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THEMATIC ISSUE

Benthic fluxes and nitrate reduction activity in a marine park (Northern San Jorge Gulf) from Patagonia Argentina

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Abstract The Northern San Jorge Gulf (NSJG) was declared Coastal Marine Park in 2008 with the goal of preserving its biodiversity. *In situ* benthic fluxes experiments were performed using an opaque chamber in Sara Creek (SC) and Malaspina Creek (MC). Moreover, ex situ nitrate reduction activity was assessed using a continuous flow-through system. Benthic fluxes in MC, showed a consumption of oxygen (794 ± 196 mg m^{-2} h^{-1}) and nitrate (103 ± 68 µmol m^{-2} h^{-1}) by sediment and release of ammonium (175 ± 60 µmol m^{-2} h^{-1}), phosphate (66 ± 10 µmol m^{-2} h^{-1}) and silicate (116 ± 66 µmol m^{-2} h^{-1}) towards the water column. In SC, the same pattern was observed and the fluxes values were 375 ± 132, 128 ± 1, 76 ± 12, 39 ± 24 and 133 ± 81, respectively. Only, ammonium and oxygen fluxes showed significant differences between sites, due to the highest organic matter content and the particular hydrodynamic conditions in MC. These are the first benthic flux measurements made within the protected area. Nitrate uptake rate, based on Michaelis–Menten kinetic, was K_m: 155.3 µM and V_{max}: 0.053 µM g^{-1} h^{-1} in SC and K_m: 131.2 µM and V_{max}: 0.039 µM g^{-1} h^{-1} in MC. Considering the nitrate dynamic we concluded that the sediments both SC and MC act as a sink of nitrate. This ion could be principally used as a terminal electron acceptor during the oxidation process of organic compounds in sediments of NSJG.

Keywords Benthic flux chamber · Sediment–water interface · Nitrate-reduction · Continuous flow-through system · Marine Park · Argentinean Patagonia

Introduction

Different sources are responsible for the input of nutrients in the water column of a coastal system: external discharges (e.g., domestic and industrial sewages or agriculture activities) and benthic recycling occurring in sediments. The role of marine sediments as a source or sink of nutrients is controlled by environmental conditions (Graca et al. 2006), and relative rates of several aerobic and anaerobic occurring processes. Nitrogen cycling in sediment is complex due to the different assimilative and dissimilative processes involved. The most important processes which regulate the nitrogen bioavailability in coastal systems are the ammonification of sedimentary organic matter, the dissimilative reduction of nitrate, the assimilative reduction of nitrate, the atmospheric nitrogen fixation, the anaerobic oxidation of ammonium, nitrification, and denitrification (Bange et al. 2005; Risgaard-Petersen 2004; Risgaard-Petersen et al. 2005; Francis et al. 2007). The nitrification–denitrification coupled is essential because it involves the output of nitrogen (as gas nitrogen) (Risgaard-Petersen 2004; Macreadie et al. 2006). Therefore, the couple nitrification–denitrification activity acts as a natural process counteracting the eutrophication (Macreadie et al. 2006; Dalsgaard et al. 2012; Lansdown et al. 2012). Contrarily, the organic matter remineralization

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(i.e., which produces ammonium) is one of the main sources of bioavailability nitrogen in the coastal environments (McGlathery et al. 2004).

On the other hand, nitrate reduction is an important pathway related to nitrogen cycling and oxidation. Microbiological reduction of nitrate to ammonium occurs under anaerobic conditions, and it is carried out mainly by chemoheterotrophic bacteria (Sánchez and Sanabria 2009). In this sense, nitrate is used as terminal electron acceptor during the oxidation of organic compounds (Maier et al. 2000). Nitrate reduction activity in sediments can be measured using a continuous flow-through sediment–water system (Northby 1976). This method allows that the metabolites formed during the process are continuously removed from the reactor, and thus do not disturb bacterial metabolic activity. The reactor represents a microcosm with some controlled variables (e.g., temperature, nitrate concentration, flux rate) that allow us to determine the kinetic constants which are used to estimate the natural functioning (Esteves et al. 1986b; Torres et al. 2012).

