

SEDIMENT DENITRIFICATION, DNRA AND ANAMMOX RATES IN TROPICAL FLOODPLAIN LAKE (PANTANAL, BRAZIL)

Viviane Figueiredo Souza¹, Ana Lúcia Santoro¹, Margaretha van Weerelt¹ & Alex Enrich-Prast^{1*}

¹Universidade Federal do Rio de Janeiro (UFRJ), Centro de Ciências da Saúde (CCS), Instituto de Biologia, Departamento de Ecologia, Laboratório de Biogeoquímica, Av. Carlos Chagas Filho, 373, Inter-blocos A-F, Caixa Postal: 68016, Ilha do Fundão - Cidade Universitária, Rio de Janeiro, RJ, Brasil, CEP:21941-971.

Email: vivi.bioufrj@hotmail.com, analusantoro@gmail.com, m.weerelt@gmail.com, aenrichprast@gmail.com*

ABSTRACT

The rates of denitrification, dissimilatory nitrate reduction to ammonia (DNRA) and anammox were evaluated on a typical lake of Pantanal wetlands using the isotope pairing method. Denitrification rates were low at all studied sites, ranging from 3,4 to 34,5 $\mu\text{MN}_2\text{m}^{-2}\text{h}^{-1}$. The rates of O_2 consumption in the sediment were two to three orders of magnitude higher, ranging from 2630 to 3880 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$. A parallel incubation showed that as 50% of added nitrate was reduced to N_2 through denitrification and that anammox activity was negligible. From this result we estimated that the anaerobic reduction of nitrate is equal between denitrification and DNRA processes. Although microorganisms that carry out denitrification and DNRA were present in the lake, as seen in one experiment performed in this study, the rates were very low. Both denitrification and DNRA in this lake are regulated mainly by very low nitrate concentrations ($<5\mu\text{M}$).

Keywords: denitrification; DNRA; ANAMMOX; Pantanal; oxygen consumption.

RESUMO

TAXAS DE DESNITRIFICAÇÃO, RDNA, E ANAMMOX NO SEDIMENTO DE UMA PLANÍCIE ALAGÁVEL TROPICAL (PANTANAL, BRASIL). As taxas de desnitrificação, redução dissimilatória de nitrato a amônia (RDNA) e anammox foram avaliadas em um típico lago da planície de inundação do Pantanal usando o método de pareamento isotópico. As taxas de desnitrificação foram baixas em todos os locais estudados, variando de 3,4 a 34,5 $\mu\text{MN}_2\text{m}^{-2}\text{h}^{-1}$. As taxas de consumo de O_2 no sedimento foram duas ou três ordens de magnitude maior, variando de 263 a 3880 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$. Uma incubação paralela mostrou que 50% do nitrato adicionado foi reduzido a N_2 através da desnitrificação e que a atividade da anammox foi negligenciada. Através desse resultado, nós estimamos que a redução anaeróbica de nitrato é equivalente entre os processos de desnitrificação e RDNA. Apesar dos microorganismos que realizam a desnitrificação e RDNA estarem presentes no lago, como visto no experimento realizado no estudo, as taxas foram muito baixas. Tanto a desnitrificação quanto a RDNA nesse lago, são regulados principalmente pela baixa concentração de nitrato ($<5\mu\text{M}$).

Palavras-chave: desnitrificação; RDNA; ANAMMOX; Pantanal; consumo de oxigênio.

RESUMEN

TASAS DE DENITRIFICACIÓN, RDNA Y ANAMMOX EN LOS SEDIMENTOS UN LAGO TROPICAL DE PLANICIE INUNDABLE (PANTANAL, BRASIL). Las tasas de denitrificación, reducción desasimilatoria del nitrato en amonio y Anammox fueron evaluadas en un lago típico de los humedales de Pantanal, usando el método de emparejamiento isotópico. Las tasas de denitrificación fueron bajas en todos los sitios de estudio, variando desde 3,4 hasta 34,5 $\mu\text{MN}_2\text{m}^{-2}\text{h}^{-1}$. Las tasas de consumo de O_2 en los sedimentos fueron dos a tres órdenes de magnitud más altas, desde 2630 hasta 3880 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$. Una incubación paralela mostró que hasta un 50% del nitrato añadido fue reducido a N_2 a través de denitrificación y que la actividad Anammox fue mínima. A partir de este resultado se estimo que la reducción anaeróbica del nitrato es igual

entre los procesos de denitrificación y RDNA. Aunque los microorganismos que llevan a cabo la denitrificación y la RDNA estaban presentes en el lago, tal como se observó en un experimento llevado a cabo en el marco de este estudio, las tasas fueron muy bajas. Tanto la denitrificación y el DNRA en este lago están reguladas por las muy bajas concentraciones de nitrato ($<5\mu\text{M}$).

Palabras clave: denitrificación; RDNA; ANAMMOX; Pantanal; consumo de oxígeno.

INTRODUCTION

Understanding the interactions and different pathways of the nitrogen processes is a key component to understand and quantify the relative importance of this element on ecosystems structure and function (Enrich-Prast 2005). Nitrogen is one of the key nutrients in regulating ecosystem production (Zehr *et al.* 2000) and its excess can promote eutrofication (Schindler *et al.* 2008). Nitrous oxide (N_2O) is a greenhouse gas that can also destroy the ozone layer (Galloway *et al.* 2008). N_2O can be produced as a byproduct of nitrification or denitrification.

Denitrification is a process carried out by facultative anaerobic bacteria and it consists of dissimilatory anaerobic reduction of oxidized elements of nitrogen (NO_2^- and NO_3^-) to atmospheric dinitrogen (N_2) and/or nitrous oxide (N_2O) (Schmidt *et al.* 2000). This process occurs in areas with low oxygen concentration or totally anoxic, located immediately below the oxic zone, since this area is the production of nitrite and nitrate via nitrification, subsidizing the denitrifying activity (Kuschik *et al.* 2003). The result is the loss of nitrogen from the system because most organisms cannot assimilate N_2 as nitrogen source so this gas is released into the atmosphere (Nielsen & Glud 1996, Menges *et al.* 1997).

