 displayed similar rates, higher than those in S3 and S4, during the NUPW season, whereas during the upwelling period DNRA rates decreased from S1 to S4. Significant positive correlations were found between DNRA and TOC and Dw whereas negative correlations were observed between DNRA and Dn. (Table S2, Supplementary Material). The comparison of the share of nitrate consumption between Dtot and DNRA indicated that the latter process was responsible for a major, similar fraction of nitrate consumption (60 to 65%) at stations S1 and S2 in both UPW and NUPW seasons. The contribution of DNRA to nitrate uptake was smaller and consistent in the two seasons at station S3 (15–17%) and S4 (29–31%).
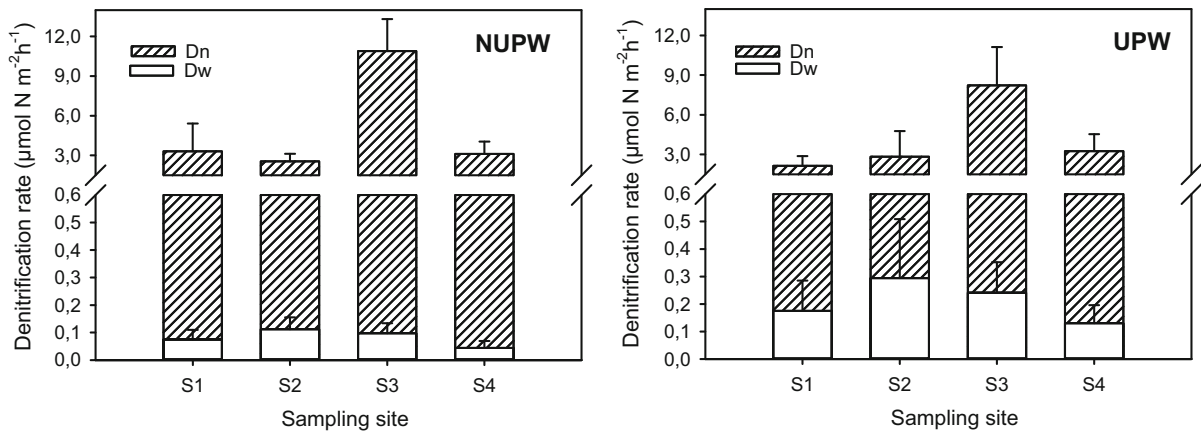
Fig. 6 Rates of denitrification of water column nitrate (Dw) and of nitrate produced via nitrification (Dn) measured in the dark at the 4 stations during the non upwelling (NUPW) and upwelling (UPW) season. Averages (n = 12) and standard deviations are reported
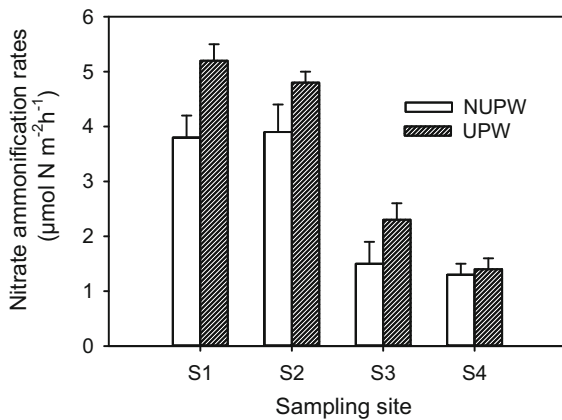

Fig. 7 Dark rates of nitrate ammonification (DNRA) measured at the 4 stations during the non upwelling (NUPW) and upwelling (UPW) season. Averages (n = 4) and standard deviations are reported

Nutrient fluxes at the sediment–water interface

Dissolved inorganic N, P and Si fluxes were low as compared to what expected from oxygen demand and the elemental composition of sediments and did not follow clear patterns (Fig. 8). Fluxes of NO_3^- and NH_4^+ were significantly different between sampling periods and among stations (Table 3). The effect of season was stronger for NO_3^- while the effect of the interaction was important for NH_4^+. NO_3^- flux dominated DIN fluxes at S1 in both seasons, and at S2 and S4 only in the UPW season. Significant positive correlations were found between NO_3^- and O_2 fluxes (Table S2, Supplementary Material).