In shallow coastal environments with low water renewal, the nutrient exchange in the sediment–water interface plays a major role in the metabolism and nutrient cycling (Niencheski and Jahnke 2002). Organic matter generated in the water column and from mainland input is deposited on surface sediments. The most important biogeochemical processes take place in sediments, where the density of microorganisms in the top layer is several orders of magnitude higher than in the water column (Sundbäck and McGlathery 2005). The nutrient mobilization into porewater and consequently from the porewater to the water column is strongly influenced by several factors which vary spatially and seasonally (Sakamaki et al. 2006; Graca et al. 2006). Because of that, the benthic fluxes represent the dynamic link between the sediment and the water column. This exchange in the sediment–water interface is due to physical processes of molecular diffusion, water advection and sediment resuspension, being also biologically influenced by processes, such as bioturbation and bioirrigation (Burdige 2006; Sakamaki et al. 2006; Fanjul et al. 2007). The bioturbation can cause an increase in one order of magnitude in benthic fluxes (Burdige 2006), which impacts particularly, on the nitrogen cycle processes (Nielsen et al. 2004; Webb and Eyre 2004; Michaud et al. 2006; Fanjul et al. 2007), such as nitrification and denitrification (Nielsen et al. 2004). Additionally, the magnitude of benthic exchange fluxes and the vertical concentration profile in sediments is influenced by the changes in nutrient concentrations of overlying water, water temperature, hydrodynamic conditions, and sediment conditions (Risgaard-Petersen 2004; Sakamaki et al. 2006). In addition, the redox potential in sediment can be modified by other factors, such as seasonal variations associated with hypoxia and anoxia, changes in the intensity of bioturbation, and meteorological factors (Sakamaki et al. 2006; Fanjul et al. 2007).

As a result of chemical transformations and processes occurring in sediments, some nutrients are released into the water column and other nutrients are incorporated into the sediment (Niencheski and Jahnke 2002; Sakamaki et al. 2006; Torres et al. 2009; Gil et al. 2014). Therefore, this is considered relevant to determine the magnitude of benthic fluxes, to understand the sediment contribution towards the water column, because it may support the development of primary producers (Niencheski and Jahnke 2002). Nitrogen is the limiting nutrient of the primary production in several coastal environments worldwide and the environments from Patagonia, Argentina are not an exception (Charpy-Roubaud et al. 1983; Paparazzo et al. 2010). The aims of this study are to determine in situ benthic fluxes of dissolved oxygen and dissolved inorganic nutrients, and to estimate in vitro nitrate reduction activity in sediment of two sites from Northern San Jorge Gulf (NSJG). This study is valuable because it is the first of its type performed within Coastal Marine Park.

Materials and methods

Study area

The study was conducted in a coastal area of the NSJG located in Patagonia, Argentina (Fig. 1). It has a large diversity of environments including the presence of numerous islands and islets. This area is widely inhabited by several species of seabirds, marine mammals, seaweeds and benthic invertebrates, which are valuable for feeding of resident and migratory seabirds (Yorio 2001). To conserve biodiversity and natural resources, the area was protected by National Law (No. 26446) creating the Inter-jurisdictional Coastal Marine Park "Patagonia Austral" (ICMPPA) which is the first National Marine Park in Argentina (Yorio 2001).

The NSJG is one of the most productive areas of Patagonia. Several economic activities, such as fisheries, seaweed and guano exploitations take place in the region. However, the main economic activity in this zone is the oil production which represents one of the most important crude oil production fields in Argentina. Therefore, the principal hazard of this ecosystem is the oil spill associated with the transportation of petroleum in the San Jorge Gulf (Commendatore et al. 2000; Yorio 2001; Gatto et al. 2005). In this context, this situation justifies baseline studies to identify the changes which may occur in the future. The Sara Creek (SC) and Malaspina Creek (MC) are located in the NGSJ in Chubut province (Argentina). Sara Creek has