The dissimilatory reduction of nitrate to ammonia (DNRA) is a process carried out by strictly anaerobic fermentative bacteria (Philippot & Germon 2005) that compete for substrate with denitrifying bacteria, since both are heterotrophs, occupy the anoxic zone and use nitrate as electron acceptor (Poulin *et al.* 2007). What will determine the density of the respective bacteria and which process will prevail is the proportion between the availability of organic carbon and the electron acceptors (Enrich-Prast 2005, Scott *et al.* 2008). The greater the ratio of the carbon/electron acceptor, the highest percentage of nitrate will be used by DNRA bacteria. This process does

not export nitrogen from an ecosystem but instead, feeds the nitrogen cycle, because its final product is ammonia, an easily assimilated molecule by the biota (An & Gardner 2002, Scott *et al.* 2008).

ANAMMOX is the process of anaerobic oxidation of ammonia using nitrite as electron acceptor and it is mediated by obligatory anaerobic bacteria (Dalsgaard *et al.* 2005). In this process there is no degradation of organic matter but oxidation of ammonia to atmospheric nitrogen, in order to obtain energy. Studies indicate the ANAMMOX process is an important process of removing nitrogen from marine ecosystems (Dalsgaard *et al.* 2003, Kuypers *et al.* 2003, Engstrom *et al.* 2005) and it is responsible for up to half the total loss of this nutrient in the oceans (Dalsgaard *et al.* 2005).

The processes connected to the nitrogen cycle in tropical ecosystems are still poorly known (Serra *et al.* 2004, Piña-Ochoa & Alvares-Cobelas 2006) especially in flood plains (Scott *et al.* 2008). These areas are extremely important for biodiversity as they have a significant number of plants and animals adapted to flooded environment. In addition, they exert control over the hydrological dynamic of rivers and its tributaries, retaining excess water that reaches the ecosystem during the flooding period. Tropical regions have large numbers of wetlands due to geological, hydrological and climate conditions (Serra *et al.* 2004) and these ecosystems are often limited by nitrogen (Hordijk *et al.* 1986). A good example of this type of ecosystem, in the tropics, is the Pantanal, where studies focusing on processes of the nitrogen cycle in aquatic environments have not been performed yet.

The purpose of this study was to quantify and evaluate the relative importance of the anaerobic processes that use nitrite/nitrate as electron acceptor denitrification, DNRA and ANAMMOX in the sediment of a lake located at the largest world tropical wetland (Pantanal, Brazil), during wet period of the year (2001) of severe drought.

MATERIAL AND METHODS

This study was conducted in the lake “Baia das Pedras”, located at the floodplain of the Mato Grosso’s Pantanal, in West Central Brazil (Figure 1). The Pantanal is the world largest floodplain system with an annual variation in water level. This large biome is part of the Upper Paraguay basin formed

by the Paraguay River and its tributaries. During the flooding period the waters are retained and stored allowing the flow from the spring to the plain. The natural vegetation also influences this process, acting as a natural filter to retain sediment, nutrients and particulate organic matter arising out of rivers, during high water, connect with bays and lakes (Alho & Gonçalves 2005).

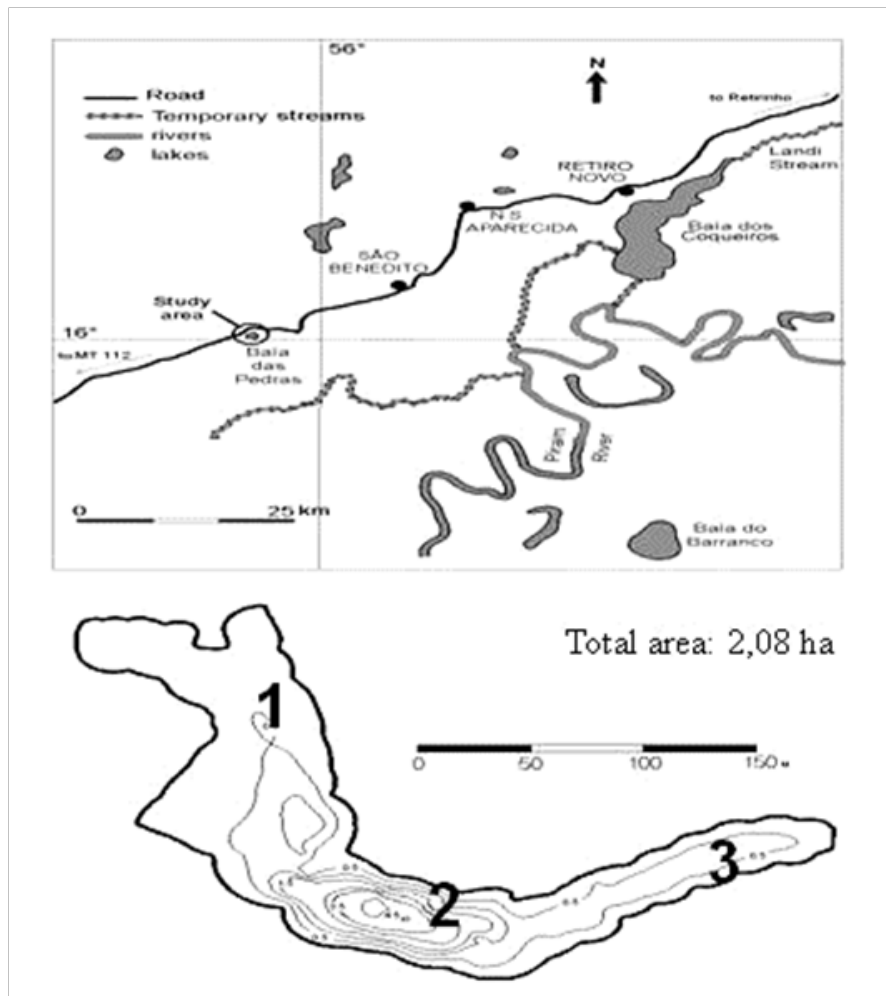


Figure 1. Baía das Pedras, Pantanal Mato-grossense, Brazil. Geographical position, sampled points (1-Pedra, 2-Buraco and 3-Coqueiro) and bathymetric data during the drought period, in September of 1999 (Adapted of Nogueira *et al.*, 2002).

