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Sediment Denitrification and Nutrient Fluxes in the San José Lagoon, a Tropical Lagoon in the Highly Urbanized San Juan Bay Estuary, Puerto Rico

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Abstract Direct measurements of net N_2 production, oxygen, and inorganic nutrient fluxes at the sediment–water interface were conducted in the San José Lagoon (SJL), a tropical coastal lagoon located at the San Juan Bay Estuary, Puerto Rico, that is heavily impacted by anthropogenic activities. Sediment of the SJL had high organic matter (average=16.7 %; SD=9.0), pore water ammonium (541 μM±422), and pore water soluble reactive phosphorus (SRP) (57 μM±48) concentrations. High fluxes of oxygen (−1999 μmol m^{-2} h^{-1}±2180) and N_2–N (192 μmol m^{-2} h^{-1}±315) fluxes were observed. Similarly, high fluxes of ammonium (770 μmol m^{-2} h^{-1}±701) and SRP fluxes (167 μmol m^{-2} h^{-1}±138) were measured, with moderate nitrate fluxes (−40 μmol m^{-2} h^{-1}±53) mostly directed into the sediment. The dissolved inorganic nitrogen and soluble reactive phosphorus ratio (DIN/SRP) was low relative to the Redfield ratio and suggested that the sediment would support SJL as a nitrogen-limited system. An experiment with higher nitrate concentration resulted in a significant response ($p<0.05$) of denitrifying microbes and ammonium fluxes to a 100-μM KNO_3 addition. Net N_2 production was an important sink in the nitrogen balance of the SJL, though most remineralized N was released as ammonium. Efforts to mitigate eutrophication in the SJL require consideration of the substantial role that sediments play in biogeochemical cycles.

Keywords Sediment N_2 production · Nutrient cycling · Tropical urban estuary

Introduction

In recent decades, coastal tropical ecosystems have been impacted by human activities, showing signs of eutrophication due to high nutrient loads from agriculture (Downing et al. 1999; Devlin and Brodie 2005; Borbor-Cordova et al. 2006; Atapattu and Kodituwakku 2009), aquaculture ponds (Heilskov and Holmer 2001; Prasad and Ramanathan 2008; Nguyen et al. 2012; FAO 2012; Molnar et al. 2013; Herbeck et al. 2011, 2013), and urbanization (UNEP 1999; Gabric and Bell 1993; Vandeweerd et al. 1997; Corredor et al. 1999; Fang 2000; Martinelli et al. 2006; Pagliosa et al. 2005; Dai et al. 2006; Nixon et al. 2007; Larsen and Webb 2009). In contrast to temperate systems, tropical aquatic ecosystems have high solar radiation resulting in limited seasonal temperature ranges; in shallow areas, mangrove forest, seagrass bed, and coral reef habitats are characterized by high productivity, rich biodiversity, and valuable ecosystem services (Nagelkerken 2009). The tropics encompass about 40 % of the land surface

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of the Earth and 50 % of world population (Schultz 2005; Nagelkerken 2009).

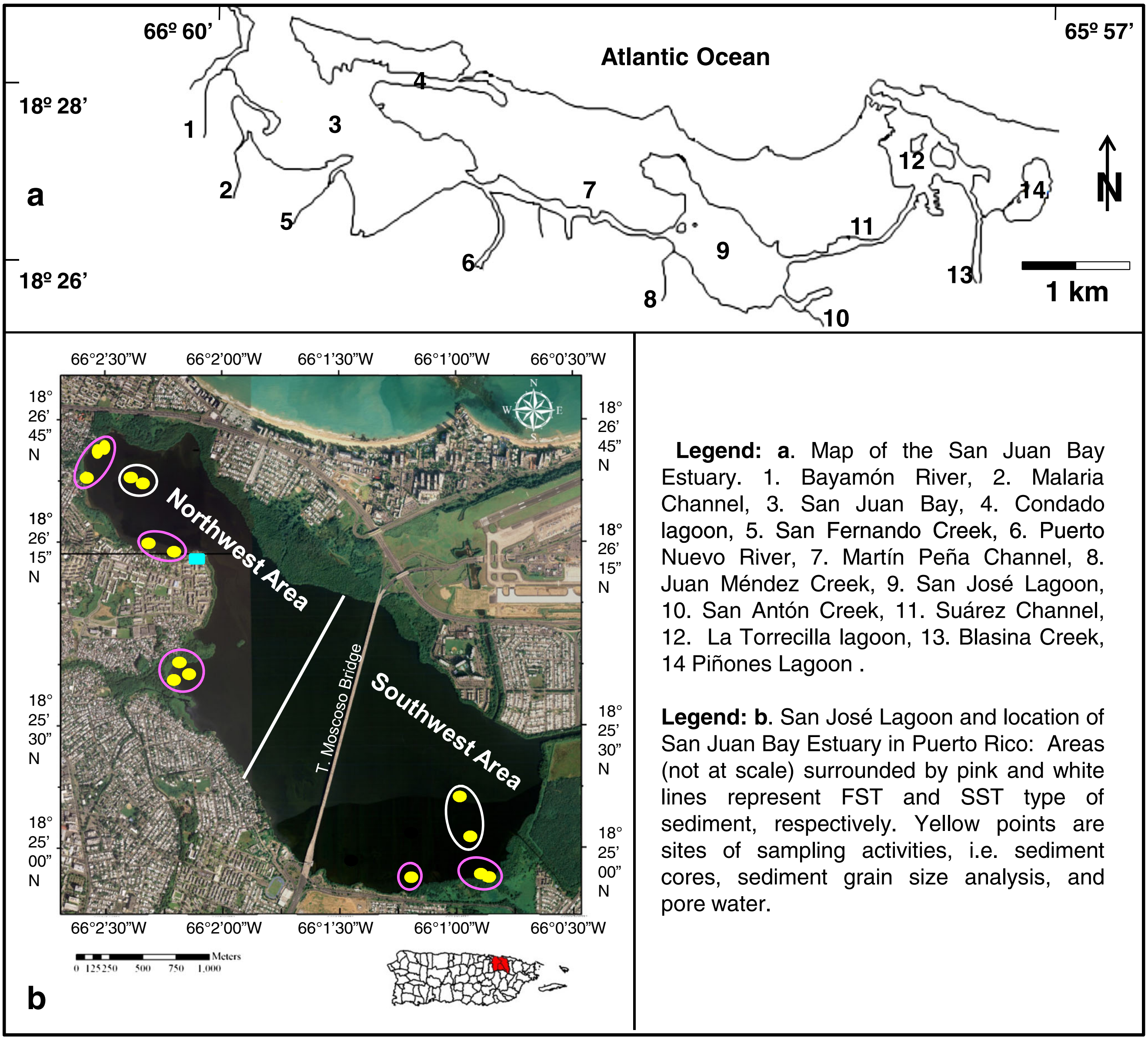
Investigations in nutrient-impacted subtropical and temperate coastal waters show that sediments are important sites for nutrient cycling (Dunn et al. 2012; Zimmerman and Benner 1994; Trimmer et al. 1998; Teixeira et al. 2010; Rowe et al. 1975), driven by microbial transformations of autochthonous and allochthonous organic matter. Sediment biogeochemical processes are strongly affected by proximal physicochemical parameters such as temperature, oxygen status, salinity, and pH (Boynton and Kemp 1985; Seitzinger 1991; Gardner et al. 1991; Gao et al. 2012), with larger forcing functions such as climate, tidal range, freshwater inputs, turbidity, and water residence time (Seitzinger 1987; Nixon et al. 1996; Cowan and Boynton 1996; Nedwell and Trimmer 1996; Dettmann 2001; Tobias et al. 2003; Kemp et al. 2005; McGlathery et al. 2007). All these factors influence the role of sediments as traps, sources, sinks, buffers, and filters for nutrients (Nowicki and Oviatt 1990). Although there have been advances in the study of nutrient dynamics in tropical coastal sediments (Corredor and Morell 1989; Morell and Corredor 1993; Mosquera et al. 1998; Cerco et al. 2003; Dong et al. 2011;Nguyen et al. 2012), overall the responses of coastal tropical ecosystem to elevated nutrient loads have been poorly documented (Downing et al. 1999; Galloway et al. 2008). Such nutrient loading is a particular concern because of the oligotrophic nature of tropical coastal ecosystems such as coral reefs (Larsen and Webb 2009) and the lack of depurative mechanisms for these nutrients, suggesting that tropical systems may be more susceptible to eutrophication than their temperate counterparts (e.g., Nixon et al. 1996; Corredor et al. 1999). For instance, Morell and Corredor (1993) and Mosquera et al. (1998) observed a limited removal of the mineralized fraction of the organic matter through denitrification, i.e., the reduction of nitrate to N_2O or N_2 under anoxic conditions (Tiedje 1994; Zunft 1997). These authors suggested that denitrification is severely limited by the low oxygen saturation levels in water columns due to high temperature and the inhibitory effect of sulfide on nitrification. Other researchers, working in Asia and Australia, have also reported that denitrification was not the main mechanism of available nitrogen attenuation due to the high temperature, relatively low water column nitrate concentrations, and oxygen deficits in the sediments (Dong et al. 2011; Dunn et al. 2012). In contrast, dissimilatory nitrate reduction to ammonium (DNRA; reviewed by Giblin et al. 2013) has the potential to be a dominant nitrogen cycling pathway in both tropical (Morell and Corredor 1993; Dong et al. 2011) and subtropical sediments (An and Gardner 2002; Gardner and McCarthy 2009). The environmental conditions favoring DRNA over denitrification in tropical sediments include high rates of organic matter decomposition and high sulfide concentrations (Joye and Hollibaugh 1995; An and Gardner 2002; Gardner et al. 2006; Dong et al. 2011). If DRNA is a dominant nitrate pathway in tropical sediments, increased nitrogen loads in coastal tropical ecosystems can enhance nitrogen recycling and primary production (Gardner et al. 2006; Giblin et al. 2013), reinforcing eutrophication processes.