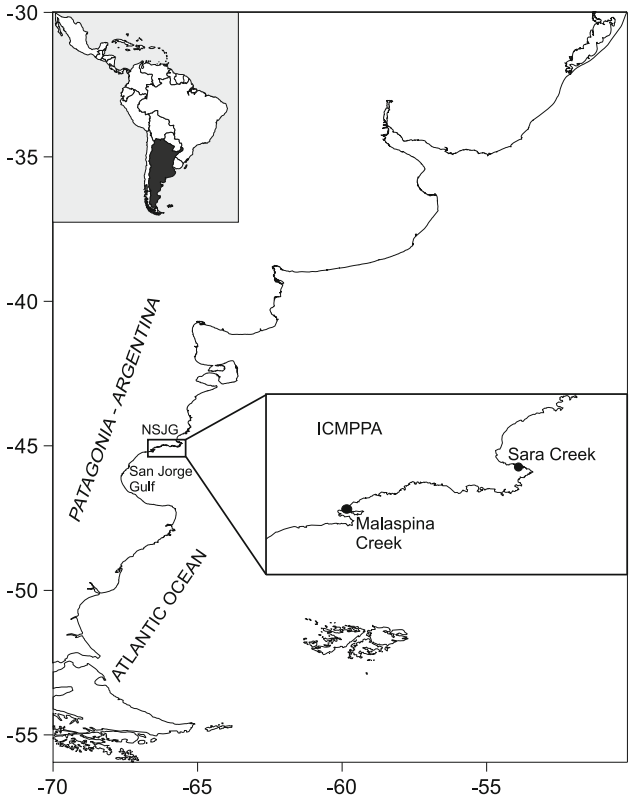

Fig. 1 Map of the study area in the coastal zone from Northern San Jorge Gulf (NSJG). Sara and Malaspina Creeks are located within the Inter-jurisdictional Coastal Marine Park "Patagonia Austral" (ICMPPA) from Argentinean Patagonia

an area of approximately 0.3 km^2 and Malaspina Creek 34.4 km^2 (Gatto et al. 2005). The last Creek is a semi-closed and low-energy environment favorable for the deposition of particles. Thus, the produced and recycled material within the system remains in the region for long periods of time (Marinho et al. 2013). The observed semi-diurnal tide amplitude is 4.26 m (SHN 2013). Marinho et al. (2013) determined the highest proportions of fine material (silt–clay) in five sampling sites from MC (9.75–36.04 %) and in one sampling site from SC (4.68 %), likewise, they found a total organic matter contents up to 3.5 % (MC) and 0.42 % (SC). The sediment in MC showed anoxic conditions, dark color and unpleasant odor, and was positively correlated with the highest content of fine material (Marinho et al. 2013).

Sediment sampling

Sediment samples were collected manually in both Creeks, in October 2012. At each site, 15 samples were collected manually with acrylic cores of 45 mm internal diameter and 30 cm length, every 2 m and along the low tide line. Subsequently, the superficial layer (<1 cm) was separated in situ using spatulas and they were mixed in a composite sample, which was placed in hermetic plastic containers. A layer of 2 cm of seawater was added in situ in each plastic container and they were stored at 4 °C in dark conditions. Experiments in laboratory started ten days after the sample collection by measuring nitrate reduction activity.

In situ benthic flux chamber experiments

Benthic fluxes were measured in coastal sites with an average depth of 0.8 m from SC and MC. An opaque PVC flux chamber was employed which enclosed 9.70 L of bottom water over a 0.09 m^2 area of sediment surface (Esteves et al. 1986a; Torres et al. 2009; Gil et al. 2014). The water inside the chamber was homogenized by means of a magnetic stirring bar, to avoid the formation of concentration gradients. Temperature and dissolved oxygen were registered in the enclosed volume every 5 min using a Yellow Spring Instruments (YSI—Model 58). To analyze dissolved inorganic nutrients, water samples (60 ml) were aspirated through a syringe from the inside of the chamber every 30 min, by means of 2 mm internal diameter tubing. Dissolved inorganic nutrients were measured employing a Skalar autoanalyzer according to Strickland and Parsons (1972). The experiences lasted 2 h and were carried out by triplicate. The magnitude and direction of benthic fluxes were calculated using the following equation according to Esteves et al. (1986a):

$$F_b = (X - X_0) \cdot V_c / S_c \cdot t \quad (1)$$

where, F_b is the dissolved oxygen flux (mg m^{-2} h^{-1}) or nutrient flux (μmol m^{-2} h^{-1}) across the sediment–water interface; X_0 and X are the solute concentrations within the chamber at start and "t" time, respectively; V_c is the water volume inside the chamber, and S_c is the sediment surface area covered by it. Benthic fluxes of dissolved oxygen and nutrients were estimated considering the concentration changes within the chamber in the first hour. Positive and negative values of F_b correspond to fluxes out of sediments and into sediments, respectively. Temperature and dissolved oxygen were measured in situ in bottom seawater using a multi-probe sensor (YSI—Model 58).