Figura 1. Baía das Pedras, Pantanal Mato-grossense, Brasil. Posição geográfica, pontos de coleta (1-Pedra, 2-Buraco e 3-Coqueiro) e dados batimétricos durante o período de seca, em Setembro de 1999 (Adaptado de Nogueira *et al.*, 2002).

Baía das Pedras has 2 acres of extension and belongs to the sub-region of the Poconé’s Pantanal (16°24’S and 56°10’W). It was originated from an abandoned meander that resulted in a lake, as a result of the fluvial river dynamics of this area. During the sampling period there was no evidence of connection with another aquatic environment (Figure 1) because was the end of wet period and the level water had lowered.

We collected five replicates of sediment samples in points Pedra (1) and Coqueiro (3) and two replicates in point Buraco (2), shown in Figure 1. The sediment samples were collected in plexiglass cores (3,5cm in diameter and 10cm high), one time during the wet period in September 2001. This year was had a drastic drought and, during the high water period, the level water was atypical, being lower (Galdino *et al.* 2002). The cores were transported to the laboratory

keeping the sediment-water interface intact. In the laboratory, the cores were placed in small aquarium with 10 centimeters of water above the cores to keep

the oxic conditions in water over the sediment. The water circulation was enhanced by the use of small magnetic stirrers.



Figure 2. Picture of the schematic incubation system showing the aquarium with water and sediment samples from Baía das Pedras during the stabilization time.

Figura 2. Foto esquemática do sistema de incubação mostrando o aquário com amostras de água e sedimento da Baía das Pedras, durante o período de incubação.

After the stabilization period of 3 hours, the initial concentration of oxygen in the water column was determined (Oximeter Mettler). The cores were closed and the final concentration of oxygen was obtained after 2 hours of incubation. The rates of oxygen consumption in the sediment were calculated according to Dalsgaard *et al.* (2000).

After the final measurement of oxygen, the cores went through a new stabilization period of two hours in order to determine the actual rates of denitrification in the sediment (Nielsen 1992). Different volumes of a solution $\text{Na}^{15}\text{NO}_3$ (5mM) were added in each of the cores and the water column was mixed with the use of magnetic stirrers. Five minutes after the addition of this solution, water samples were retrieved for an initial analysis of the rate of $^{15}/^{14}\text{NO}_3^-$. After a two hour incubation period, the final water aliquots were removed and placed in exetainers (Labco. Co) with ZnCl_2 , to prevent any microbial activity and these conditions were suitable for further mass spectrometry analysis of the proportions of $^{28}\text{N}_2$, $^{29}\text{N}_2$, $^{30}\text{N}_2$ (Carbo Erba). Subsequently, samples of N_2 , in its different isotopic forms were removed with an injection of helium gas in the exetainers, forming a headspace in the vials. After shaking, the N_2 was transferred to the

headspace, allowing its removal for analysis by mass spectrometry.

The resulting isotopic compositions are $^{14}\text{N}^{14}\text{N}$ ($^{28}\text{N}_2$), $^{14}\text{N}^{15}\text{N}$ ($^{29}\text{N}_2$) and $^{15}\text{N}^{15}\text{N}$ ($^{30}\text{N}_2$). The amount of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ isotopes produced was calculated using its proportions, according to Nielsen (1992). The potential denitrification rate (^{15}D) was calculated through the sum of the concentrations of ^{15}N forms produced: $^{15}\text{D} = (^{29}\text{N}_2) + 2 (^{30}\text{N}_2)$. The rate of real denitrification (^{14}D), which represents the denitrification $^{14}\text{NO}_3^-$ already present in the medium or produced by nitrification, was based on the calculation of the potential denitrification and in the concentrations of the isotopic forms, which derived from a binomial distribution: $^{14}\text{D} = (^{29}\text{N}_2) / 2 (^{30}\text{N}_2) \times ^{15}\text{D}$.

The relative rates of denitrification, DNRA and ANAMMOX was assessed by carrying out potential incubations with the first cubic centimeters of sediment surface of each of the sampling sites. After homogenization, these sediments were transferred to 6ml exetainers and different forms of inorganic nitrogen were added according to the process studied. Finally the bottles were filled with anoxic water (previously bubbled with N_2).

To evaluate the relative rates of denitrification and DNRA, 50 μ M of an anoxic solution of $^{15}\text{NO}_3^-$ was added and the amount of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ isotopes produced was calculated according to Nielsen (1992). In this comparison between denitrification and DNRA, we assumed that the production of isotopes $^{29}\text{N}_2$ and $^{30}\text{N}_2$ was derived from the denitrification and the percentage of $^{15}\text{NO}_3^-$, which was not reduced to such isotopes, as having been reduced to $^{15}\text{NH}_4^+$ via DNRA.

The relative rates of ANAMMOX were evaluated adding 500 μ M of $^{15}\text{NH}_4^+$ and 50 μ M $^{14}\text{NO}_2^-$ to the exetainers with anoxic sediment (as described above) and its relative activity was estimated from the production of $^{29}\text{N}_2$, after 48 hours of incubation, through same procedure used for the other two above processes, using a solution of ZnCl_2 and helium for later analysis by mass spectrometry.

All statistical tests were performed in GraphPad Prism 4.0 program. The normality of data was evaluated using the Kolmogorov-Smirnov test and were analyzed using parametric tests. We compared the relative rates of denitrification, DNRA and ANAMMOX in the sampling sites using T-Student test ($p < 0,05$). The relationship between the rates of denitrification and oxygen consumption were evaluated using a Pearson correlation coefficient test. The comparison between the processes of actual of denitrification, oxygen consumption and DNRA ANAMMOX were made only in sites Pedra and Coqueiro, because we had five replicas of sediment samples from these two points and only two replicas from Buraco, which no enable to us use statistical analysis.

Table 1. Average values (surface, 70, 140 and 220 depth centimeters) of water limnological characteristics from Baía das Pedras lake (Adapted of Nogueira *et al.* 2002).