Ammonium fluxes dominated inorganic N exchanges at S3 in both seasons, while at S2 and S4 they were relevant only in November. Fluxes of NO_2^- were negligible and are not reported.

DIP fluxes were generally low and ranged from − 0.56 to 0.17 µmol P m^{-2} h^{-1}. They displayed a significant temporal variation whereas there was no significant difference among stations (Table 3). Significant spatial and seasonal effects were determined also for SiO_2 fluxes, that ranged from − 3.19 µmol Si m^{-2} h^{-1} at S3 during the UPW season to 7.04 µmol Si m^{-2} h^{-1} measured at S3 during the NUPW season. Sediments were sources of SiO_2 at S1 and S2 in both seasons and at S3 and S4 during the NUPW season, whereas SiO_2 was taken up at S3 and S4 during the UPW season.

Multivariate analysis

The PCA analysis allowed to identify a subset of sedimentary properties (TOC, $\delta^{13}C$ and $\delta^{15}N$) and of nutrient fluxes (O_2, N_2, NH_4^+, NO_3^-, DIP, SiO_2, Dw, Dn and DNRA) with essential information for the RDA analysis. TOC, $\delta^{13}C$ and $\delta^{15}N$ were the most significant variables explaining 41.90% of changes in the nutrient fluxes in the two axes of RDA that were statistically significant (p < 0.001, Table S3 and Table S4 in Supplementary Material). Fluxes of NH_4^+, SiO_2 and DNRA were directly correlated with the increase of TOC while Dn and NO_3^- were inversely correlated (Fig. 9 and Fig. S2 in Supplementary Material). TOC and $\delta^{13}C$ were not correlated
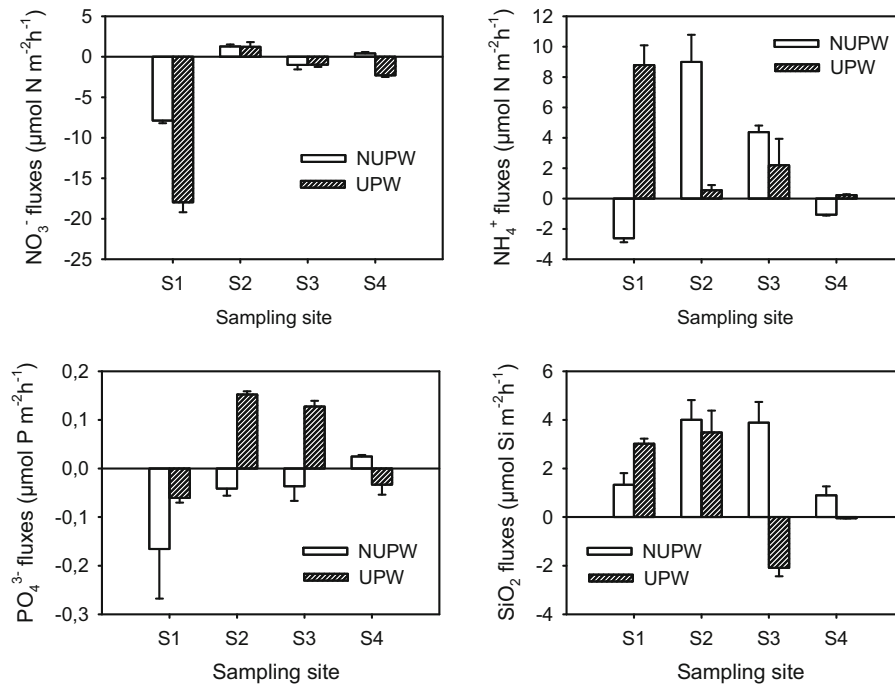
Fig. 8 Dark fluxes of inorganic nutrient measured at the 4 stations during the non upwelling (NUPW) and upwelling (UPW) season. Averages (n = 5) and standard deviations are reported
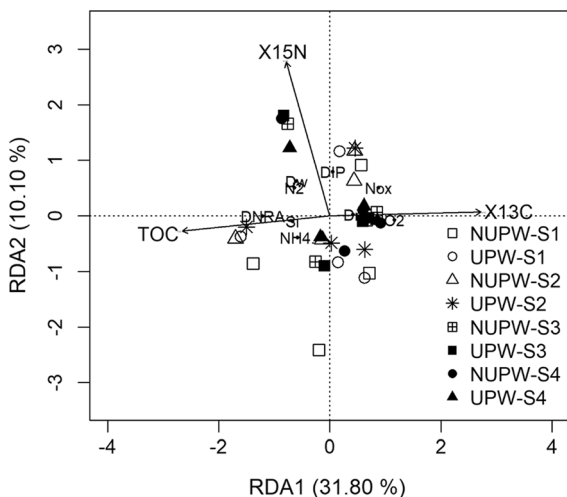