In vitro nitrate reduction activity determination

Nitrate reduction activity was determined in vitro using the continuous flow-through system method developed by Northby (1976) and adapted to microbiological processes by Esteves et al. (1986b) (more details in Torres et al. 2012). The nitrate reduction rates ($R_cNO_3^-$) defined as function of the dry weight of sediment into the reactor was obtained as follows:

$$R_cNO_3^- = R_c/W = J_0(X_0 - X_i)/W \quad (2)$$

where, J_o flow of initial seawater solution enriched with nitrate (KNO_3 was used) (l h^{-1}); X_i and X_o is the input and output nitrate concentrations (μmol l^{-1}) respectively, R_c is the nitrate consumption (μmol h^{-1}); and W is the dry weight of sediment in the reactor (g). Negative values of $R_cNO_3^-$ correspond to nitrate uptake by microbial community.

Experimental design by measuring nitrate reduction activity

In laboratory experiences three reactors and enriched seawater with different nitrate concentrations (25, 100, 200, 400 μM) were used. The seawater supplied to the reactors was previously filtered, enriched with nitrate and sterilized in autoclave during 20 min under 1.5 atmospheres to obtain low dissolved oxygen levels. Immediately before the beginning of each experience, the sediment samples were homogenized in an oxygen free glove box filled with inert gas (N_2). The sediment (43.3 ± 7.6 g dry weight) inserted inside of the reactors was retained on a glass-frit (10 μm porosity). The low oxygen atmosphere in each reactor was maintained supplying nitrogen gas. A temperature of 17 ± 0.5 °C was maintained by water circulation around each reactor using a thermostatic bath (Mgw Lauda Kzr). The experiences were run in dark conditions to avoid the activity of photosynthetic microorganisms. The flux of enriched seawater was 36 ml h^{-1} through a Technicon peristaltic pump. The experiences lasted 9 h. After the first 4 h (enough time to achieve stability of the system, Esteves et al. 1986b; Torres et al. 2012) and every 30 min, a fraction of 15 ml was taken from the reactor outflow. Ammonium concentration was measured immediately in a subsample of 5 ml, according to Strickland and Parsons (1972). The remaining volume was stored at −20 °C to analyze nitrate (NO_3^-) and nitrite (NO_2^-) concentrations following the Strickland and Parsons (1972).

Kinetic constants determination

The $R_cNO_3^-$ values were plotted as function of initial nitrate concentration in the reactor, and double reciprocal relationship between them was applied. The Michaelis–Menten function (Michaelis and Menten 1913) was fitted to the data by least-square regression:

$$V = V_{\max} \cdot X/K_m + X \quad (3)$$

where V is the nitrate uptake rate ($R_cNO_3^-$) (μmol g^{-1} h^{-1}), V_{max} is the maximum uptake rate (μmol g^{-1} h^{-1}), X is the nitrate concentration (μmol l^{-1}), and K_m is the half-saturation constant (μM).

Statistical analysis

Comparative analyzes of benthic fluxes were carried out by means of Kruskal–Wallis test (by ranking) after verifying the absence of homoscedasticity. Significance was set at $p < 0.05$. All the statistical tests were performed using the InfoStat software package (InfoStat 2008).

Results

Nutrient exchange in sediment–water interface

In bottom water dissolved oxygen concentrations were 12.1 ± 1.6 mg l^{-1} and 9.8 ± 0.7 mg l^{-1} in SC and MC, respectively. Average temperature was 12.7 ± 0.7 °C and 14.0 ± 0.7 °C in the same places. The evolution of dissolved oxygen and dissolved inorganic nutrient concentrations in seawater enclosed by chamber are showed in Fig. 2. The in situ benthic fluxes measured in SC displayed a consumption of dissolved oxygen (375 ± 132 mg m^{-2} h^{-1}) and nitrate (128 ± 1 μmol m^{-2} h^{-1}) by the sediment. Conversely, a release of ammonium (76 ± 12 μmol m^{-2} h^{-1}), phosphate (39 ± 24 μmol m^{-2} h^{-1}) and silicate (133 ± 81 μmol m^{-2} h^{-1}) towards the near-bottom water layer was determined. The same pattern was found in MC. This site showed sediment consumption of dissolved oxygen (794 ± 196 mg m^{-2} h^{-1}) and nitrate (103 ± 68 μmol m^{-2} h^{-1}), and liberation of ammonium (175 ± 60 μmol m^{-2} h^{-1}), phosphate (66 ± 10 μmol m^{-2} h^{-1}) and silicate (116 ± 66 μmol m^{-2} h^{-1}) into the water column. Highest ammonium and dissolved oxygen fluxes were found in MC. They show significant differences ($p < 0.05$) in comparison with those from SC. The rest of the nutrient fluxes do not displayed significant differences ($p > 0.05$) between sites.