Tabela 1. Média dos valores de características limnológicas da água da Baía das Pedras (Adaptado de Nogueira *et al.* 2002).

Depth (m)	Secchi disk (m)	pH	Temperature (°C)	Electric Conductivity (uS/cm)	PO_4^- (uM)	NO_3^- (uM)
3,8	0,3	6,3	24,5	3,8	< 0,05	0,2

RESULTS

Some of the limnological data characterizing the Baía das Pedras lake (Table 1) and are described by Nogueira *et al.* (2002).

All data were normally distributed (Kolmogorov-Smirnov, $p < 0,05$) and parametric tests were applied. The actual rates of denitrification, oxygen consumption and the relative rates of

denitrification, DNRA and anammox presented no significant differences between the studied sampling sites (T-Student test, $p < 0,05$). The increasing concentrations additions of $^{15}\text{NO}_3^-$ in the sediment from Pedras and Coqueiro sites caused an increase on the values of ^{15}D but not the on the ^{14}D values, indicating that the method was applied correctly and that the actual rates of denitrification in the environment were measured (Figure 3).

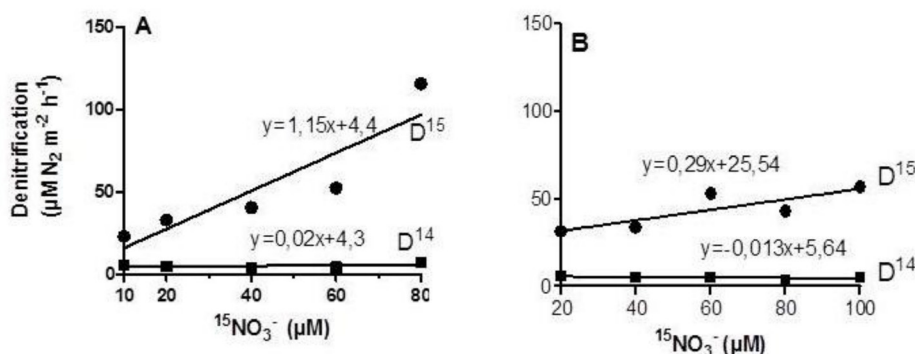


Figure 3. Real (D14) and Potential (D15) denitrification rates in the sediment from Pedra (A) and Coqueiro (B) sampling regions.
Figura 3. Taxas de desnitrificação Real (D14) e Potencial (D15) do sedimento das regiões amostradas, Pedra (A) e Coqueira (B).

The actual rates of denitrification in the sediment were low and similar between Pedras and Coqueiro sites, ranging between 6,8 and 14 $\mu\text{MNm}^{-2}\text{h}^{-1}$. The highest rates of this process were observed at Buraco area, varying between 52,6 and 69 $\mu\text{MNm}^{-2}\text{h}^{-1}$ (Figure 4A). The three sampling sites had similar values of oxygen consumption in the sediment, varying between 2,63 and 3,88 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$ (Figure 4B).

The actual rates of denitrification and oxygen consumption in the sediment were not significantly correlated (Pearson, $p < 0,05$) in laboratory experiment

(Figure 4C). The denitrification rates in the studied ecosystem were among the lowest values obtained from literature that also used the isotopic pairing technique (Table 2).

The relative contribution of each process to the consumption of nitrate was similar in the three sampling sites and the rates of denitrification and RDNA were equally responsible for 50% of its consumption. The relative contribution of ANAMMOX in the nitrite reduction was very low, ranging between 0,0 and 0,07% (Table 3).

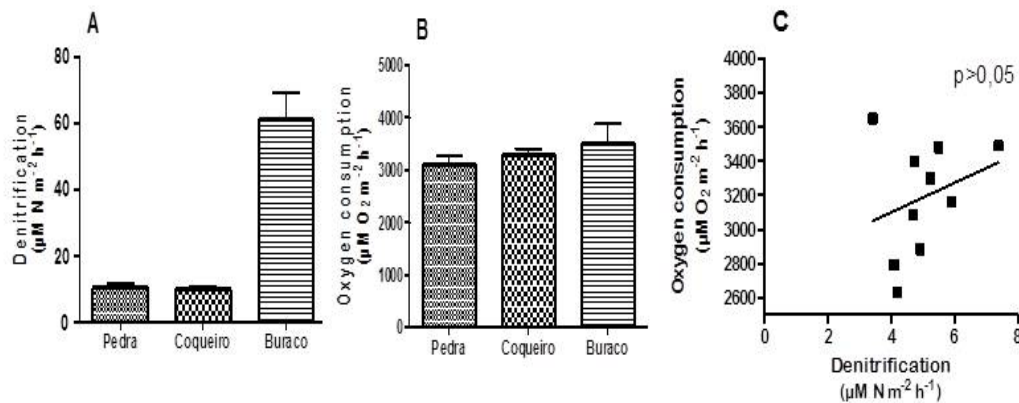


Figure 4. A) Denitrification rates; B) Oxygen consumption rates and C) correlation between denitrification and oxygen consumption in Baía das Pedras. Values in Pedra and Coqueiro stations represent average \pm SD, ($n=5$). Values in Buraco station are based on an average of 2 measurements only.

Figura 4. A) Taxa de desnitrificação; B) Taxa de consumo de oxigênio e C) correlação entre desnitrificação e consumo de oxigênio na Baía das Pedras. Valores das estações Pedra e Coqueiro representam média \pm DP, ($n=5$). Valores da estação Buraco são apenas a média de 2 medidas.

DISCUSSION

The rate of oxygen consumption in the sediment varied between 2634 and 3882 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$, being quite high compared to those obtained in temperate aquatic environments (1125 $\mu\text{M O}_2\text{m}^{-2}\text{h}^{-1}$, Hordijk *et al.* 1987, between 62,5 and 187,5 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$, Silverberg *et al.* 2000, between 1000 and 2000 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$, An & Gardner 2002, between 141 and 1890 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$, Tomaszec & Czerwieniec 2003, between 1300 and 1800 $\mu\text{MO}_2\text{m}^{-2}\text{h}^{-1}$, Poulin *et al.* 2007). These high oxygen consumption rates at the studied lake can be attributed to the fact that this environment receives, when the level of water is very high and there are connections to other aquatic systems, and accumulates large amounts of organic matter as a consequence of its location, inside a flood plain, supporting an intense decomposing activity in the sediment. Another important factor in the efficiency of the processes of decomposition is the higher temperatures that

stimulate decomposition (Tomaszec & Czerwieniec 2003, Marotta *et al.* 2009). The Pantanal is a biome that has temperatures relatively high throughout the year in comparison to temperate environments and due to the high availability of organic matter, oxygen consumption is intense, explaining the high values observed.