The San José Lagoon in Puerto Rico represents an extremely eutrophic urban end member that is under-represented in the tropical literature. This ecosystem is useful to contrast the transformations and fate of the large nutrients inputs to sediment nutrient cycling assessments in coastal waters of both tropical and temperate regions. We hypothesize that high rates of terrestrial loading of organic matter and nitrate, in combination with high sediment temperatures, will result in high rates of sediment nitrogen turnover, with sediment redox conditions influencing the interplay between denitrification and DNRA. Specifically, the objectives of this investigation were to (1) quantify fluxes of nutrients (DIN, SRP) and gases (O_2, N_2) from the sediment cores taken from the San José Lagoon and determine the role of the denitrification in a nitrogen balance of a coastal tropical lagoon influenced by intensive human activities and (2) determine the efficiency of denitrifying bacteria in reducing added nitrate to N_2 in the sediments cores of the San José Lagoon. Experimental addition of nitrate will help indentify controls of denitrification and competing processes such as DNRA.

Materials and Methods

The SJBE basin lies on the subtidal area of the flat coastal plain located within an alluvium valley. It comprises four lagoons (Condado, San José, La Torrecilla, and Piñones) and the San Juan Bay (Fig. 1). In their pristine conditions, these water bodies were predominantly surrounded by mangrove wetlands (Seguinot-Barbosa 1983; Ellis and Gómez-Gómez 1976). Historically, the SJBE has had the highest density of inhabitants and major industrial activities in Puerto Rico (Juncos et al. 2001). In 2000, the population living in the SJBE basin was about 622,000 habitants, and 67 % of the basin area was urban (Juncos et al. 2001; SJBE-IMCP 2001). The SJBE receives freshwater from Bayamon and Puerto Nuevo rivers and the creeks Juan Méndez, San Antón, Blasina, among other unnamed and small creeks. For decades, as a result of the unplanned urbanization and deficiencies in the fluvial and wastewater treatment systems, these streams and lagoons have been impacted by the direct discharges of domestic and industrial wastewaters from the combined-sewer overflows, faulty sewers lines, and point and nonpoint sources of pollution (Gómez-Gómez et al. 1983; Webb and Gómez-Gómez 1998; Lugo et al. 2011).

The SJL is the largest lagoon in the SJBE; its surface area and average water depth are 4.6×10^6 m^2 and 1.5 m, respectively (Bunch et al. 2001; Cerco et al. 2003). The SJL is a



Legend: **a**. Map of the San Juan Bay Estuary. 1. Bayamón River, 2. Malaria Channel, 3. San Juan Bay, 4. Condado lagoon, 5. San Fernando Creek, 6. Puerto Nuevo River, 7. Martín Peña Channel, 8. Juan Méndez Creek, 9. San José Lagoon, 10. San Antón Creek, 11. Suárez Channel, 12. La Torrecilla lagoon, 13. Blasina Creek, 14 Piñones Lagoon .

Legend: **b**. San José Lagoon and location of San Juan Bay Estuary in Puerto Rico: Areas (not at scale) surrounded by pink and white lines represent FST and SST type of sediment, respectively. Yellow points are sites of sampling activities, i.e. sediment cores, sediment grain size analysis, and pore water.

Fig. 1 Map of the San Juan Bay Estuary and location of the sampling sites in SJL. The *blue rectangle* shows where sediment incubations were performed

relatively isolated lagoon connected to the Atlantic Ocean through Canal Suárez, a narrow, ~3-km-long channel. Tidal oscillation at SJL is ~5 cm (Bunch et al. 2001). The SJL receives freshwater from the largest creeks Juan Méndez and San Antón and through several small tributaries (Gómez-Gómez et al. 1983; Bunch et al. 2001). The land cover over the catchment area of the Juan Méndez and San Ant ón creeks is mostly urban, reaching up to 73 % in Juan Méndez and 69 % in San Antón. These tributaries also receive a great amount of untreated domestic wastewater, which is exported to the SJL. It is calculated that nutrient yields exported by Juan Méndez Creek to SJL are as high as 326, 0.2, and 66 kg km^2 year^{-1} of NH_4^+–N, NO_3^-–N, and SRP, respectively. Similarly, San Antón Creek contributes with high nutrient loads to SJL, i.e., 204, 0.2, and 35 of NH_4^+–N, NO_3^-–N, and SRP to the SJL, respectively. An important point source of pollution for the SJL is the Baldorioty de Castro Pump Station. This stormwater station discharges into SJL stormwater generated from a sector of the metropolitan area in San Juan. It is estimated that 2.8×10^4 of NH_4^+–N and 4.5×10^3 kg year^{-1}of NO_3^-–N, mostly coming from domestic wastewater through the deficient septic systems around this storm water pump, are discharged into the SJL.

Field Sampling Activities

All sampling activities were conducted along a northeast to southeast transect in SJL (Fig. 1). Incubation experiments, comprising 15 individual undisturbed, permanently submerged sediment cores (ambient cores), were conducted on four dates in 2010 (January 26–27, October 24, and November 06) and two dates in 2011 (March 05, 21). All 15 ambient cores represented replicates of one site, namely, SJL. Each time, a total of four cores were collected representing two ambient cores with shell sediment type (SST) and fine-grained sediment type (FST) each from both NWA and SWA areas, thus obtaining the same number of ambient cores from each sampling areas. Each of these 15 ambient cores was sampled under dark and light within 24 h of collection and after a week of conditioning, thus representing four measures per core. A total of five ambient cores were discarded due to the malfunction of field sampling equipment. Physicochemical parameters were measured in situ using a Hydrolab DS5 multiparameter water quality sonde. Water samples were also collected for analysis of ammonium, nitrate, and soluble reactive phosphorus (SRP).

Sediment–Water Exchange Measurements

Sediments were collected mid-day by hand insertion of PVC incubation cores (length, 30 cm; diameter, 11.4 cm). Special care was taken to avoid the loss of the overlying water and sediment resuspension during the sampling procedure. Immediately after collection, ambient cores were sealed with black rubber caps secured with adjustable hose clamps and delivered to the “Los Laguneros” Fishermen’s Association facilities, which was used as the incubation facilities in this study (Fig. 1). In these facilities, the ambient cores were left uncapped into the incubator below the water surface and bathed with water pumped from the SJL overnight, with air pumps used to equilibrate oxygen concentration at near saturation conditions. The next morning, cores were sealed with a translucent cap with a suspended Teflon-coated stirring magnet, taking care to eliminate any bubbles (Kana et al. 1998). The magnetic stir bars were driven by an external motor-driven magnetic turntable to mix the water column without resuspension. The replacement water required during the incubations was supplied by gravity flow from a carboy containing water from the SJL. Replacement water was gravity-fed from a reservoir through a port in the top; the carboy valve was opened to allow water flows into the cores and water samples were collected.

In this study, all nutrients and gases fluxes were measured using the dark/light paired method, thus simulating both illumination under both day and night conditions. Accordingly, the ambient cores were sampled sequentially under dark and light conditions for net N_2 production, argon, oxygen, NH_4^+, NO_2^-+NO_3^- (in this study referred as NO_3^-), and SRP, with four time points under each illumination condition over ~4–5 h. After the dark and light samplings, all the cores were reopened; the air pumps turned on, and the ambient cores were maintained in the dark. After 1 week of incubation, a new round of sampling was conducted as in the previous week. Water samples for net N_2 production, argon, and oxygen were collected in 12-ml Exetainer vials (Labco Company), preserved with mercuric chloride and immediately placed under water in the refrigerator until analysis. Water samples for NH_4^+, NO_2^-+NO_3^-, and SRP were filtered through a combusted 47-mm diameter glass fiber of 0.7 pore size (Whatman grade GF/F glass microfiber filter, Whatman International Ltd) filter, collected in 60-ml high-density polyethylene (HDPE) bottles frozen until analysis. Nutrient concentrations (NH_4^+ and NO_3^-) in water samples were analyzed at the Horn Point Laboratory (University of Maryland Center for Environmental Science) using standard methods. SRP was analyzed using the ascorbic acid method following Parsons et al. (1984). Dissolved gases (O_2, N_2, and Ar) were analyzed at the Horn Point Laboratory using membrane inlet mass spectrometry (MIMS; Kana et al. 1994). The MIMS method uses the N_2/Ar ratio to determine the net denitrification, meaning that the gross autotrophic N_2 production from nitrogen fixation and anammox processes are subtracted to the gross heterotrophic denitrification.

Experiment with Higher Nitrate Concentration

To investigate the effect of higher nitrate concentration on sediment net N_2 production fluxes at SJL, an experiment was conducted on March 20–28, 2011. A total of four ambient cores (herein control cores), two from the NWA and two from the SWA (with SST and FST each), were collected in the SJL, and an initial denitrification experiment was conducted as described previously. Upon termination of the initial flux experiment, SJL water overlying the four cores was amended with KNO_3 with an increase in KNO_3^- of 100 μM. The amended cores and water were placed into a larger incubator used to maintain in situ temperature of SJL without diluting the added KNO_3^-. Cores were preincubated overnight with bubbling and sediment–water exchange of solutes and gases carried out as above.

Pore Water Collection

A 2.5-cm internal diameter plastic core tube was used to collect the upper 5 cm of surficial sediment for pore water extraction in a Hermle Z323K centrifuge operating for 5 min at 3790 G. Water samples for nutrients and pore water analysis were filtered through a combusted 0.7-μm glass fiber filter (Whatman 47 mm diameter), collected in 60-ml HDPE bottles and kept in the refrigerator until analysis. Nutrient concentration in water samples were analyzed as previously described.