Nitrate reduction activity and kinetic constants

In the steady state, nitrate concentration at the outlet of each reactor was lower than nitrate concentration in the initial solution (Table 1). Nitrate was consumed in all cases ($n = 9$) for about 41 ± 29 % respect to the nitrate concentration supplied in each treatment. Conversely, nitrite concentration in steady state was higher than nitrite concentration supplied through the initial solution. The average production of this ion—which is considered an intermediate compound in the nitrate reduction process—was 190 ± 113 % for all treatments. Similarly, ammonium concentration in steady state showed higher concentration than those in the initial solution for each treatment (Table 1). This average ammonium production (e.g., dissimilative reduction) was 808 ± 971 % for all treatments.

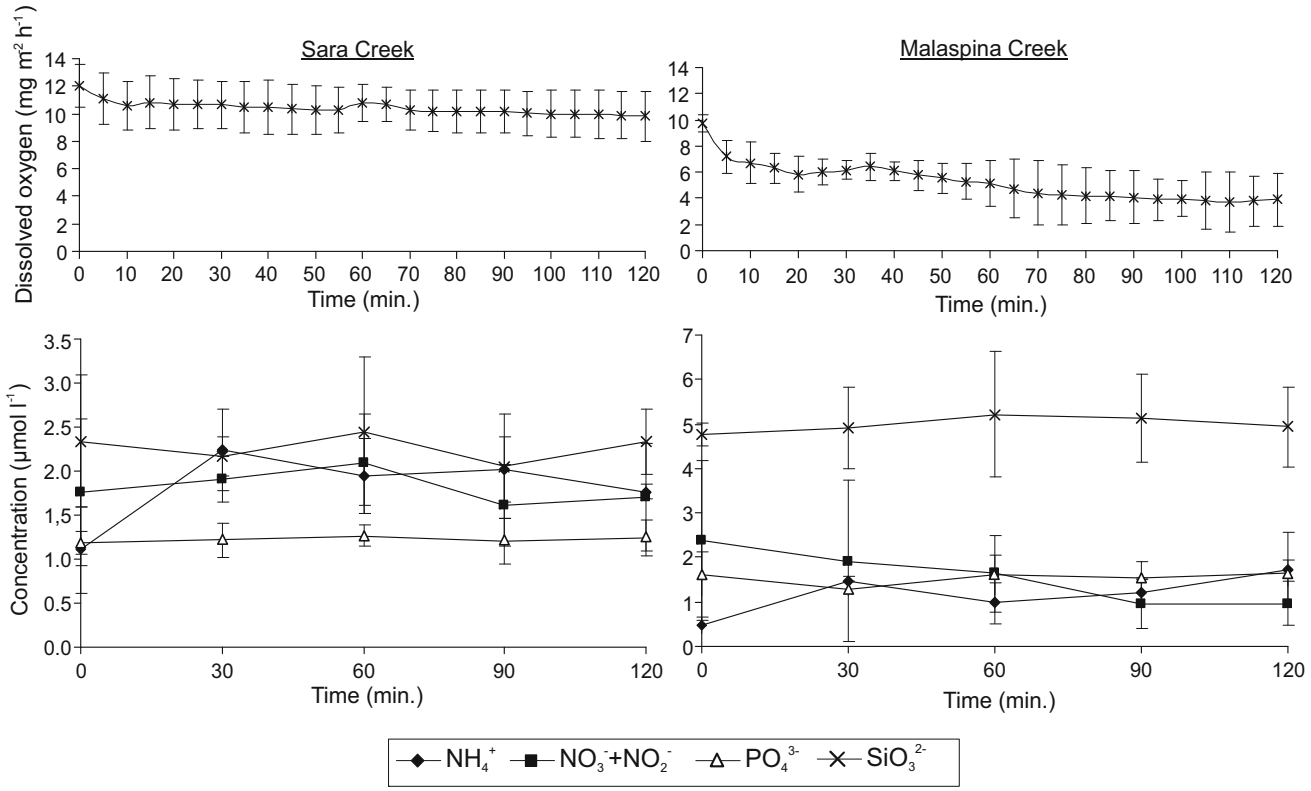

Fig. 2 Dissolved oxygen and dissolved inorganic nutrients concentrations inside of benthic chamber in Sara and Malaspina Creeks