According to Piña-Ochoa & Alvarez-Cobelas (2006), studies on sediment denitrification in tropical environments are scarce, difficult to carry out comparisons. However, studies conducted in temperate lakes, using the isotope pairing method of Nielsen (1992), showed much higher values (up to 6,15 $\text{gNha}^{-1}\text{d}^{-1}$; Table 1). The denitrification rate in this study (0,0129 $\text{gNha}^{-1}\text{d}^{-1}$) shows the degree of limitation of this process in the studied environment, which cannot be explained either by temperature nor availability of labile organic C.

The main explanation for the low denitrification rates observed in this environment is the very low

concentration of nitrate (Table 3). Denitrifying microorganisms are present in the environment, as observed by the relative potential measurements (Figure 4). The isotope pairing methodology, used in our study, is widely accepted in the literature (Nielsen 1992, Lohse *et al.* 1996, Minjeaud *et al.* 2008) and is considered appropriate for measuring actual denitrification rates.

Since most of the available nitrogen in the sediment is in the form of ammonium (Fenchel *et al.* 1998), nitrate produced exclusively in aerobic zone through nitrification, diffuses to the anaerobic zone subsidizing the denitrifying activity (Risgaard *et al.* 1998, Tomaszec & Czerwieniec 2003, Poulin *et al.* 2007, Rao *et al.* 2008). According to Dong *et al.* (2006), in environments with low availability of nitrate, nitrification can subsidize up to 75% of the total N_2 produced. Thus, in the marsh environment where there are ideal conditions for microbial processes to occur in the sediment, nitrification would happen coupled with denitrification (coupled nitrification-denitrification) allowing a high emission of nitrogen, even with low concentrations of available nitrate in the water column. However, the high rates of oxygen consumption could be inhibiting the occurrence of nitrification and indirectly limiting the activity of denitrifying bacteria.

The rates of oxygen consumption determine the depth of the aerobic zone on the surface of the sediment and consequently can regulate in different ways, the denitrifying activity, either directly by creating anoxic conditions for the occurrence of the process or indirectly by controlling the nitrification activity (Jensen *et al.* 1994). The lack of relationship between the rates of denitrification and oxygen consumption suggests an indirect regulation of denitrifying activity by the oxygen dynamics in the studied environment.

Based on the data presented in Table 2 is possible to observe that the bacteria responsible for both denitrification and DNRA processes are present and active in this environment. The activity of these organisms is directly related to the presence of labile organic matter and, as recognized in the literature, favoring the process of DNRA over denitrification, when there is a high availability of organic matter (Christensen *et al.* 2000). The Pantanal is an ecosystem with a wide availability of labile C, so it was expected a high rate of both processes. However the low nitrate availability was also limiting DNRA. The occurrence of both groups of microorganisms can be explained because denitrifying bacteria are facultative anaerobes and can grow under aerobic conditions, and DNRA bacteria are strictly anaerobes that can use other electron acceptors than nitrate (Fenchel *et al.* 1998).

Table 2. Compiled sediment denitrification rates, using the isotope pairing technique. Note that the huge majority of the data are from temperate environments.

Tabela 2. Compilação de dados de taxas de desnitrificação em sedimento, usando a técnica de pareamento isotópico. Observe que a maioria dos dados é de ambientes temperados.

Name	Rate (g N ha ⁻² d ⁻¹)	Data source
Lake Sobygaard So (Denmark)	152	Risgaard-Petersen <i>et al.</i> (1998)
Bay Aarhus (Denmark)	1344	Nielsen & Glud (1996)
Coastal ecosystem Gullmar Fjord (Sweden)	22,2	Sundbäck <i>et al.</i> (2004)
Coastal ecosystem Svalvard island (Norway)	1,9	Blackburn <i>et al.</i> (1996)
Estuary Young Sound (Denmark)	6,3	Risgaard & Glud (2004)
Estuary Patuxent River (USA)	299	Jenkins & Kemp (1984)
Estuary St. Lawrence (Canada)	11,1	Wang <i>et al.</i> (2003)
Lake Ringsjön (Sweden)	100,8	Svensson <i>et al.</i> (2001)
Baltic Sea (Gulf of Finland)	54,6	Tuominen <i>et al.</i> (1998)
Baltic Sea (Gulf of Finland)	74,2	Tuominen <i>et al.</i> (1998)
Bering Sea	6048	Haines <i>et al.</i> (1981)

Continuaton Table 2

Name	Rate (g N ha ⁻² d ⁻¹)	Data source
Bothnian Sea	42	Tuominen <i>et al.</i> (1998)
North Sea	44,4	Lohse <i>et al.</i> (1996)
Wadden Sea (Germany)	20,2	Jensen <i>et al.</i> (1996)
Wadden Sea (Germany)	201,6	Jensen <i>et al.</i> (1996)
Green Creek (Antarctica)	50,4	Gooseff <i>et al.</i> (2004)
Sugar Creek (USA)	403,2	Böhlke <i>et al.</i> (2004)
Branch Walker (USA)	40	Mulholland <i>et al.</i> (2004)
Arctic Sea (Norway)	19,6	Blackburn <i>et al.</i> (1996)
Baltic Sea (Gulf of Finland)	54,6	Tuominen <i>et al.</i> (1998)
Baltic Sea (Gulf of Finland)	74,2	Tuominen <i>et al.</i> (1998)
Wetland Baía das Pedras (Brazil)	0,0129	This Study

Table 3. Relative potential contribution of denitrification, dissimilatory nitrate reduction to ammonia (DNRA) and anaerobic ammonium oxidation (ANAMMOX) measured in terms of nitrate amendment in the three sampled points, in Baía das Pedras.