Solid Phase Analysis

After collection of the 0–5-cm sediment horizon in a 4-cm diameter tube, the sediment grain size distribution was determined by wet sieving following Sheldrick and Wang (2008). Samples were suspended in deionized water, followed by wet sieving into six grain sizes: very coarse sand (2.36–1.00 mm), coarse sand (1.00–0.50 mm), median sand (0.5–0.25 mm), fine sand (0.25 mm–125 μm), very fine sand (63–45 μm), and silt and clay (<45 μm). Sediment organic matter content was measured as loss on ignition (Heiri et al. 2001).

Calculations and Statistics

Sediment nutrient fluxes were calculated from the slope of the gas and solute concentrations over time:

$$F = \frac{\Delta C}{\Delta t} \times \frac{V}{A}$$

where F is the flux (μmol m^{-2} h^{-1}), $\frac{\Delta C}{\Delta t}$ is the slope of the concentration change over time in the overlying water (μmol L^{-1} h^{-1}), V is the volume of the overlying water (L), and A is the area of the incubated cores (m^{-2}). Each sediment core was sampled seven times: three in the dark, one at the dark/light transition, and three in the light. Two regressions were performed on each time series, with separate light and dark incubation regressions with four time points each. A total of 90 % of the regression analyses had R^2 regression coefficients>0.70. All regression slopes were corrected for water column-only gas and nutrient changes.

Net Daily Oxygen Flux Estimation

The net daily oxygen flux was the sum of direct community respiration, reoxidation of reduced species, and benthic microalgal photosynthesis. Sediment oxygen fluxes measured in dark and light incubations were extrapolated to 24 h using the light/dark period lengths for the day of the year. The net daily oxygen flux was calculated with the equation suggested by Thorbergsdóttir et al. (2004), as follows:

$$J_{\mathrm{T}} = J_{\mathrm{D}} L + J_{\mathrm{N}}(24\text{–}L)$$

where J_{T} (mmol O$_2$m^{-2} day^{-1}) is the net daily oxygen flux, J_{D} is the calculated oxygen fluxes in light sediment cores (mmol O$_2$m^{-2} h^{-1}), J_{N} is the calculated oxygen fluxes in dark sediment cores (mmol O$_2$m^{-2} h^{-1}), and L refers to the hours of the daylight. Sediment denitrification efficiency (DE) was calculated as a fraction of the ΣN fluxes lost through denitrification during the decomposition of the organic matter follows:

$$\mathrm{DE} = \mathrm{N_2–N}/\left[\mathrm{N_2–N} + \mathrm{NH_4^+} + \mathrm{NO_3^-}\right]$$

The flushing rate was calculated according to Geyer et al. (2000) as follows:

$$T_{\mathrm{f}} = V_{\mathrm{SJL}}/Q_{\mathrm{ebb\ \ tide(1\ \ day)}}$$

where V_{SJL} represents the volume of the SJL estimated as the product of the lagoon surface area and its average depth (Bunch et al. 2001), and Q is the average net volume of water in dry and wet season during the ebb tide flowing from SJL to the La Torrecila Lagoon (Gómez-Gómez et al. 1983; Webb et al. 1998). Accordingly, the residence time calculated was:

$$T_{\mathrm{f}} = 6.9 \times 10^6\ \ \mathrm{m^3}/127{,}000\ \ \mathrm{m^3\ \ day^{-1}} = 54\ \ \mathrm{days}$$

Nitrogen Inventory Calculations

The SJL nitrogen inventory (Fig. 2) presented in this study averaged nitrogen loading data from tributaries and the BCPS and the nitrogen fluxes at the water–sediment interface reported by Cerco et al. (2003), and the nitrogen fluxes ($\mathrm{N_2}$–N, $\mathrm{NH_4^+}$, $\mathrm{NO_3^-}$) at the water–sediment interface presented in this study, scaled up to kilograms of N per day.

To investigate the differences of oxygen, ammonium, nitrate, SRP fluxes, and net $\mathrm{N_2}$ production between light and dark incubations, paired t tests were performed. A simple linear regression, Pearson, and Spearman correlations analysis were performed on $\mathrm{NH_4^+}$ versus DIN flux relationships and nitrification versus net $\mathrm{N_2}$ production. A Kruskal–Wallis test was performed to test the relationship between total net DIN and net oxygen fluxes in sediment cores. To explore the response of sediment cores to higher nitrate on oxygen, ammonium, and $\mathrm{N_2}$ production, a paired t test and Wilcoxon signed rank test were performed. Prior to statistical analysis, all data were tested to meet the assumption of normality and considered significant at the $p<0.05$ level. Minitab version 15 statistical package and Origin 7 technical graphing software were used in all analyses. For all fluxes presented in this study, positive and negative values indicate net fluxes into the water column and into the sediment, respectively.

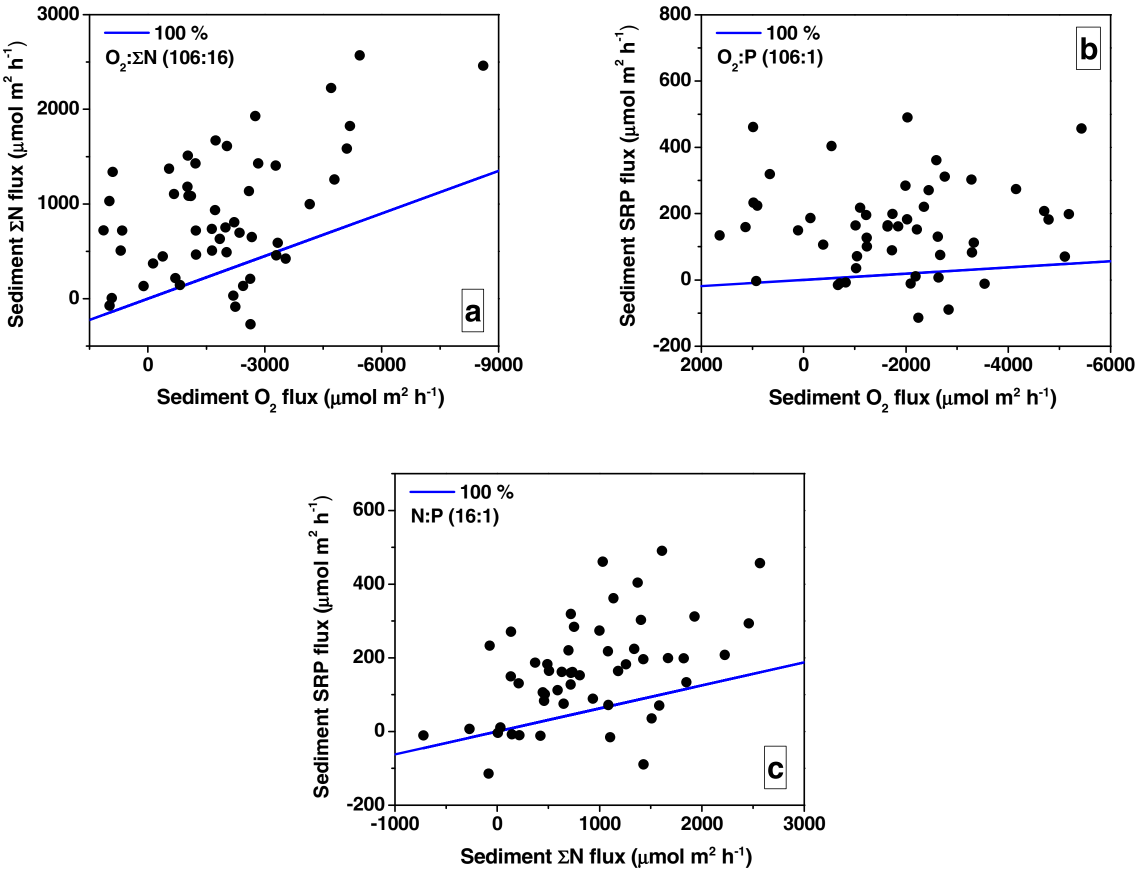


Fig. 2 **a** Relationship between the sum of all net N fluxes versus net sediment oxygen fluxes. The Redfield ratio of C/N (106:16), assuming to be identical to the O_2/N ratio. **b** Soluble reactive phosphorus versus sediment oxygen consumption. The 100 % line represents the Redfield ratio of C/P (106:1), assumed to be identical ratio between O_2 and phosphorus. **c** The SRP fluxes versus the sum of the N fluxes for the individual ambient cores. The *blue line* represents the Redfield ratio of ΣN/SRP (16:1)

Results

Physicochemical Characteristics of SJL

The SJL water temperature during this study averaged 28.3 °C (SD=1.8), with highest temperature values recorded on October 23, 2010 (30.7 °C), followed by November 05, 2010 (29.6 °C), and March 20, 2011 (28.2 °C), with the lowest in January 2010 (25.6 °C). The highest average salinity was observed in March 2011 (10.3) and in November 2010 (5.9), while October 2010 (3.3) and January 2011 (1.4) had the lowest values. Most of the pH values were between 6.9 and 8.4; the highest mean pH was recorded in October 2010 (8.4) and the lowest in January 2011 (6.9). Dissolved oxygen ranged from 1.3 to 11.9 mg/L. Oxygen concentrations were lowest in January 2010 (2.7 mg/L) and November 2010 (5.1 mg/L); March 04, 2011 and October 2010 had the highest average oxygen concentrations (9.6 and 7.4 mg/L, respectively.). During the ambient denitrification experiment, nutrient concentrations were relatively higher, particularly ammonium and SRP. The SWA had higher ammonium (190.2 μM NH_4^+± 217) and SRP (14.2 μM±11.4) concentrations than those found at the NWA (29.3 μM±29.1, and 4.9 μM±2.2 for ammonium and SRP, respectively). Nitrate concentrations in both NWA (3.4 NO_3^-±2.0) and SWA (2.5 μM NO_3^-±2.1) were similar in magnitudes.