By applying Eq. 2, estimated nitrate reduction activity ($R_cNO_3^-$) in SC ranged between −0.0005 to −0.0222 µmol g^{-1} h^{-1}, whereas in MC varied between −0.0018 and −0.0264 µmol g^{-1} h^{-1} (Table 2). The relationship between estimated $R_cNO_3^-$ and nitrate initial concentration, and double reciprocal relationship between those parameters to SC are showed in Fig. 3a, c, respectively. The same relationships to MC are displayed in Fig. 3b, d, respectively. Nitrate uptake rates exhibited saturation kinetics, as according to the predicted by the Michaelis–Menten model (Eq. 3). Kinetic parameters were V_{max}: 0.0533 µmol g^{-1} h^{-1} and K_m: 155.3 µM in SC, and V_{max}: 0.0391 µmol g^{-1} h^{-1} and K_m: 131.2 µM in MC.

Discussion

Dissolved oxygen concentrations in the surficial seawater were near saturation at both studied sites and were occasionally supersaturated due to wind and breaking waves. The dissolved oxygen and nitrate consumption, and the release of ammonium observed in both creeks are indicative of impacted or eutrophic sediments. This could indicate that oxygen in sediment is insufficient for organic matter mineralization, although in the water column high dissolved oxygen levels were found; and also, the nitrate is used as terminal electron acceptor. On the other hand, the significant higher ammonium and dissolved oxygen fluxes measured in MC than in SC are in agreement with the highest organic matter content in the former. The phosphate fluxes were released from sediments of both Creeks, probably favored by the dissolution of adsorption iron oxyhydroxide substrates in anoxic conditions (Graca et al. 2006). According to Niencheski and Jahnke (2002), the silicate fluxes obtained in this study were inversely related to water column silicate concentration. Moreover, silicate fluxes exhibited the same order of magnitude than those obtained by Niencheski and Jahnke (2002) in three sites from the estuarine region of Patos Lagoon (Brazil). These authors, determined benthic fluxes in stations located in areas along the lagoonal coast (<1.5 m depth) with generally unobstructed water movement. Moreover, in these sites, the sediment was dominated by sand (>70 %), low porosity, and were located away from the urban areas.

Benthic fluxes measured in SC were in the identical direction and the same order of magnitude (for oxygen, nitrate and phosphate) and two order of magnitude lower (for ammonium) than those obtained by Torres et al. (2009) in a semi-closed system from southern Argentina.

Table 1 Sediment and solutions used in each experiment with the reactor

No. experience	Site	Weight of wet sediment (g)			Concentration in the initial solution (μmol l^{-1})			Average concentration in the liquid percolated at steady state (μmol l^{-1})								
								Nitrate			Nitrite			Ammonium		
		A	B	C	Nitrate	Nitrite	Ammonium	A	B	C	A	B	C	A	B	C
1	SC	36.79	37.20	34.94	38.11	1.43	2.59	3.31	21.06	33.55	1.67	4.28	3.33	50.57	18.46	15.46
2	SC	46.79	49.38	57.66	110.78	2.49	3.15	10.72	21.42	17.26	10.4	8.12	5.81	33.53	37.90	29.38
3	SC	39.86	41.09	46.22	177.41	3.60	3.03	183.07‘	175.91	119.52	5.68	5.39	7.98	4.86	6.76	24.98
4	SC	36.65	28.56	39.02	405.86	5.95	9.08	342.76	351.71	317.95	9.13	6.50	18.32	32.80	69.08	40.32
5	MC	36.65	28.56	39.02	51.28	0.85	1.57	36.37	29.11	27.81	1.96	2.69	3.87	5.07	9.17	11.12
6	MC	51.16	56.35	55.50	86.01	2.73	3.17	31.03	40.12	25.92	12.01	7.66	11.92	8.07	9.16	8.74
7	MC	35.29	49.44	45.63	186.94	4.13	9.39	182.24	176.20	192.94	8.61	13.77	12.05	15.91	21.62	16.88
8	MC	47.45	44.86	43.98	322.06	3.29	0.37	226.08	247.38	169.41	10.53	13.63	16.46	12.13	10.83	12.31