Tabela 3. Contribuição potencial relativa da desnitrificação, redução dissimilatória de nitrato à amônia (RDNA) e oxidação anaeróbica do amônio (ANAMMOX), medidos em termos de alteração de nitrato em três pontos de coleta, na Baía das Pedras.

	Denitrification (%)	DNRA (%)	ANAMMOX (%)
Pedra	49,6	50,4	0,07
Coqueiro	48,90	51,1	0,00
Buraco	52,00	48	0,02

Low concentrations of nitrate, in general being indicative of low concentration of nitrite also, observed in the bay are also the probable explanation for the nearly absent ANAMMOX activities, since this is a process dependent on nitrite and ammonium (Kartal *et al.* 2007). Furthermore, the chemosynthetic microorganisms involved in this process, may have been outcompeted by other nitrate utilizing anaerobes, since heterotrophic processes prevail in environments with excessive load of organic matter (Dalsgaard *et al.* 2005). Another factor that may be regulating this process is the temperature. According to Rysgaard & Glud (2004), in marine environments, the ideal ANAMMOX temperature is below 15°C and the efficiency of ANAMMOX bacteria can significantly decrease at 30°C. It is possible to speculate that in the Pantanal, due to

high temperatures and low nitrite concentrations, ANAMMOX is probably not a relevant process.

A comparison of denitrification and oxygen consumption rates would indicate the relative contribution of denitrifying bacteria to all mineralization of organic matter. Converting both processes in terms of carbon dioxide (CO₂) production, we can estimate that denitrification has a low participation (less than 0,5%) in all organic matter mineralization in the sediment. But more studies, in other periods of year, are necessary for corroborate this data.

ACKNOWLEDGMENTS: The authors are grateful to CNPq, CAPES and FAPERJ for the financial support, scholarships to Viviane Figueiredo Souza and Ana Lucia Santoro and a research fellowship to Alex Enrich-Prast. We would like to thank Dr. Flavia Barros Nogueira and her students for her invaluable support during sampling.

REFERENCES

- ALHO, C.J.R. & GOLÇALVES, H.C. 2005. *Biodiversidade do Pantanal: Ecologia e Conservação*. Editora UNIDERP; Campo Grande, MS. 143p.
- AN, S. & GARDNER, W.S. 2002. Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). *Marine Ecology Progress Series*, 237: 41-50, <http://dx.doi.org/10.3354/meps237041>
- BLACKBURN, T.H.; HALL, P.O.J.; HULTH, S. & LANDEN, A. 1996. Organic-N loss by efflux and burial associated with a low efflux of inorganic N and with nitrate assimilation in Arctic sediments (Svalbard, Norway). *Marine Ecology Progressive Ser.*, 141: 283-293, <http://dx.doi.org/10.3354/meps141283>
- BÖHLKE, J.K.; HARVEY, J.W. & VOYTEK, M.A. 2004. Reach-scale isotope tracer experiment to quantify denitrification and related processes in a nitrate-rich stream, midcontinent United States. *Limnology and Oceanography*, 49: 821-838, <http://dx.doi.org/10.4319/lo.2004.49.3.0821>
- CHRISTENSEN, P.B.; RYSGAARD, S.; SLOTH, N.P.; DALSGAARD, T. & SCHWAERTER, S. 2000. Sediment mineralization, nutrient fluxes, denitrification and dissimilatory nitrate reduction to ammonium in an estuarine fjord with sea cage trout farms. *Aquatic Microbial Ecology*, 21: 73-84, <http://dx.doi.org/10.3354/ame021073>
- DALSGAARD, T.; NIELSEN, L.P.; BROTHAS, V.; VIAROLI, P.; UNDERWOOD, G.J.C.; NEDWELL, D.B.; SUNDBÄCK, K.; RYSGAARD, S.; MILES, A.; BARTOLI, M.; DONG, L.F. ; THORNTON, D.C.O.; OTTOSEN, L.D.M.; CASTALDELLI, G. & RISGAARD-PETERSEN, N. 2000. *Protocol handbook for NICE – Nitrogen Cycling in Estuaries: a project under the EU research programme: Marine Science and Technology (MAST III)*. National Environment Research Institute, Silkeborg, Dinamarca, 66p.
- DALSGAARD, T.; CANFIELD, D.E.; PETERSEN, J.; THAMDRUP, B. & ACUÑA-GONZÁLES, J. 2003. N₂ production by the anammox reaction in the anoxic water column of Golfo Dulce, Costa Rica. *Nature*, 422: 606-608, <http://dx.doi.org/10.1038/nature01526>
- DALSGAARD, T.; THAMDRUP, B. & CANFIELD, D.E. 2005. Anaerobic ammonium oxidation (anammox) in the marine environment. *Research in Microbiology*, 156: 457-464, <http://dx.doi.org/10.1016/j.resmic.2005.01.011>
- DONG, L.F.; NEDWELL, D.B. & STOTT, A. 2006. Sources of nitrogen and nitrous oxide formation in sediments of the hypernitrified Colne, the nitrified Humber, and the oligotrophic Conwy estuaries, United Kingdom. *Limnology and Oceanography*, 51: 545-557, http://dx.doi.org/10.4319/lo.2006.51.1_part_2.0545
- ENGSTROM, P.; DALSGAARD, T.; HULTH, S. & ALLER, R.C. 2005. Anaerobic ammonium oxidation by nitrite (anammox): Implications for N₂ production in coastal marine sediments. *Geochimica et Cosmochimica Acta*, 69(8): 2057-2065, <http://dx.doi.org/10.1016/j.gca.2004.09.032>
- ENRICH-PRAST, A. 2005. Caminhos do Nitrogênio em Ecossistemas aquáticos Continentais. Pp. 209-227. In: F. Roland, D. Cesar, & M. Marinho (eds.). Lições de Limnologia. Rima, São Carlos, SP. 532p.
- FENCHEL, T.; KING, G.M. & BLACKBURN, T.H. 1998. *Bacterial Biogeochemistry: The ecophysiology of mineral cycling*. Second edition. Academic Press. Copenhagen, Dinamarca. 307p.
- GALDINO, S.; VIEIRA, L.M.; OLIVEIRA, H. DE. & CARDOSO, E.L. 2002. O mais longo e intenso ciclo de cheia do Pantanal. In: II Simpósio de Recursos Hídrico do Centro-Oeste – SIMPORH. 2002. Campo Grande. Águas do centro-oeste: a fronteira é hídrica. Campo Grande: ABRH-MS: UFMS, 2002. CD-ROM (trab 037).
- GALLOWAY, J.N.; TOWNSEND, A.R.; ERISMAN, J.W.; BEKUNDA, M.; CAI, Z.; FRENEY, J.R.; MARTINELLI, L.A.; SEITZINGER, S.P. & SUTTON, M.A. 2008. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. *Science*, 320: 889-892, <http://dx.doi.org/10.1126/science.1136674>
- GOOSEFF, M.N.; MCKNIGHT, D.M.; RUNKEL, R.L. & DUFF, J.H. 2004. Denitrification and hidrologic transient storage in a glacial meltwater stream, McMurdo Dry Valleys, Antarctica. *Limnology and Oceanography*, 49: 1884-1895, <http://dx.doi.org/10.4319/lo.2004.49.5.1884>
- HAINES, J.R.; ATLAS, R.M.; GRIFFITHS, R.M. & MORITA, R.Y. 1981. Denitrification and nitrogen fixation in Alaskan continental shelf sediments. *Applied Environment Microbiology*, 41: 412-421.
- HORDIJK, C.A.; SNIEDER, M.; VAN ENGELEN, J.J.M. & CAPPERBERG, T.E. 1987. Estimation of bacterial nitrate reduction rates at in situ concentrations in freshwater sediments. *Applied and Environmental Microbiology*, 53(2): 217-223.
- JENKIS, M.C. & KEMP, W.M. 1984. The coupling of nitrification and denitrification in two estuarine sediments. *Limnology and Oceanography*, 29: 609-619, <http://dx.doi.org/10.4319/lo.1984.29.3.0609>