Fig. 9 RDA plot showing the angles vectors representing sedimentary properties and those nutrient fluxes reflect their (linear) correlation (scaling 1)

with $\delta^{15}N$, whereas there was a strong negative correlation between TOC and $\delta^{13}C$. $\delta^{15}N$ showed slightly higher correlation with N_2, DIP and Dw than with other fluxes. Results from the RDA align with what described in previous paragraphs and confirm

that $\delta^{15}N$ is a poor predictor of the effects of the outfall on the benthic compartment. Two-way PERMA-NOVA showed highly significant differences over the stations, seasons and interaction on nutrient fluxes, Dw, Dn and DNRA. Pairwise test pointed out that stations and interaction were significantly different from each other (Table 4 and Table S5 in Supplementary Material).

Discussion

Sedimentary features

Carbon isotopic signature along the transect as tracer of anthropic impact

Carbon isotopic signature along the analyzed transect traced the spatial variation of anthropogenic TOC, TP and TIP in sediments, as reported elsewhere by Church et al. (2006), Gao et al. (2008) and Dang et al. (2018). In Taganga Bay the most depleted average $\delta^{13}C$ values were found in the sediments with the highest oxygen average uptake, chemically reduced conditions (very

Table 4 Results of two-way PERMANOVA to test the effects of the factors sampling season and stations on nutrient fluxes, Dn, Dw and DNRA

Independent variable	F	Adjusted R^2	P	Pairwise
Season	65.889	0.12	**< 0.001**	
Station	134.295	0.72	**< 0.001**	S1–S3, S1–S4, S2–S3, S2–S4 S3–S4
Season:Station	22.210	0.12	**< 0.001**	Table S5. Supplementary Material

negative redox potential and buildup of free sulphides in the pore water, and presence of *Beggiatoa* mats), suggesting a large, measurable impact of the organic matter delivered from the outfall. The average $\delta^{13}C$ values at S1 and S2 were similar to or more depleted than values reported in marine sediments impacted by untreated and primary treated sewage effluents in Portugal, Canada and China (− 24.0 to − 25.4‰, Sampaio et al. 2010; Burd et al. 2013; Li et al. 2016). In contrast, the less impacted sediments of station S4 showed the most enriched average $\delta^{13}C$ values, lowest oxygen average uptake, oxidized sediments without free sulphides in the upper horizon and higher relative percentage of marine material.