Solid Phase and Pore Water Chemistry

The sediment cores with SST sediment were mostly composed of very coarse sand (32.5 %) and coarse sand (29.1 %) with smaller contributions from medium sand (11.0 %) and fine sand (14.9 %). The silt and clay fraction was very small (0.8 %). In contrast, sediment sites close to the shore of the lagoon had >50 % of grain size in very fine sand (29.1 %) and fine sand (28.6 %), followed by coarse sand (26.6 %), median sand (8.5 %), very coarse sand (6.9 %), and silt and clay (0.3 %). The average content of organic matter in FST was almost threefold higher (23.4 %; ±5.9) than that calculated at the SST (8.9 %±4.5; median, 7.7). Pore water ammonium (527.2 μM NH_4^+±451.2) and SRP (62.2 μM±54.0) concentrations were high in both SST and FST environments. In contrast, nitrate concentrations were low (Table 1).

Table 1 Average±one standard deviation (±SD) and median of nutrient concentration in pore water and organic matter content in shell sediment type (*SST*) and fine sediment type (*FST*) in northwest area (*NWA*) and southwest area (*SWA*)of the SJL sampled in May 2011, and surface water sampled between January 2010 and March 2011

San José lagoon				
Sediment type	Organic matter (%)	NH_4^+ (µM)	NO_3^- (µM)	SRP (µM)
		Porewater		
SST				
Average	8.9±4.5	460.9±245.2	4.2±5.4	43.1±26.7
Median	7.7	475.6	1.0	38.8
FST				
Average	23.4±5.9	572.7±479.0	3.9±5.4	62.0±54.0
Median	23.5	411.7	0.5	41.9
Overall average	16.7±9.0	540.7±422.2	4.0±5.0	56.6±47.9
Overall median	17.0	460.0	0.8	41.9
		Surface water		
NWA				
Average		28.5±30.4	3.7±1.8	4.7±2.3
Median		18.4	3.4	4.9
SWA				
Average		190.1±217.8	2.5±2.1	14.2±11.4
Median		54.4	3.0	8.0
Overall				
Average		79.0±138.7	3.3±1.9	7.7±7.7
Median		26.9	3.3	5.2

Net Oxygen Fluxes

The average oxygen fluxes in ambient cores were relatively high (Table 2). No significant relationship was observed (paired *t* test, $p>0.05$) between dark and light oxygen fluxes. Average oxygen fluxes were higher in cores with SST sediment than in cores with FST sediment. Similarly, oxygen uptake in ambient cores incubated in dark with both FST and SST sediments were higher than the ambient cores incubated in light. Of a total of 17 oxygen flux measurements, six cores having FST sediment showed positive daily oxygen fluxes, while in cores with SST sediment, three measures showed net positive oxygen fluxes from a total of seven. The average dark sediment oxygen fluxes were threefold higher than that that obtained in light incubations.

Net Soluble Reactive Phosphorus Fluxes (µmol m^{-2} h^{-1})

SRP fluxes were extremely high (Table 2). The average net flux value was 167±138; median=162. A total of 19 (35.2 %) and 18 (33.3 %) of 54 SRP flux measurements were <100 and between 200 and 400 µmol m^{-2} day^{-1}, respectively. There was no significant difference in the average of SRP flux between dark and light incubations (paired *t* test, $p>0.05$). Ambient cores with SST sediment showed higher SRP fluxes in both incubation dark and light (dark, average=237±158; median=202; light, 206±SD 126; median=224) than in ambient cores with FST sediment (dark, 121±135; median, 141; light, average, 52.8±340; median, 127). Eight SRP data values were between 7 and 90, while nine data values were negative. In both FST and SST sediment types, the average SRP flux of ambient cores incubated in light was lower than those measured in dark.

Sediment Nitrogen Cycling

Net Ammonium Fluxes (µmol NH_4^+ m^{-2} h^{-1})

In general, ambient cores showed very high ammonium fluxes consistently directed out of the sediment (average=770±701; median=695; Table 2). The 25.9 and 53.7 % levels of all measured ambient cores corresponded to the ammonium fluxes ranging from 1000 to 2000 and 100 to 900, respectively. A significant difference (paired *t* test, $p<0.05$) was found in sediment ammonium fluxes between ambient cores incubated in the dark (average=938±835; median=775.6) and under light conditions (average=654±525; median=594) incubations. The highest average ammonium fluxes were calculated for the ambient cores in both dark (1085±1076; median=1435) and light treatments (average=796±705; median=504) with SST sediment. Although FST ammonium fluxes were 876±738 (median=763, dark) and 523±408 (median=467; light) lower than those with SST sediment, they were also very high.

Net Nitrate Fluxes (µmol NO_3^- m^{-2} h^{-1})

In contrast to ammonium and SRP fluxes, fluxes of nitrate were generally directed into the sediment (Table 2). The average nitrate flux of all sampled ambient cores was −39.9±53.5; median=−23.8. Although nitrate fluxes were higher in dark incubations (−62.5±56.8; median=−44.7) than in light incubations (−29.5±52.8; median=−16.6), this difference was not significant (paired *t* test, $p>0.05$). The average of nitrate fluxes (average=−92.5±91.1; median=−71.1) measured in ambient cores with SST was three times higher than the fluxes obtained in ambient cores with FST sediment (−32.9±48.4; median=−16.0). An even higher nitrate flux was found in cores with SST sediment in both dark and light incubations (dark, −127.2±96.5; median, −126.1; light, −63.0±65.8; median=−43.1) than those obtained in both FST sediment in dark and light incubations (dark, −58.7±55.1; median=−42.5; light, −12.1±25.6; median=−10.4).

Table 2 Average±one standard deviation (± SD), and median of nutrients, oxygen, and net N_2 production in shell sediment type (SST) and fine sediment type (FST) of the SJL between January 2010–March 2011

Statistical parameter	Incubation/ sediment types	O_2 Sediment flux (µmol m^{-2} h^{-1})	NH_4^+	NO_3^-	N_2–N	SRP
Average	Dark	−3122±2072	938±835	−62±57	266±258	177±154
Median		−2594	776	−45	157	182
Average	Light	−856±1657	654±525	−29±53	125±351	152±115
Median		−708	594	−17	41	149
Average	Dark+light	−1999±2180	770±701	−40±53	192±315	167±138
Median		−1999	695	−24	100	162
Average	Dark/FST	−2932±1985	827±695	−59±55	239±220	121±135
Median		−2445	741	−42	157	141
Average	Light/FST	−762±1342	523±408	−12±26	119±367	53±340
Median		−824	467	−10	37	127
Average	Dark+light/FST	−1847±2000	700±615	−33±48	179±304	98±258
Median		−1686	645	−16	98	142
Average	Dark/SST	−3573±2341	1131±950	−127±96	329±342	237±158
Median		−2699	1386	−126	191	202
Average	Light/SST	−1292±2508	884±705	−63±66	187±311	206±126
Median		−384	504	−43	100	224
Average	Dark+light/SST	−2360±2592	1000±803	−92±91	223±349	221±131
Median		−2366	1012	−71	111	201

Net N_2 Production (µmol N_2–N m^{-2} h^{-1})

In general, net N_2 production was relatively high (Table 2); while these rates are generally considered denitrification, there remains the possibility that annamox may contribute some of the N_2 while N fixation could consume N_2. Average N_2–N fluxes including all ambient cores were 192.3±315.4 (median=100). Ambient cores incubated in the dark showed consistently higher net N_2 production than ambient cores incubated under light conditions, although the difference was not significant (paired *t* test, $p>0{,}05$). The highest net N_2 production was calculated in cores with SST sediment in dark incubations (average=329±342; median=191), while ambient cores with FST sediment reached a N_2–N flux average of 239±220; median=157. Similarly, in light incubations, ambient cores with SST sediment showed higher net N_2 production (182±311; median=100) than those ambient cores with FST sediment (119±367; median=37).

The magnitude of the measured N_2–N fluxes may be divided into four groups: (1) 17 % apparent negative rates ranging from −363 to −8.2, all from ambient cores ($n=27$) under light incubations; (2) N_2–N fluxes ranging from 8 to 99 (34 %); (3) cores with net N_2 production fluxes between 100 and 400 (32 %); and (4) a group with net N_2 production between 500 and 1000 (17 %). The highest net N_2 production in ambient core was 1113.9 measured in ambient conditions with FST sediment in March 2011.

Sediment Denitrification Efficiency

The DE, including all ambient cores, averaged 20.9 %, which is similar to those calculated at ambient cores with FST (20.4 %) and SST (20.2 %). The highest DE was calculated for ambient cores with SST sediment in dark conditions (24.7 %).

Stoichiometric Relationships of Sediment Fluxes

The Redfield ratio can be considered a guide to the ratios of nutrient elements remineralized from decomposing algae in aquatic sediments. There was no significant relationship (Kruskal–Wallis test, $p>0.05$) between total net sediment dissolved inorganic nitrogen ($\Sigma N = NH_4^+$–N, NO_3^-–N and N_2–N) and net oxygen fluxes in ambient cores, with most of the individual ambient cores (68.5 %) above the Redfield ratio line $O_2/\Sigma N$ (106:16) (Fig. 2a). A similar relationship was observed between net SRP fluxes and net sediment oxygen fluxes, with 70.4 % of the individual ambient cores exceeding the Redfield ratio of $106O_2/1P$ (Fig. 2b). For ΣN and SRP, most of the ambient cores are above the line, meaning that these ambient cores appeared to have an excess of SRP, while those falling below the line are deficient in the flux of remineralized P (Fig. 2c).