- JENSEN, K.; SLOTH, N.P.; RISGAARD-PETERSEN, N.; RYSGAARD, S. & REVSBECH, N.P. 1994. Estimation of nitrification and denitrification from microprofiles of oxygen and nitrate in model sediment systems. *Applied and Environmental Microbiology*, 60: 2094-2100.
- JENSEN, J.P.; JENSEN, M.H. & KRISTENSEN, E. 1996. Nitrification and denitrification in Wadden Sea sediments (Konigshafen, Island of Sylt, Germany) as measured by IPT and isotope dilution. *Aquatic Microbiology Ecology*, 11: 181-191, <http://dx.doi.org/10.3354/ame011181>
- KARTAL, B.; KUYOERS, M.M.M; LAVIK, G.; SCHALK, J.; OP DEN CAMP, H.J.M.; JETTEN, M.S.M. & STOUS, M. 2007. Anammox bacteria disguised as denitrifiers: nitrate reduction to dinitrogen gas via nitrite and ammonium. *Environmental Microbiology*, 9: 635-642.
- KUSCHK, P.; WIEXNER, A.; KAPPELMEYER, U.; WEIX-BRODT, E.; KASTNER, M. & STOTTMEISTER, U. 2003. Annual cycle of nitrogen removal by a pilot-scale subsurface horizontal flow in a constructed wetland under moderate climate. *Water Research*, 37: 4236-4242, [http://dx.doi.org/10.1016/S0043-1354\(03\)00163-5](http://dx.doi.org/10.1016/S0043-1354(03)00163-5)
- KUYPERS, M.M.M.; SLIEKERS, A.O.; LAVIK, G.; SCHMID, M.; JORGENSEN, B.B.; KUENEN, J.G.; DAMSTÉ, J.S.S.; STROUS, M. & JETTEN, M.S.M. 2003. Anaerobic ammonium oxidation by anammox bacteria in the Black Sea. *Nature*, 422: 608-610, <http://dx.doi.org/10.1038/nature01472>
- LOHSE, L.; KLOOSTERHUIS, H.T.; RAAPHRST, W. & HELDER, W. 1996. Denitrification rates as measured by the isotope pairing method and by the acetylene inhibition technique in continental shelf sediments of the North Sea. *Marine Ecology Progressive Series*, 132: 169-179, <http://dx.doi.org/10.3354/meps132169>
- MAROTTA, H.; DUARTE, C.M.; SOBEK, S. & ENRICH-PRAST, A. 2009. Large CO₂ disequilibria in tropical lakes. *Global Biogeochemical cycles*, 23: 1-4, <http://dx.doi.org/10.1029/2008GB003434>
- MENGIS, M.; GACHTER, R. & WEHRLI, B. 1997. Sources and sinks of nitrous oxide (N₂O) in deep lakes. *Biogeochemistry*, 38: 281-301, <http://dx.doi.org/10.1023/A:1005814020322>
- MINJEAUD, L.; BONIN, P.C. & MICHOTÉY, V.D. 2008. Nitrogen fluxes from marine sediments: quantification of the associated co-occurring bacterial processes. *Biogeochemistry*, 90: 141-157, <http://dx.doi.org/10.1007/s10533-008-9241-4>
- MULHOLLAND, P.J.; VALETT, H.M.; WEBSTER, J.R.; THOMAS, S.A.; COOPER, L.W.; HAMILTON, S.K. & PETERSON, B.J. 2004. Stream denitrification and total nitrate uptake rates measured using a field ¹⁵N tracer addition approach. *Limnology and Oceanography*, 49: 809-820, <http://dx.doi.org/10.4319/lo.2004.49.3.0809>
- NIELSEN, L.P. 1992. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microbiology Ecology*, 86: 357-362, <http://dx.doi.org/10.1111/j.1574-6968.1992.tb04828.x>
- NIELSEN, L. P. & GLUD, R.N. 1996. Denitrification in a coastal sediment measured *in situ* by the nitrogen isotope pairing technique applied to a benthic flux chamber. *Marine Ecology Progress Series*, 137: 181-186, <http://dx.doi.org/10.3354/meps137181>
- NOGUEIRA, F.S.; SILVA, R.L.; SILVA, A.J.; SOUZA, M.D & BACHEGA, I. 2002. Seasonal and diel limnological differences in a tropical floodplain lake (Pantanal of Mato Grosso, Brazil). *Acta Limnologica Brasiliensis*, 14 (3): 17-25.
- PHILIPPOT, L. & GERMON, J.C. 2005. Contribution of bacteria to initial input and cycling of nitrogen in soils. *Soil Biology*, 3: 159-176, http://dx.doi.org/10.1007/s10073-540-26609-7_8
- PIÑA-OCHOA, E. & ALVARES-COBELAS, M. 2006. Denitrification in aquatic environments: a cross-system analysis. *Biogeochemistry*, 81: 111-130, <http://dx.doi.org/10.1007/s10533-006-9033-7>
- POULIN, P.; PELLETIER, E. & SAINT-LOUIS, R. 2007. Seasonal variability of denitrification efficiency in northern salt marshes: An example from the St. Lawrence Estuary. *Marine Environmental Research*, 63: 490-505, <http://dx.doi.org/10.1016/j.marenvres.2006.12.003>
- RAO, A.M.F.; MCCARTHY, M.J.; GARDNER, W.S. & JAHNKE, R.A. 2008. Respiration and denitrification in permeable continental shelf deposits on the South Atlantic Bight: N₂:Ar and isotope pairing measurements in sediment column experiments. *Continental Shelf Research*, 28: 602-613, <http://dx.doi.org/10.1016/j.csr.2007.11.007>
- RISGAARD-PETERSEN, N.; NIELSEN, L.P. & BLACKBURN, T.H. 1998. Simultaneous measurement of benthic denitrification, with the isotope pairing technique and the N₂ flux method in a continuous flow-through system. *Water Research*, 32(11):3371-3377, [http://dx.doi.org/10.1016/S0043-1354\(98\)00121-3](http://dx.doi.org/10.1016/S0043-1354(98)00121-3)
- RYSGAARD, S. & GLUD, R.D. 2004. Anaerobic N₂ production in Arctic sea ice. *Limnology and Oceanography*, 49: 86-94, <http://dx.doi.org/10.4319/lo.2004.49.1.0086>
- SCHMIDT, I.; ZART, D. & BOCK, E. 2001. Effects of gaseous NO₂ on cells of *Nitrosomonas eutropha* previously incapable of