The sedimentary gradients determined along the transect of anthropogenic organic matter input are consistent with those indicated by Hargrave et al. (2008). These authors provide a nomogram for benthic organic enrichment zonation based on Eh and total free sulphides, conforming to our findings, with sediments at S1 and S2 chemically reduced (Eh < − 150 mv), grossly polluted (∼ 75% of terrigenous OC) and with *Beggiatoa* mats clearly visible on the sediment surface. The appearance of white *Beggiatoa* mats on sediments is a consequence of the increase (S^{2-} above 500–1000 μM) and vertical migration of free sulfides in pore water (Hargrave et al. 2008) which may explain the production of NH_4^+, due to DNRA (Preisler et al. 2007). Sediments from station S3 possibly showed a transitory organic enrichment, oxic type B (∼ − 50 < Eh < 100) with a lower relative proportion of terrigenous OC. Sediments from station S4 had positive redox potential and there were no indications of anaerobic metabolism end-products accumulation in the pore water, suggesting low impact.

The influence of sewage effluent on sedimentary features, as evidenced by carbon isotopes signature, was clearly detectable at 750 m (S3) and 1800 m (S4)

far from the outfall (∼ 45% and ∼ 20% contribution of terrestrial organic carbon, respectively). These results suggest that the analysis of sediments reveals a much more spatially extended impact of the outfall in the study area than previously evaluated, at least by a factor of 3. In fact, according to García (2013), the maximum extension of an area affected by SMSO discharge is 600 m, if the concentration of fecal coliforms in the water column is considered. Terrigenous OC at S4 may possibly reflect a high background value in Taganga Bay, due to the influence and/or mixing of wastewater outfall with other anthropogenic sources such as riverine inputs, surface runoff and harbor activities due to currents and transport process.

The significant interactions between the effects produced by the submarine outfall and the upwelling might be partly due to the overlapping of the UPW and the high touristic season, when the organic and nutrient loads generated by the town of Santa Marta peak. It can be expected that the further increase of the loads generated by the outfall may act in synergy with the effects of the upwelling on particulate matter, nutrient and oligoelements mobilization. The two-end-member mixing model we employed supports this statement as it suggests during the UPW season an increase of terrestrial OC at S1 and S2 and an increase of marine OC at S3 and S4. Our interpretation for what observed close to the outfall is that the increase of the sewage loads generated during the highest tourist season of Santa Marta city (December–January) produces a much large, local effect than that generated by the UPW. On the contrary, the marine OC increment calculated for the outer stations is the result of the dominant net effect of the UPW-associated particulate transport.

The isotopic signature of N in sediments suggests multiple co-occurring processes

The nonlinear variation of δ^{15}N along the analyzed transect likely reflects the complexity of the biogeochemical N-cycle (Bedard-Haughn et al. 2003; Meyers 1997). Such complexity might be exacerbated in impacted ecosystems, where the level of fractionation can be affected by short-term temporal or spatial variability of dominant microbial N transformations (Bedard-Haughn et al. 2003). The average δ^{15}N values at S1 and S2 were similar or more enriched than values reported in other impacted areas in Portugal, Canada and China (2.1 to 5.2‰, Sampaio et al. 2010; Burd et al. 2013; Li et al. 2016). δ^{15}N-enriched values were also observed at S3, where total denitrification rates were nearly 3–4 times higher than at the other stations. This is in agreement with the preferential consumption of ^{14}N by denitrifiers (Mariotti et al. 1981; Bedard-Haughn et al. 2003; Alkhatib et al. 2012; Rooze and Meile 2016). Slightly depleted δ^{15}N values at S4 could be due to N_2 fixation rates (Bedard-Haughn et al. 2003).

What emerges from our seasonal results is that processes as denitrification, leading to higher δ^{15}N signatures, and N_2-fixation, leading to lower δ^{15}N signatures, may vary over relatively short time and spatial scales. Net N_2 fluxes in fact were mostly negative during the NUPW season, when the benthic system need to import reactive nitrogen for microbial growth, to sustain heterotrophic processes in organic-enriched sediments. During the UPW season, only a few months later, nitrate concentrations in the water column nearly doubled (from 1.10 to 1.89 μM) and organic inputs from terrestrial and marine sources increased. Under these circumstances the net fluxes of N_2 were reversed in 3 out of 4 stations, suggesting that denitrification exceeded-fixation.