The O_2/N_2–N ratio shows that individual ambient cores are mostly above the Redfield ratio line (6.6:1). These data suggest that the remineralized N was released to the water column as NH_4^+ (Fig. 3a). The relationship between $NH_4^+$and ΣDIN fluxes showed a consistent pattern of ambient cores falling around the 1:1 line (Fig. 3b). There was a positive Spearman correlation fit between NH_4^+–N and the ΣDIN fluxes. Most of the individual ambient cores were centered around the 1:1 line (Fig. 3b), meaning that sediment NO_3^- fluxes were about zero or negative, indicating a net loss to denitrification; there was a strong positive Pearson correlation between net sediment NH_4^+ and net dissolved inorganic nitrogen fluxes. The DE was distributed equally above and below the line marking the 25 % (Fig. 3c). There was also a strong positive Pearson correlation between nitrification and net N_2 production (Fig. 3d) with the slope, suggesting that the nitrate for net N_2 production is primarily derived from nitrification. In terms of the average of nutrient ratio calculations, the ΣN/SRP (5.6), O_2/ΣN (2.2), and O_2/SRP (12.2) ratios were consistently lower than that expected from the Redfield ratio. The exception was the O_2/N_2–N (10.4) ratio, which showed a higher value than expected by the Redfield ratio, if denitrification was 100 % efficient.

Fluxes with Elevated Nitrate

Most of the oxygen fluxes data collected during the experiment with higher nitrate were relatively high and directed into the sediment (Table 3), but there was no significant difference (paired *t* test, $p<0.05$) in the net oxygen fluxes between cores before and after 100 μM KNO_3 addition. In fact, the average net oxygen fluxes measured in control cores were very similar to that obtained in cores with added 100 μM KNO_3. In dark incubations, both control cores and cores with added 100 μM KNO_3 showed very high negative oxygen fluxes, 5 and 11 times higher than those obtained in light incubations, respectively. In the light incubations, lower oxygen fluxes occurred because out of nine sampled cores, five of them (with both FST and SST sediments) showed positive fluxes, i.e., net oxygen production, possible due to photosynthetic activity (Table 4).

Ammonium fluxes amended with 100 μM KNO_3 were significantly higher (paired *t* test, $p<0.05$) than nonamended control cores (Fig. 4). The average flux of ammonium with

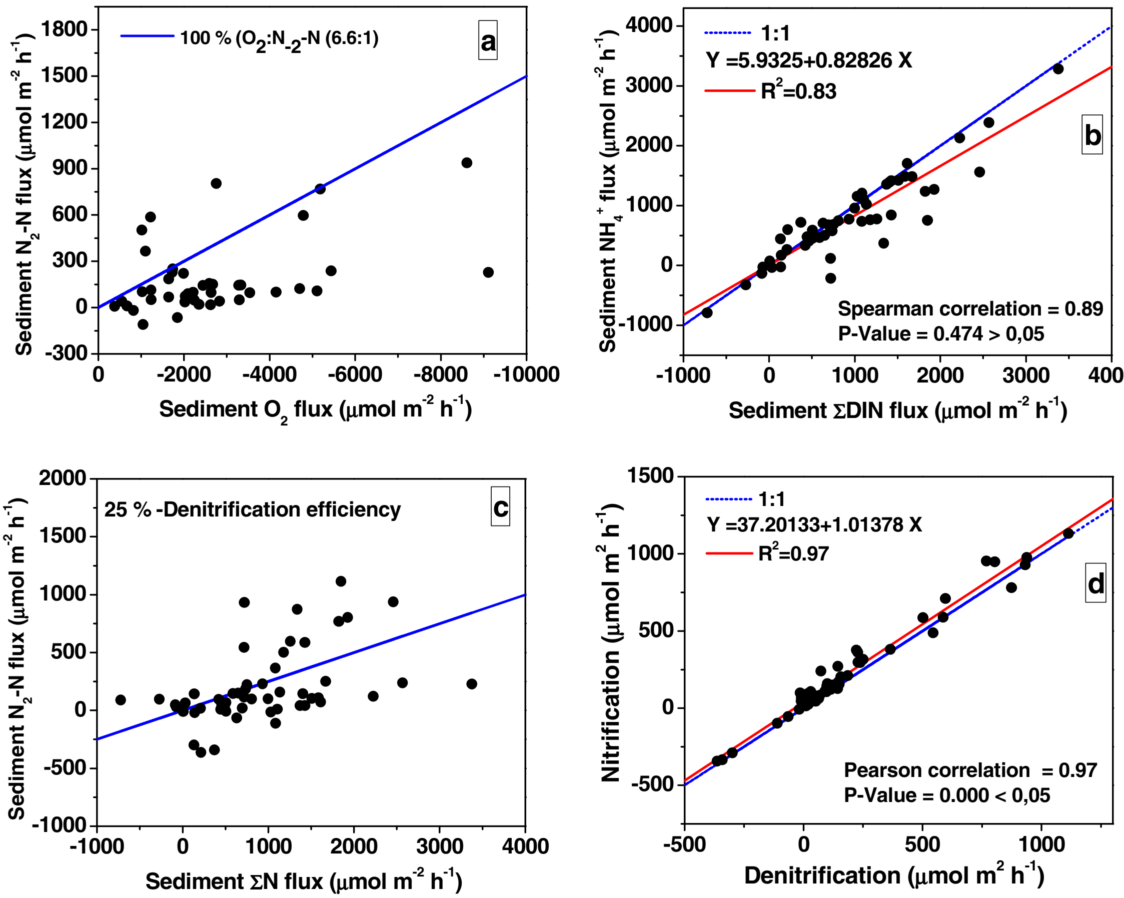


Fig. 3 **a** Plot of sediment net N_2 production versus net sediment oxygen fluxes. The 100 % line represents a Redfield ratio of O_2/N (6.6:1). **b** Plot of the dissolved inorganic nitrogen (*DIN*) versus sediment ammonium fluxes. The 1:1 line means that sediment NO_3^- fluxes were about zero or negative, meaning a net loss to denitrification; this figure also shows positive Pearson correlation fit between net sediment NH_4^+ and net dissolved inorganic nitrogen (*DIN*) fluxes. **c** Plot of sediment net N_2 production versus sum of the net dissolved inorganic nitrogen (ΣN) fluxes. **d** Plot of sediment net N_2 production versus nitrification (net N_2–N minus net NO_3^-)

Table 3 Average±one standard deviation (±SD), and median of nutrients, oxygen, and net N_2 production calculated in sediment cores before and after amended with 100 μM KNO_3 in the nitrate addition experiment in the SJL between March–April 2011

Statistical parameter	Nitrate amended cores/incubation	O_2	NH_4^+	NO_3^-	N_2–N	SRP
		Sediment flux (μmol m^{-2} h^{-1})				
Average	Before/dark	−3699±2496	698±1148	−21.7±24.4	221±295	126±193
Median		−2542	699	−18	105	53
Average	After/dark	−4435±2761	1869±823	−1534±871	882±484	99.2±180
Median		−3349	1737	−1481	839	30
Average	Before/light	−688±2081	841±706	−46.0±40.2	21.5±82.3	147±168
Median		13	843	−42	1.3	75
Average	After/light	376±1008	1062±542	−1152±621	566±337	268±253
Median		157	1070	−1055	492	197
Average	Before/dark+light	−2193±2711	770±924	−34±34	121±233	136±175
Median		−2168	843	−24	94	71
Average	After/dark+light	−2029±3194	1466±792	−1343±757	724±435	184±229
Median		−1295	1476	−1213	649	130

added 100 μM KNO_3 was about twofold higher than the average in control cores, while ammonium fluxes in cores incubated under light conditions in both the control and experimental treatments were similar (Table 3). As expected, high nitrate uptake was observed cores with added 100 μM KNO_3. Average fluxes with added 100 μM KNO_3 were unambiguously higher than those of the control cores. The average of nitrate fluxes was very similar in cores with added 100 μM KNO_3 in both dark and light incubations. Conversely, nitrate fluxes were twofold higher in control cores incubated with light than those control cores in dark incubation (Table 3). The addition of 100 μM KNO_3 increased nitrate uptake significantly (Wilcoxon signed rank test, $p<0.05$) and increased the net N_2 production of the experimental cores (Table 3). The

Table 4 Average±one standard deviation (±SD), and median of nutrients, oxygen, and net N_2 production calculated at the cores with both shell sediment (SST) and fine sediment (FST) types in the nitrate addition experiment in the SJL between March–April 2011

Statistical parameter	Sediment type/ incubation	O_2	NH_4^+	NO_3^-	N_2–N	SRP
		Sediment flux (μmol m^{-2} h^{-1})				
Average	FST/dark	−2121±393	1449±415	−815±465	468±168	15.9±71
Median		−2017	1584	−882	473	−15
Average	FST/light	−440±361	1168±629	−701±350	350±203	109±73
Median		−480	1208	−623	276	114
Average	SST/dark	−6748±1836	2289±969	−2253±416	1297±246	183±228
Median		−7473	1860	−2281	1319	110
Average	SST/light	−1191±683	957±510	−1603±486	781±316	428±275
Median		−1303	1020	−1713	886	340
Average	FST/dark+light	−2778±4433	1623±1010	−1928±544	1039±381	305±268
Median		−1862	1597	−1939	1019	269
Average	SST/dark+light	−1281±965	1308±516	−758±386	409±184	62±83
Median		−1295	1457	−663	358	54
Average	FST/overnight	−1273±1324	996±568	−578±398	339±210	15±54
Median		−1216	908	−459	252	0
Average	FST/after a week	−1288±650	1620±197	−938±321	478±147	109±86
Median		−1295	1588	−944	473	130
Average	SST/overnight	−3013±5162	1744±1456	−1679±550	964±472	401±342
Median		−3146	1473	−1769	1017	357
Average	SST/after a week	−2544±4365	1502±473	−2178±471	1114±318	210±164
Median		−1862	1597	−2186	1082	247

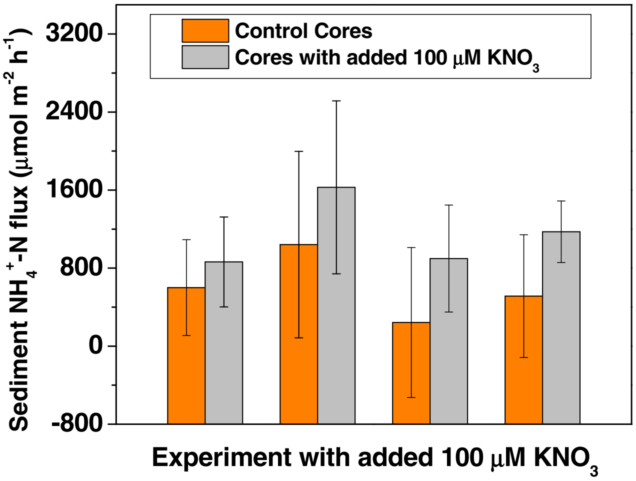


Fig. 4 Sediment net ammonium fluxes measured in ambient cores of the San José Lagoon on March 2011. There was a significant difference (paired *t* test, $p<0.05$) in net ammonium fluxes between cores before and after the addition of 100 μM KNO_3

average net N_2 production rates under elevated nitrate were almost four times greater than that obtained in control cores (Fig. 5).