Likewise, ammonium flux in MC was one order lower than that measured by Torres et al. (2009), while the others nutrient fluxes were in the equal direction and in the same order. This could be explained by the wide differences in relation to the organic matter content and anoxic conditions in the sediments of both sites. Moreover, dissolved oxygen fluxes measured in this study were one order of magnitude higher than those obtained by Gil et al. (2014) in four coastal stations of Nueva Bay from Argentinean Patagonia. Nitrate and phosphate fluxes determined in this study presented opposite direction with respect to those obtained by Gil et al. (2014).

In general, ammonium fluxes determined in this study showed one order of magnitude lower, than those measured by Gil et al. (2011) in an urban polluted subantarctic coastal environment (Ushuaia bay, Tierra del Fuego Island, Argentina). The dissolved oxygen, phosphate, and nitrate fluxes determined in this study exhibited the same order of magnitude, than those obtained by Gil et al. (2011). Finally, our fluxes were higher or equal to those reported in worldwide marine coastal environments, e.g., Clavero et al. (2000), Graca et al. (2006), Lansard et al. (2008) in Europe, Eyre and Ferguson (2002) in Australia, Grenz et al. (2010) in New Caledonia, Niencheski and Jahnke (2002) in South America, and Sakamaki et al. (2006) in Asia.

The $R_cNO_3^-$ and kinetic parameters adjusted to Michaelis–Menten model measured in both Creeks were of the same order of magnitude; showing not major differences. In addition, V_{max} and K_m parameters obtained in this study were the same order than those measured by Montes (1994) (V_{max}: 25.6 × 10^{-3} μmol g^{-1} h^{-1}, K_m: 48 μM) (in impacted sediment with hydrocarbons and 2 % of organic matter), and by Esteves et al. (1986b) (V_{max}: 168 × 10^{-3} μmol g^{-1} h^{-1}, K_m: 78 μM), in sediments with 1 % of organic matter content. Furthermore, Montes (1994) reported inhibition of this activity using a nitrate concentration of 500 μmol l^{-1}. Nevertheless, both parameters were lower than those measured by Torres et al. (2012) (V_{max}: 2.7 μmol g^{-1} h^{-1}, K_m: 1028 μM) in sediments from a highly eutrophic subantartic environment with 19 % of organic matter (Encerrada bay, Tierra del Fuego Island). An increase in ammonium concentrations was observed in each treatment. However, significance of dissimilative reduction of nitrate relative to denitrification cannot be assessed using this methodology.

Based on the kinetic constants and the range of nitrate concentration in seawater (SC: 1.0 and 2.6 μmol l^{-1}, MC: 0.9 and 4.4 μmol l^{-1}) obtained in this study, and into to the average density of sediment taken from Marinho et al. (2013) (SC: 1.74 g cm^{-3} and MC: 1.69 g cm^{-3}), it is possible to estimate the in situ nitrate flux in the sediment–water interface per surface unit (∼1 m^2). Assuming that

Table 2 Determination of nitrate reduction rates applying Equ. 2

No. exp. Rc NO3 (μmol g h)	Site	A	B	C
1	SC	−0.0011	−0.0007	−0.0005
2	SC	−0.0056	−0.0057	−0.0072
3	SC	−0.0190	−0.0169	−0.0187
4	SC	−0.0203	−0.0222	−0.0193
5	MC	−0.0018	−0.0019	−0.0018
6	MC	−0.0124	−0.0137	−0.0137
7	MC	−0.0144	−0.0150	−0.0145
8	MC	−0.0252	−0.0221	−0.0264