The stoichiometry of organic C and N

The sedimentary ratios of TOC and TN have been widely used as proxies to elucidate the source and fate of organic matter in marine sediments (Meyers 1997; Li et al. 2016; Wang et al. 2018). Generally, TOC:TN ratios higher than 15 characterize terrigenous material (Meyers 1997; Wang et al. 2018) whereas values close to 6–8 are generally associated to fresh phytoplankton detritus (e.g. from upwelling waters, as determined in

our study area by Bayraktarov and Wild 2014). Ratios at S1, S2 and S3 indicate a mixture of terrestrial and marine sources, whereas at S4 the high TOC:TN ratio is unexpected. S4 is far from the outfall and has the lowest relative proportion of terrigenous organic carbon; its high TOC:TN ratio can be explained by settling of micro- and macroalgae growing on reef substrate due to anthropogenic nutrient input and upwelling, as reported by Vega-Sequeda et al. (2008) at Punta Venado, near station S4. High TOC:TN ratios in algal organic matter can be due to N-limitation, supported by negative N_2 fluxes, enhanced synthesis of lipid-rich organic matter or enhanced preservation of carbon-rich components and degradation of nitrogen-rich components (Meyers 1997). Similarly to δ^{15}N, TOC:TN ratios have limitations as tracers of organic pollution and do not allow to discriminate organic matter sources to marine sediments due to the complexity of N-cycle biogeochemical processes (Bedard-Haughn et al. 2003; Meyers 1997).

Metabolic rates

Oxygen and nutrient fluxes

Regardless the origin of the organic matter to sediments and despite marked decrease of bottom water temperatures during the UPW season, the OC input from the marine side stimulated or maintained elevated oxygen uptake in all stations. Bayraktarov and Wild (2014) found that from 75 to 79% of the annual organic matter input to the sediments of Santa Marta coastal area was supplied during UPW, with higher inputs at exposed than at sheltered sites. They also reported higher oxygen uptake at sheltered sites due to lower water currents promoting particles sedimentation, which is consistent with oxygen uptake rates at S4 that were 26 and 23% higher than at S3 in the NUPW and UPW seasons, respectively.

Sedimentary oxygen uptake peaked at S1 with rates comparable or even higher than those reported for estuaries and marine ecosystems that receive high loadings of organic matter (Table 5). At station S1 rates of oxygen and nitrate consumption were nearly 4 and 12 times higher than those measured far from the outfall, respectively. As sediment sieving revealed the absence of significant contribution of macrofauna to benthic processes, we speculate that most oxygen consumption was microbial or chemical and that most

Table 5 Fluxes of O$_2$ and nutrients and rates of denitrification, N$_2$ Fixation and DNRA from different estuarine zones (μmol m^{-2} h^{-1})