Six out of eight N_2–N flux rate values ranged from 600 to 2000 μmol m^{-2} h^{-1}, while two cores showed N_2–N fluxes between 200 and 500 μmol m^{-2} h^{-1}. Average flux rates measured after the nitrate addition were higher in cores after a week of incubation than that obtained after overnight incubation. Even higher N_2–N fluxes differences were found between in SST sediment cores after a week of incubation compared to overnight incubation. A similar pattern was also observed in FST sediment, where average and median N_2–N fluxes after a week of incubation were higher than those in cores measured after overnight incubation (Table 4).

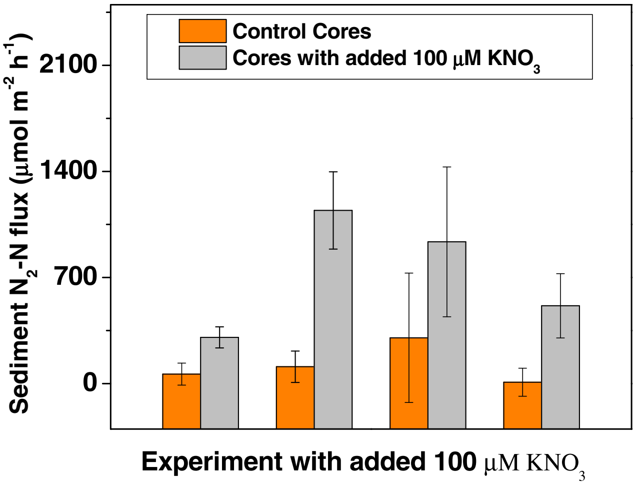


Fig. 5 Sediment net N_2–N flux measured in ambient cores of the San José Lagoon on March 2011. There was a significant difference (Wilcoxon signed rank test, $p<0.05$) in net N_2–N fluxes between cores before and after the addition of 100 μM KNO_3

Effect of Higher Nitrate on the Ammonium, Nitrate, and N_2–N Relationships

High NH_4^+ fluxes were observed before and after cores were amended with 100 μM KNO_3 (Fig. 4). Although there was a poor linear correlation ($R^2=9.13$) between increased NO_3^- concentration in the water column and the NH_4^+ fluxes after the 100 μM KNO_3 addition, this relationship tended towards linearity as NO_3^- fluxes increased (Fig. 6a). In contrast, the net N_2 production systematically increased after 100 μM KNO_3 addition (Fig. 6b), and a clear linear response ($R^2=0.86$) was obtained. The added KNO_3 (Fig. 6c) resulted in about fivefold higher net N_2 production when nitrate fluxes were below −1500 NO_3^- μmol m^{-2} h^{-1}, while above −1500 NO_3^- μmol m^{-2} h^{-1}, NH_4^+ and net N_2 production were similar.

Net Soluble Reactive Phosphorus Fluxes

Generally, net SRP fluxes were very high. The average SRP fluxes measured in cores with added 100 μM KNO_3 were not significantly different than those obtained in control cores (Wilcoxon signed rank test, $p<0.05$). The average net SRP fluxes in control cores in dark incubations were higher than that calculated after a 100 μM KNO_3 addition. However, in light incubations, cores with added 100 μM KNO_3 showed higher net SRP fluxes than that obtained in control cores. Of a total of 36 measured cores, there were eight instances with negative SRP fluxes, including an exceptional value of −1275 μmol m^{-2} h^{-1}. These negative data were measured in both control cores and cores with added 100 μM KNO_3 in light and dark incubations (Table 3).

Discussion

The surface water of the both NWA and SWA showed similar values for physicochemical parameters (Table 1). In general, these values may change drastically in response to both diel variation and runoff input after rainfall events. The highest observed salinity was in October, coinciding with the lowest monthly rainfall total collected over the study (11.2 mm). Alternatively, rainfall in January, November, and March ranged between 199.6 and 281.2 mm diluting the salinity. Eyre and Balls (1999) have suggested that the hydrological regime and the insolation are important physical forces that shape both physicochemistry and nutrient dynamics in tropical estuaries.

As a characteristic feature of heavily impacted coastal systems, the SJL receives nutrients inputs not only from its tributaries but also from both combined sewage and storm water systems, which discharges untreated wastewater to the lagoon, and from nonpoint sources of pollution coming from its

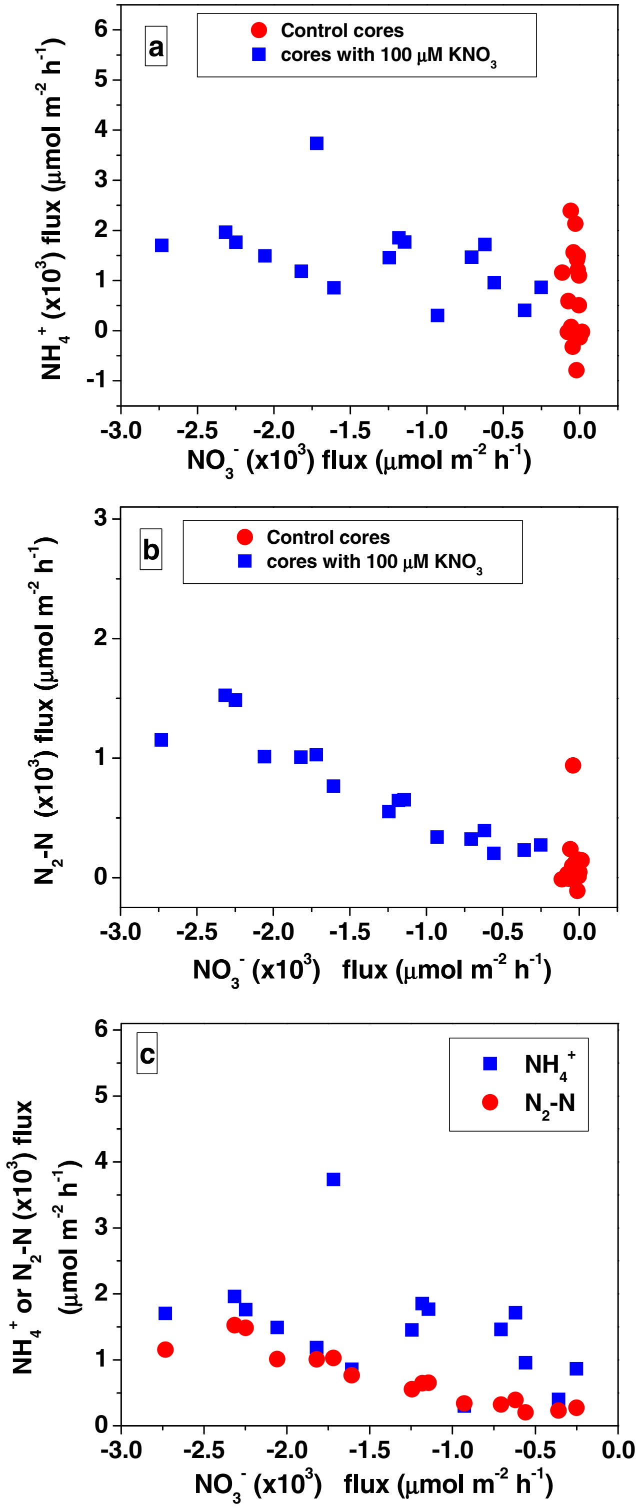


Fig. 6 Relationships between ammonium, nitrate, and net N_2 production after the addition of 100 µM KNO_3

watershed. The concentrations of ammonium and SRP in surface water around the SWA were seven and three times higher than those measured at the NWA. Spatial differences in nutrient concentrations may change depending on the timing of the flushed wastewater from the different sides of the lagoon. The USEPA (2007) has estimated that 18–23 % of the runoff flushed to the SJL from the BCPS, located around the NWA, is in the form of raw sewage. With such high nutrient concentrations, we might expect higher surface water chlorophyll *a* concentrations than commonly observed in the SJL (9.2–23. 6 µg/L; Webb and Gómez-Gómez 1998). Attenuation of chlorophyll *a* concentrations in the SJL may result from filtration by mussels (*Perna* spp.), particularly abundant around both NWA and SWA areas, and other physical factors such as water mixing and turbidity (Kennedy et al. 1996). Chlorophyll *a* concentrations can change dramatically after rainfall events, with peak chlorophyll *a* concentrations of 200 µg/L (Webb and Gómez-Gómez 1998).

The high organic matter content in both FST (23.4 %±5.9) and SST (8.9 %±4.5) may reflect high inputs of mangrove organic matter, diffuse inputs of organic matter from the watershed, and from the invasive aquatic plant water hyacinth (*Eichhornia crassipes*) that enters the SJL mainly in runoff through the Quebrada Juan Méndez. It is also possible that the restricted water circulation observed in the SJL as well as its shallow depth could promote the accumulation of organic matter, particularly in areas with higher content of fine sands like FST (Webb and Gómez-Gómez 1998). The organic matter content in the sediment of the SJL was comparable (16.7 %±9.0) to values reported by Mosquera et al. (1998) for La Parguera, Puerto Rico (12.6 %).