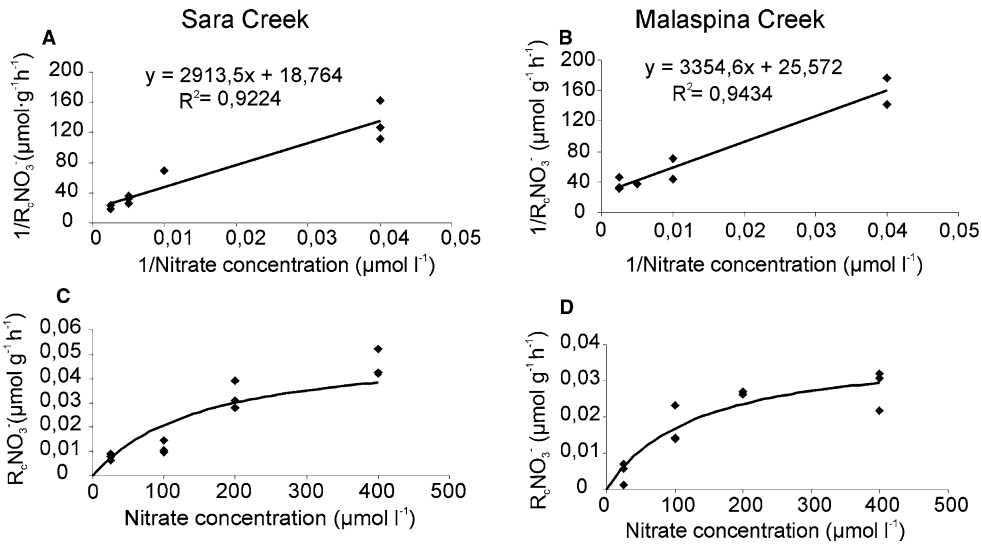

Fig. 3 **a** and **b** Double reciprocal plot of the nitrate concentration vs. nitrate reduction activity obtained to Sara Creek and Malaspina Creeks, respectively; **c** and **d** Nitrate reduction activity ($R_cNO_3^-$) as function of initial nitrate concentration (*black point*); and curve was fitted by use of the Michaelis–Menten equation (*line*) to Sara and Malaspina Creeks, respectively

total nitrate reduction in sediment of SC and MC is restricted to superficial layer of 0.015 m we may expect nitrate fluxes between 9–23 and 6–29 μmol m^{-2} h^{-1}, respectively. These values, in the same costal environment, are one order of magnitude lower than fluxes obtained in situ through opaque benthic chambers (128 ± 1 and 103 ± 68 μmol m^{-2} h^{-1}). Torres et al. (2012) applied both methods on sediments with high anthropogenic influence from Encerrada Bay. They did not find significant differences between the fluxes when both methodologies were applied. This results could be explained by the characteristics of the studied sediment in Encerrada bay which had a high percentage of organic matter (19 %), lower average density (1.26 ± 0.07 g cm^{-3}), a porosity average of 63.1 ± 4.7 %, and predominantly thin granulometry largely contrasting with the sediment used in this study. Another reason could be the higher temperature in which the laboratory experiences were performed (17 ± 0.5 °C), where the in situ seawater temperature was a few degrees lower (12 ± 0.7 °C in SC and 14 ± 0.7 °C in MC). Based on this, we expected to find greater fluxes. Therefore, we believe that these differences are the main reason why we cannot compare the results obtained using both methods.

According to Kaspar (1983), the denitrification process in several sediment types represent between 70 and 95 % of total nitrate consumption. Considering this, for a maximum nitrate flux (23 μmol m^{-2} h^{-1} in SC and 29 μmol m^{-2} h^{-1} in MC), the amount of released molecular nitrogen via denitrification process would be between 16 and 22 μmol m^{-2} h^{-1} in SC and between 20 and 28 μmol m^{-2} h^{-1} in MC, respectively. This may produce a system nutrient loss of about 97–132 and 13870–19,418 kg N monthly in SC and MC, respectively.

The employed methodology in laboratory experiences allowed us to estimate nitrate uptake rate. It also provided information about the use of the nitrate as terminal electron acceptor in an environment without anthropogenic impact. However, molecular nitrogen production (N_2) could not be adequately explained by the methodology applied in this study.

Future investigation should include the analysis of other electron acceptors as Mn-oxides, Fe-oxides, SO_4^{2-} and

CO_2, for a better understanding of the processes involved in degradation of labile organic matter, considering that these are unknown in coastal marine environments from Patagonia, Argentina.

Conclusions

The nitrate flux from the water column toward the sediment would be principally used as a terminal electron acceptor during the oxidation process of organic matter. We conclude that both Sara and Malaspina Creeks act as a sink of nitrate. More research is needed to understand if the reduced nitrate is used for denitrification (N_2 production) or another process to improve knowledge on nitrogen cycling in these environments.

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