Site	Zone	O$_2$	NO$_3^-$	NH$_4^+$	DIP	SiO$_2$	DIN	Dt	N-fixation	DNRA	Method[e]	References
Boston Harbor, Massachusetts[a]	Temperate	-291.67 to -6250	–	–	-16.67 to 325.00	–	-1.25 to 883.33	167	–	–	Stoichiometric	Giblin et al. (1997)
Whites Point Los Angeles County sewage outfall[b]	Temperate	-875.00 to -958.33	-20.83 to -41.67[c]	87.50–137.50	11.25–42.92	75.00–166.67	–	–	–	–	–	Berelson et al. (2002)
Laguna Madre and Baffin Bay, Texas	Sub-tropical	–	3.8 ± 3.0	110 ± 17	–	–	–	32 ± 5	58 ± 5	41 ± 13	ICCF/MIMS N2:Ar ^{15}NO$_3^-$ addition	Gardner et al. (2006)
Sabine Lake, Texas	Sub-tropical	–	1.29 ± 5.07	34.7 ± 23.1	–	–	–	30.1 ± 10.1	49.7 ± 14.4	0.91 ± 0.81		
East Matagorda Bay, Texas	Sub-tropical	–	11.1 ± 3.2	23.2 ± 4.8	–	–	–	22.5 ± 4.4	2.8 ± 2.1	3.6 ± 0.6		
Nueces Estuary, Texas	Sub-tropical	–	0.7 ± 1.1	38.7 ± 16.8	–	–	–	40.0 ± 6.6	19.1 ± 7.4	10.3 ± 4.0		
Florida Bay, USA	Sub-tropical	–	-2.0 to 2.0	7–540	0.1–13	–	–	0–350	–	10–240	MIMS	Gardner and McCarthy (2009)
Mae Klong, Thailand	Tropical	-3800 ± 300	–	–	–	–	–	0–7.4	–	0.3–22.8	Acetylene Block	Dong et al. (2011)
Cisadane, Indonesia	Tropical	-5800 ± 400	–	–	–	–	–	0–103	–	0–1137		
Vunidawa-Rewa, Fiji	Tropical	-3900 ± 400	–	–	–	–	–	0–2.6	–	0.2–10.2		
Moreton Bay, Australia	Sub-tropical	-257 ± 32 to -988 ± 35	0–35	-10 to 60	–	–	–	6.5 ± 2–32 ± 5	40–67	–	MIMS N2:Ar	Ferguson and Eyre (2012)
Little Lagoon, Alabama, USA	Sub-tropical	-342 to -439	-93 to -121	45–110	-1.0 to 1.0	–	–	13–55	1.6–2.6	1–168	MIMS	Bernard et al. (2015)
Golfo de Mexico, hypoxic zone	Sub-tropical	-408 to -1800	-26.59 ± 11.49	77.4 ± 14.6	3.29 ± 1.66	–	75.81 ± 16.13	2–492	0–147 ± 39	1.12 ± 0.44–31.4 ± 10.8	ICCF/MIMS N2:Ar, ^{15}NO$_3^-$ addition	McCarthy et al. (2015)
Waquoit Bay, Massachusetts	Temperate	-997 ± 97[d]	ND	540 ± 51[d]	34 ± 8[d]	–	–	0–28 ± 14	49 ± 8–103 ± 4	5.7 ± 4.8	ICCF /MIMS N2:Ar ^{15}NO$_3^-$ and ^{30}N$_2$ additions	Newell et al. (2016)

Table 5 continued

Site	Zone	O_2	NO_3^-	NH_4^+	DIP	SiO_2	DIN	Dt	N-fixation	DNRA	Method[e]	References
Taganga Bay—Caribbean Colombia	Tropical	−1018.4 to 6311.0	−21.36 to 2.83	−3.36 to 13.43	−0.56 to 0.17	−3.19 to 7.04	−12.3 to 14.8	1.27–14.87		1.00–5.40	ICSI/MIMS N_2:Ar, $^{15}NO_3^-$ addition	This study

[a]Nutrient fluxes values at the site where sewage sludge was directly pumped before implementing wastewater treatment in Boston Harbor, Massachusetts. The average annual oxygen consumption was 5541.67 μmol O_2 m^{-2} h^{-1}

[b]Nutrient fluxes were measured by free-vehicle benthic chamber devices (landers). The wastewater discharge is located at 60 m. Receiving medium temperature between 11 and 14 °C

[c]Nitrate flux

[d]Incubation 5 h

[e]ICCF intact core continuous-flow incubations, ICSI intact core static incubations, MIMS membrane inlet mass spectrometry

small scale variability was due to the distribution of *Beggiatoa* colonies, patchily colonizing surface sediments at S1 and S2.