Environmental Controls on the Sediment Oxygen Balance

The average dark oxygen fluxes measured in this study were twofold higher than observations by Cerco et al. (2003). Our oxygen fluxes were also higher than many literature rates (Fisher et al. 1982, 917–3500 m^{-2} h^{-1}; Hammond et al. 1985, 542–917 m^{-2} h^{-1}; Boynton et al. 1991, 1042–3000 m^{-2} h^{-1}; Cowan and Boynton 1996, 125–1708 m^{-2} h^{-1}) suggesting a large supply of labile organic matter to SJL sediments.

The net daily sediment oxygen flux was negative indicating that SJL sediments are net heterotrophic. The importance of anaerobic metabolism in mangrove sediments has been widely observed (Gattuso et al. 1998; Kristensen et al. 2000; Lyimo et al. 2002; Valdes-Lozano et al. 2006; Prasad and Ramanathan 2008), but it should be noted that the high oxygen fluxes directed to the sediment in SJL are enhanced by the high organic matter content of the sediment, sewage inputs through the BCPS, and high pathogen loading (389×10^{15} MPN day^{-1}; Cerco et al. 2003). In addition, the relatively invariable high water temperature and restricted circulation promotes high respiration rates from efficient particulate trapping, thus exacerbating the sediment oxygen deficit.

Environmental Controls on Sediment Phosphorus Cycling

Our average SRP fluxes (average=159.2±138.1, median=178.2 μmol m^{-2} h^{-1}) were higher than the highest flux reported by Cerco et al. (2003) for SJL (106.2, average=8.7 μmol PO_4^{3-}–P m^{-2} h^{-1}). The similar SRP fluxes in light and dark incubations found in SJL suggest that the high SRP fluxes calculated in this study may be explained by the high SRP concentration in pore water (56.6 μM SRP±47.9), which was higher than those reported for other coastal water such Joyuda Lagoon in Puerto Rico (11.3 μM $PO_4^{3-}$$m^{-2}$ h^{-1}, Corredor and Morell 1993) and Ría Lagartos in Mexico (3.72 μmol $PO_4^{3-}$$m^{-2}$ h^{-1}; Valdes-Lozano and Real 2004). Even the extremely high phosphate fluxes (100 μmol PO_4^{3-}–P m^{-2} h^{-1}) reported for Boston Harbor (Tucker et al. 2002), and for New York–New Jersey Harbor (133 μmol SRP m^{-2} h^{-1}, ANS 1995) are lower than or comparable to the SRP fluxes reported in this study. Some geochemical factors, such as interaction of Fe, S, and P cycles, low oxygen concentration, and high temperature in sediments may enhance the release of P to surface water (Jensen et al. 1995; Jordan et al. 2008). In this study, the O_2/SRP ratio was lower (O_2/SRP=12.2) than that expected if all SRP was derived from the organic matter decomposition (Fig. 2b). Deviation from the O_2/SRP may occur because of the conversion of Fe oxides to FeS or FeS_2, thus minimizing of the Fe-oxide adsorption of P (Jensen et al. 1995; Jordan et al. 2008). Another way to assess the P dynamics at the sediment–water interface is to quantify the relationship between ΣN and SRP, considering as a reference the Redfield ratio of N (16)/P (1). These data suggest that P desorption from solids contributes to high P fluxes (Fig. 2c). Aluminum, calcium, and manganese may also play an important role in P desorption or sequestration in estuarine sediment (Maher and DeVires 1994; Pant and Reddy 2001); P inputs to the lagoon may have both organic and inorganic origins. The 100 μM KNO_3 addition experiment did not inhibit the release of SRP fluxes from the sediment as shown elsewhere (Tirén and Pettersson 1985).

Environmental Controls on Sediment Nitrogen Cycling

The high ammonium fluxes observed in this study, as well as the high ammonium concentration in porewater, are consistent with the high rates of sediment metabolism suggested by the measured high O_2 uptake rates. Similar patterns were reported by Corredor and Morell (1993), who concluded that high temperature and high rates of organic matter sedimentation limits oxygen penetration in mangrove lagoon sediments in Puerto Rico, thus hindering the process of nitrification and promoting anaerobic respiratory pathways. Similarly, Kemp et al. (1990), working at the Chesapeake Bay, associated the “dramatic reduction” of nitrification and enhanced ammonium recycling to a combination of factors, i.e., higher organic matter accumulation, minimal oxygen penetration, and elevated summer temperatures. More recently, Abell et al. 2011 and Beman 2014 have demonstrated that the activity and abundance of nitrifier communities can be negatively affected by light and low oxygen concentrations in two subtropical estuaries of Australia and Mexico, respectively. The persistent smell of sulfide emitted from the sediments during coring of sediments at the SJL suggests that sulfate reduction may result in an underestimation of the metabolism by the O_2 fluxes, but also suggest potential poisoning of nitrifying microbes (Joye and Hollibaugh 1995; An and Gardner 2002). Under such conditions, DNRA may be an important fate for oxidized nitrogen. A prevalence of ammonium pathways has been cited as of particular importance in tropical (Dong et al. 2011) as well as in subtropical (An and Gardner 2002; Gardner and McCarthy 2009) estuaries. The increased efflux of ammonium during nitrate addition experiment is consistent with this process.

In this study, average net N_2 production rates were higher in dark than in light incubations, although not significantly. Such patterns may be explained by the oxygen production during the photosynthesis increasing diffusive distances for nitrate to anaerobic zones as well as promoting competition for ammonium between nitrifiers and autotrophs. In fact, this pattern has been consistently reported by Sundbäck et al. 2000; Jørgensen and Sørensen 1988; Risgaard-Petersen et al. 1994; Risgaard-Petersen 2003. The preferential use of oxygen rather than nitrate as terminal acceptor during the organic matter oxidation by the facultative denitrifying bacteria (Zunft 1997; Gottschalk 1986) may also explain the lower denitrification flux under light conditions in SJL.

Also interesting is the observation that during the light incubations, 14 % of ambient cores showed negative denitrification flux. This could be indicating sediment N_2 fixation in SJL. In effect, patches of benthic algal mat were observed in both NWA and SWA areas, just around the population mussels (*Perna* spp.). However, these data should be analyzed with caution, since it may reflect an artifact related to the difference in solubility of N_2 and Ar in oxygen bubbles produced during the photosynthesis, though bubbles were not observed in the water column of the cores. Indeed, oxygen concentrations during light incubations were below saturation.

To compare our net N_2 production with other tropical, subtropical, and temperate estuarine and coastal systems, and to understand the factors controlling denitrification rates, denitrification rates reported for these systems are listed (Table 5). However, it should note the variety of analytical methods used to measure denitrification flux, which may affect their interpretation and comparison among those flux (Seitzinger 1988; Cornwell et al. 1999). The average net N_2 production calculated at this study are at the higher end of the range previously reported for tropical, but lower than those reported for subtropical and temperate systems. From a review of literature

Table 5 Net N$_2$ production (μmol N$_2$–N m^{-2} h^{-1}) reported for selected coastal sediments in different region of the world

Estuarine/coastal systems	Method	Denitrification flux (μmol N$_2$–N m^{-2} h^{-1})	Reference
Tropic/subtropic			
San José Lagoon, PR	MIMS	266^{a} (18.4–937.9) 125^{b} (8.3–1113.9) 192^{c} (8.3–1113)	This study
Joyuda Lagoon, PR	AIT	36^{a} (0.74–160)	Morell and Corredor 1993
La Parguera, PR	AIT	74.9^{a} (9.7–183.0)	Corredor and Morell 1994
Galveston Bay, USA	TCD	n/r (0–11.7)^{a}	Zimmerman and Benner 1994
La Parguera, PR	AIT	n/r (0.03–2.30)^{a}	Mosquera et al. 1998
Brunswick, Aus	MIMS	n/r (15.5–131.0)^{a}	Ferguson et al. 2004
Southern Moreton Bay, Aus	MIMS	n/r (38.5–206.0)^{a}	Eyre et al. 2011
Mae Klong, Thai	IPT	n/r (0–7.4)^{a}	Dong et al. 2011
Cisadane, Ind	IPT	n/r (0–103)^{a}	
Vunidawa-Rewa, Fiji	IPT	n/r (0–2.6)^{a}	
Coombabah Lake, Aus	IPT	0.7^{a}; 5.5^{b} (n/r)	Dunn et al. 2012
Mangrove Forest, NC	IPT	n/r (22.6–68.8)^{a}	Molnar et al. 2013
Little Lagoon, USA	MIMS	n/r (50–80)^{a}	Bernard et al. 2014
Temperate			
Waquoit Bay, USA	TCD	62.5^{a} (−4.5–356.0)	Lamontagne and Valiela 1995
Kertinge Nor Estuary, Den	IPT	n/r (<1–60)^{a,b}	Rysgaard et al. 1995
River Colne Estuary, UK	AIT	(n/r), (19–1317)^{a}	Ogilvie et al. 1997
	IPT	(n/r), (0–368.7)^{a}	
Boston Harbor, USA	TCD	27^{a} (<2.5–103.0)	Nowicki et al. 1997
Massachusetts Bay, USA		11.5^{a} (<2.5–32.0)	
Thames Estuary, UK	IPT	Inshore: n/r (0–11407)^{a} Offshore: n/r (0–27)^{a}	Trimmer et al. 2000
Colne Estuary, UK	IPT	n/r (179–530)^{a,d}, (273–1140)^{b,d} n/r (0–64.7)^{a,e}	Dong et al. 2000
	IPT	n/r (1.2–69.5)^{a,f}, n/r (1.4–23.3)^{b,f}	
Tagus Estuary, Por	IPT	n/r (20–250)^{a,b}	Cabrita and Brotas 2000
Waquoit Bay, USA	TCD	130.5^{a} (n/r); 229.0 (n/r)	LaMontagne et al. 2002
Rowley River Estuary, USA	IPT	n/r (37.5–333.3)^{a}	Tobias et al. 2003
Colne Estuary, UK	IPT	421.7^{a,d} (n/r), 53.7^{a,e} (n/r), 8.7^{a,f} (n/r)	Dong et al. 2006
Humber Estuary, UK	IPT	320^{a,d} (n/r), 0.1^{a,e} (n/r), 39^{a,f} (n/r)	
Conny, UK	IPT	n/r^{a,d} (n/r), 108^{a,e} (n/r), n/r^{a,f} (n/r)	
Boston Harbor, USA	TCD/ MIMS	n/r (92–375)^{a}	Tucker et al. 2014
Narraganset Bay, USA	MIMS	20–42^{a} (n/r)	Fulweiler and Heiss 2014