- using ammonia as an energy source. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology*, 79: 39-47, <http://dx.doi.org/10.1023/A:1010269331350>
- SCHINDLER, D.W.; HECKY, R.E.; FINDLAY, D.L.; STAIN-TON, M.P.; PARKER, B.R.; PATERSON, M.J.; BEATY, K.G.; LYNG, M. & KASIAN, E.M. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *PNAS*, 32:105. 11254-11258.
- SCOTT, J.T.; MCCARTHY, M.J.; GARDNER, W.S. & DOYLE, R.D. 2008. Denitrification, dissimilatory nitrate reduction to ammonium, and nitrogen fixation along a nitrate concentration gradient in a created freshwater wetland. *Biogeochemistry*, 87: 99-111, <http://dx.doi.org/10.1007/s10533-007-9171-6>
- SERRA, T.; FERNANDO, H.J.S. & RODRÍGUEZ, R.V. 2004. Effects of emergent vegetation on lateral diffusion in wetlands. *Water Research*, 38: 139-147, <http://dx.doi.org/10.1016/j.watres.2003.09.009>
- SILVERBERG, N.; SUNDBY, B.; MUCCI, A.; ZHONG, S.J.; ARAKAKI, T.; HALL, P.; LANDEN, A. & TENGBERG, A. 2000. Remineralization of carbon in eastern Canadian continental margin sediments. *Deep Sea Research Part II: Topical Studies in Oceanography*, 47: 699-731, [http://dx.doi.org/10.1016/S0967-0645\(99\)00123-X](http://dx.doi.org/10.1016/S0967-0645(99)00123-X)
- SUNDBÄCK, K.; LINARES, F.; LARSON, F.; WULFF, A. & ENGELSEN, A. 2004. Benthic nitrogen fluxes along a depth gradient in a microbial fjord: the role of denitrification and microphytobenthos. *Limnology and Oceanography*, 49: 1095-1107, <http://dx.doi.org/10.4319/lo.2004.49.4.1095>
- SVENSSON, J.M.; ENRICH-PRAST, A. & LEONARDSON, L. 2001. Nitrification and denitrification in a eutrophic lake sediment bioturbated by oligochaetes. *Aquatic Microbial Ecology*, 23: 177-186, <http://dx.doi.org/10.3354/ame023177>
- TOMASZEK, J.A. & CZERWIENIEC, E. 2003. Denitrification and oxygen consumption in bottom sediments: factors influencing rates of the processes. *Hydrobiology*, 504: 59-65, <http://dx.doi.org/10.1023/B:HYDR.0000008508.81690.10>
- TUOMINEM, L.; HEINANEN, A.; KUPARINEN, J. & NIELSEN, L.P. 1998. Spatial and temporal variability of denitrification in the sediments of the northern Baltic proper. *Marine Ecology Progressive Ser.* 172: 13-24, <http://dx.doi.org/10.3354/meps172013>
- WANG, F.H.; JUNIPER, S.K.; PELEGRI, S.P. & MACKO, S.A. 2003. Denitrification in sediments of the Laurentian trough, St. Lawrence Estuary, Quebec, Canada. *Estuarine Coastal and Shelf Science*, 57(3): 515-522, [http://dx.doi.org/10.1016/S0272-7714\(02\)00396-7](http://dx.doi.org/10.1016/S0272-7714(02)00396-7)
- ZEHR, J.P.; CARPENTER, E.J. & VILLAREAL, T.A. 2000. New perspectives on nitrogen fixing microorganisms in tropical and subtropical oceans. *Trends in Microbiology*, 8(2): 68-73.

Submetido em 20/04/2012

Aceito em 23/10/2012