Inorganic nutrient fluxes (ammonium, reactive Si and P) were generally low when compared to fluxes measured in different impacted coastal areas (Table 5) and did not show a clear spatial gradient as it was observed for sedimentary features, oxygen uptake rate and nitrate fluxes. The absence of a consistent trend of inorganic nutrient regeneration between stations proximal to and distant from the outfall, suggests mechanisms that are uncoupled to oxygen uptake and not redox-dependent. Alternatively, decomposition might be incomplete and lead to the release of dissolved organic forms of N and P, which were not measured in the present study. Limited efflux of inorganic N, P and Si might be a consequence of poor bioturbation by macrofauna in the Taganga Bay sediments, in turn due to organic-impacted and sulphidic sediments. Measured inorganic N, Si and P regeneration might be low as compared to expected rates (i.e. from oxygen uptake and sedimentary C:N or C:P ratios) due to the refractory macromolecular quality of the terrestrial organic carbon and to inefficient anaerobic mineralization rates. The presence of *Beggiatoa* mats at S1 and S2 and the absence of measurable Fe^{2+}, Mn^{2+} and CH_4 fluxes suggests that most of the organic matter is mineralized via suphate reduction and that large fraction of the oxygen uptake is due to reoxidation processes.

As the sewage outfall has an installed capacity of 2.5 m^3 s^{-1} (García 2013) the loads of organic matter and nutrients from the outfall are expected to increase. Under this scenario, sedimentary features and fluxes should be continuously monitored as saturation of processes retaining nutrients or replacement of denitrification by DNRA may turn sediments permanent sources of N and P to the water column. We do not think that present analytical techniques allow to trace significant increase of nutrient concentration due to sediment regeneration in the water mass, as the dilution is very large. Attempts, made in the proximities of floating fish cages, revealed no differences in nutrient concentrations along increasing distances from the cages. However, bioassays based on phytoplankton revealed much higher primary production (eutrophication, sensu Nixon 1995) in the proximity of cages. This ultimately means that sediments provide much evident and measurable impact of the outfall

than the water column dissolved nutrients, that sedimentary nutrient fluxes need to me monitored and that phytoplankton may respond to very small increase of water column nutrients (Dalsgaard and Krause-Jensen 2006).

The recent increase of phytoplankton blooms in the Santa Marta coastal area, including potentially toxigenic microalgae, such as *Pseudo-nitzschia, Anabaenopsis, Gonyaulax, Gyrodinium, Gymnodinium, Prorocentrum, Scripsiella* y *Cochlodinium* represents a warning signal about the effects of anthropogenic nutrient inputs to this system (Garcés-Ordóñez et al. 2016). Such blooms suggest subtle changes in water (and sediment) chemistry that likely affect nutrient concentrations and stoichiometry and that should be carefully considered.

Microbial N transformations

Discrepancies between measurements based on N_2/Ar and IPT have been widely discussed; N_2/Ar might overestimate true rates of denitrification due to reactions between O_2 and N_2 in the mass spectrometer ion source. As during dark incubations O_2 decreases, such reactions may lead to an apparent increase of N_2 (Eyre et al. 2002, 2013; Lunstrum and Aoki 2016). As the MIMS employed in our study was equipped with a copper reduction column maintained at 600 °C we think that most oxygen interferences were removed. Also the IPT presents some limitations depending on site-specific sediment features. At S1 and S2 for example visible mats of *Beggiatoa* might have affected the results of our denitrification measurements (Song et al. 2013). *Beggiatoa* have large intracellular pools of nitrate which prevent correct evaluation of $^{15}NO_3^-$ to $^{14}NO_3^-$ ratio in the denitrification zone, possibly leading to a slight underestimation of the nitrate reduction rates. We acknowledge that future application of the IPT in the Taganga Bay sediments with *Beggiatoa* will require to determine the vertical distribution and intracellular NO_3^- content of these microbes, together with additional experiments specifically targeting rates in individual cells (see the review by Robertson et al. (2019) on how to tackle IPT limitations).