MIMS membrane inlet mass spectrometry, *AIT* acetylene inhibition technique, *IPT* isotope pairing technique, *TCD* gas chromatography-thermal conductivity detector, *n/r* data not reported

[a] Experiment conducted in dark

[b] Experiment conducted in light

[c] Overall average

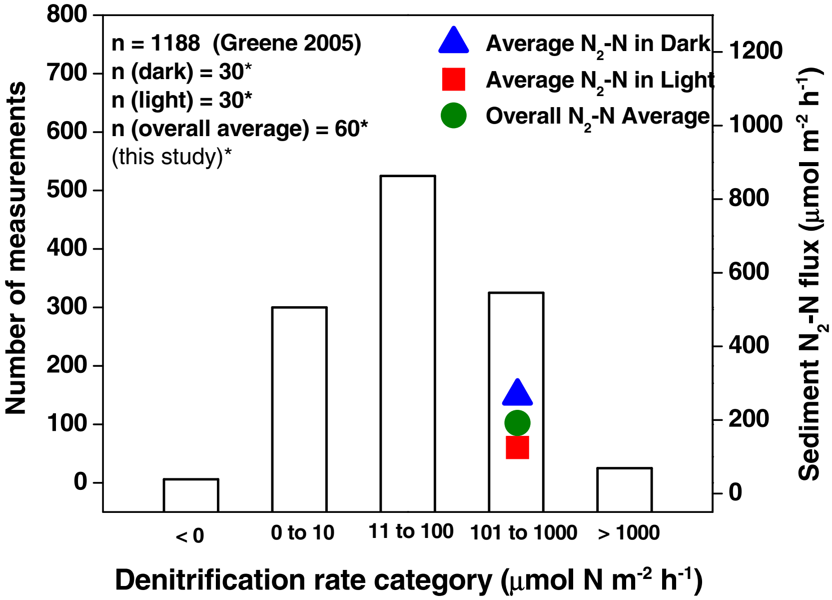
[d] Top of the estuary

[e] Middle of the estuary

[f] Bottom of the estuary

values (Greene 2005; Fig. 7), the three average measures of net N$_2$ production (in dark, light, and overall average) calculated at this study fall in the fourth range category (101–1000 μmol N$_2$–N m^{-2} h^{-1}). Despite the relatively high rates, denitrification plays a minor role in the sediment of the SJL if compared with DIN recycling. It appears that the environmental factors present in tropical coastal systems, such as high organic matter content, oxygen deficit, relatively low nitrate concentrations in both pore water and water column, sulfate-reducing microorganisms, and permanently high temperatures, favor the quantitative predominance of DRNA over denitrification. In fact, the major role of DRNA in tropical and subtropical coastal systems have been suggested by Morell and Corredor (1993) and Rivera-Monroy and Twilley (1996), and demonstrated by Gardner et al. (2006), Gardner and McCarthy (2009), Dong et al. (2011), Dunn et al. (2012), Roberts et al. 2012, and Molnar et al. (2013). In contrast, denitrification is considered as the quantitative major

Fig. 7 This figure copied and modified from Greene (2005) shows the position of all denitrification rate values reported in this in relation to other estuaries around the world. *Numbers in blue and red* represent the relative contribution of data gathered in this study to the categories

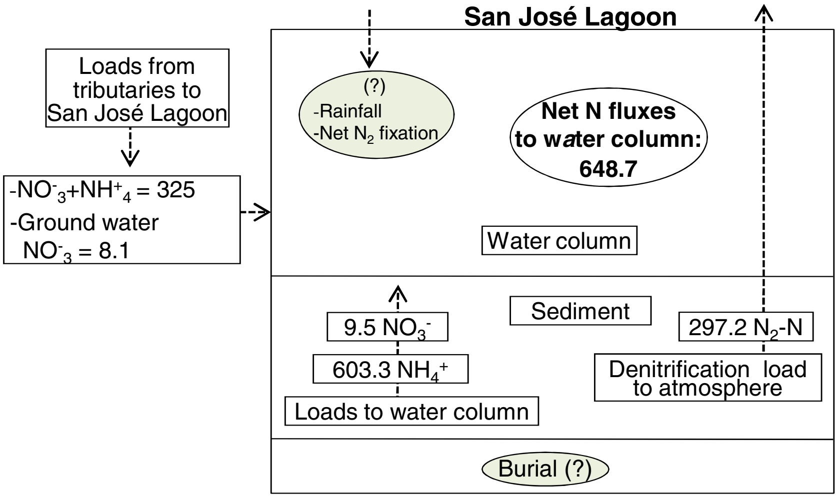


nitrate consuming process in temperate coastal systems. This is largely due to the seasonal changes in temperature and light, which can influence nitrogen cycling through the OM supply and oxygen concentration in the water column and sediment. Consequently, denitrification peaks are observed during the spring and fall, when cooler temperature and oxygen production enhance the coupled nitrification–denitrification and denitrification based on nitrate from water column, and lowest in summer coinciding with warmer temperatures, high sediment respiration, and minimal sediment oxygen concentrations (Cabrita and Brotas 2000; Jørgensen and Sørensen 1988; Kemp et al. 1990; Rysgaard et al. 1995; Cowan and Boynton 1996; Ogilvie et al. 1997; Trimmer et al. 1998). Rates of DRNA are often an important process in temperate coastal systems, but mostly in warmer seasons, under high organic matter loading, and reducing conditions (Jørgensen 1989; Gilbert et al. 1997; Christensen et al. 2000; Nizzoli et al. 2006).

Denitrification Efficiency

Denitrification efficiency is an indicator of the proportion of remineralized nitrogen converted to nitrogen gas, providing insight into the regulation of nitrogen cycling. Previously, it was shown that denitrifiers were moderately efficient in converting the available nitrate to N_2–N (Fig. 3c). This was reflected in the DE (20.9) calculated at the SJL. It appeared that this DE calculated at the SJL reflects the high organic matter content (16.7 %) in the sediment of the SJL. Nevertheless, it should note that the relative low removal efficiency calculated for the SJL (20.9) was more than twofold below of that calculated by Seitzinger (1988) for several

Fig. 8 Nitrogen loads from tributaries, and Baldorioty de Castro Pump Station and nitrogen fluxes in the interface water column-sediment of the SJL. All data scaled up to kg N day^{-1}



selected temperate estuaries (55 %) and Yoon and Benner 1992, who reported a DE of 57 % of DIN inputs in Guadalupe estuary. Several researchers have argued that, unlike temperate ecosystems, the removal of nitrogen in tropical ecosystems is limited by several factors, especially the limited capacity for the coupled nitrification and denitrification. In Puerto Rico, Corredor and Morell 1993 and Mosquera et al. 1998 reported low nitrification efficiency and a minimal role of denitrification as factors promoting the organic matter production/accumulation and the relatively high ammonium concentrations observed in the sediments of the Joyuda Lagoon and La Parguera, respectively. Other investigators, working under tropical and subtropical settings, have also reported the inhibitory effect of oxygen deficits, sulfide, and temperature on nitrification and denitrification (Joye and Hollibaugh 1995; Gardner et al. 2006; Gardner and McCarthy 2009; Dong et al. 2011). Consequently, in tropical systems DNRA dominates over denitrification in nitrogen recycling (Christensen et al. 2000; Gardner and McCarthy 2009; Dong et al. 2011; Molnar et al. 2013), while in temperate counterpart, denitrification arises as the most important process of the nitrogen pathway

Nutrient loadings in the SJL are at the hypereutrophic level, with the relative high water residence time potentially fueling nutrient recycling. This study clearly shows that high rates of sediment respiration and the consequent high ammonium fluxes result in high rates of sediment N recycling (Fig. 8). The high rates of release of ammonium help support the high water column ammonium concentrations observed in this ecosystem. It is also clear that denitrification, while not a large proportion of the sediment nitrogen effluxes, nevertheless has high rates and is an important part of the sediment nitrogen recycling despite the potential for the inhibition of nitrification by high respiration, low oxygen penetration, and production of hydrogen sulfide. The ~297 kg N day^{-1} removed by net N_2 production, while considerably smaller than the effluxes of ammonium, is similar to the direct loading of DIN from the watershed groundwater system. This also indicates that remineralization of allocthonous organic matter is necessary to support the overall observed rates of sediment N efflux.

Sediment N recycling and denitrification play a very important role in the nitrogen balance of the SJL, and any studies or models that do not account for these sediment processes will miss major processes controlling the ecology of this anthropogenically stressed tropical lagoon. Overall, the MIMS technique used in this investigation has been successfully used for the first direct quantification of net N_2 production in a tropical environment highly affected by nitrogen loads. Further research will be necessary to have a better understanding about the magnitudes and variability of nutrient and denitrification fluxes in sediments of the tropical systems heavily affected by anthropogenic activities, and unfortunately poorly studied.

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