Results from N_2/Ar ratio measurements suggest a seasonal shift between the dominance of N-fixation and denitrification in 3 out of 4 sites, that may be related to increased availability of water column

nitrate or ammonium from mineralization processes during the UPW season. A similar dynamic, regulated by large seasonal availability of reactive N, is reported by Newell et al. (2016) in the Waquoit Bay and by Zilius et al. (2018) in a coastal lagoon.

Results from the IPT suggest the co-occurrence of DNRA and denitrification in sediments, as also reported elsewhere (Burgin and Hamilton 2007; Gardner and McCarthy 2009; Newell et al. 2016; Bonaglia et al. 2014). The seasonal increase of water column nitrate was addressed to the upwelling (Arévalo-Martínez and Franco-Herrera 2008; Ramírez-Barón et al. 2010) and not to the outfall, due the huge dilution factor. Such increase was likely responsible for higher nitrate demand by organic sediments via DNRA or it may have favored the growth of primary producers competing with bacteria and potentially impacting corals (Carstensen et al. 2000; Diaz-Pulido and Garzón-Ferreira 2002; Dalsgaard 2003; Franco-Herrera et al. 2006). In Taganga Bay DNRA increased from the NUPW to UPW season in all stations.

DNRA increases with salinity and eutrophication likely due to higher sulphate reduction rates and sulphide accumulation, as shown in the present study and by others (Gardner et al. 2006; Dong et al. 2011; Bernard et al. 2015). The predominance of DNRA over denitrification in tropical estuaries may be due to both an energetic advantage of nitrate ammonifiers over denitrifiers when competing for limited nitrate, and to higher affinity for nitrate by the nitrate ammonifiers at tropical temperatures permanently > 24 °C. These explanations can be realistic for our case study, with rates of DNRA having similar patterns as those of oxygen demand and sulphide concentration in pore water.

Station S3 displayed the highest rates of denitrification in the two campaigns, likely due to moderate carbon enrichment, lower microbial oxygen consumption, absence of free sulphides in porewater and a molar ratio TOC:TN similar to Redfield ratio (8 ± 3). Ammonium availability and oxygen penetration likely drove nitrification in surface sediments, leading to high rates of coupled denitrification. At S4 denitrification rates were low likely due to the scarce availability and high competition for reactive nitrogen, resulting in net import and minimum loss.

Expected increase of organic load to the Taganga Bay sediments from the outfall and during the

UPW/touristic season will turn the benthic system more and more sulphidic. The suppression of denitrification and the increase of DNRA may ultimately lead to lower net N losses, higher NH_4^+ mobility and stimulation of algal growth, as reported in Bernard et al. (2015). Results from this study support the evidence of strong and complex relationships between variations in oceanographic conditions and biological process in the near coastal zone and of positive feedback between upwelling and impacts associated to the outfall and the touristic season (Paramo et al. 2011).

Conclusions

Sediments seem to track past and ongoing impacts from a submarine outfall better than the water column. Taganga Bay sediments allow to trace the organic pollution from a sewage outfall and reveal that the spatial extent of the impact is much larger, by a factor of 3, than what hypothesized from water column analysis of fecal coliforms. Seasonal analysis of sediment features and metabolic rates suggest also an interaction between the effects of submarine outfall and those of upwelling, due to additional nutrient and organic matter inputs from the open sea to the coastal area. The cascading effects of organic inputs, increasing respiration, sulphide build up, suppression of denitrification and increase of ammonification may dramatically impact the macrofauna community, leading to species loss over increasing areas, and stimulate the growth of algae, with negative implications for corals, water transparency and tourism. This is a real concern, as the loads released by the outfall are expected to increase. Tropical areas as Taganga Bay seem intrinsically more vulnerable to these impacts than temperate areas due to the different temperature regimes, which may easily uncouple microbial respiration and oxygen availability.

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