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Alex Enrich Prast, Ana Lucia Santoro, Rodrigo S. Coutinho, Lars Peter Nielsen and Francisco A. Esteves, Sediment Denitrification in Two Contrasting Tropical Shallow Lagoons, 2016, Estuaries and Coasts, (39), 3, 657-663.

<http://dx.doi.org/10.1007/s12237-015-0017-5>

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Postprint available at: Linköping University Electronic Press

<http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-127546>

Sediment denitrification in two contrasting tropical shallow lagoons

Running head: Denitrification in tropical lagoons

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Keywords: nitrogen, denitrification, oxygen consumption, sediment, tropical, coastal lagoons.

ABSTRACT

Sediment denitrification was monthly evaluated in two tropical coastal lagoon with different trophic states using the ¹⁵N isotope pairing technique. Denitrification rates were very low in both environments, always < 5.0 μmol N₂ m⁻² h⁻¹ and were not significantly different between them. Oxygen consumption varied from 426 to 4248 μmol O₂ m⁻² h⁻¹ and was generally 3 times higher in the meso-eutrophic than the oligotrophic lagoon. The low denitrification activity was ascribed to both low water NO₃⁻ concentrations (< 2.0 μM) and little nitrate supply from nitrification. There was no correlation of denitrification with nitrate or ammonium fluxes. Sediments in temperate environments with similar oxygen consumption rates usually presented a higher proportion of nitrification-denitrification rates. Sediment oxygen consumption was a good predictor of sediment denitrification in both studied lagoons.

INTRODUCTION

Denitrification is a dissimilatory nitrate reduction process in that nitrate or nitrite (NO_3^- or NO_2^-) is reduced anaerobically to N_2 by heterotrophic facultative anaerobic bacteria. Denitrification requires an anoxic environment and a source of both organic matter and nitrate (Mosier et al., 2002; Morley et al., 2010). As available fixed nitrogen in sediments is mostly in the form of ammonium (NH_4^+), sediment denitrification depends on local rates of nitrification (Newcomer et al., 2012) unless there is a flux of nitrate into the sediment from the overlying bottom waters.

Nitrate produced in the aerobic zone (or nitrate from the overlying water) diffuses into the suboxic zone where denitrification occurs (Nielsen et al., 1990; Risgaard-Petersen, 2003). This process combination is commonly referred to as coupled nitrification–denitrification, while denitrification supported by the physical influx of nitrate is referred to as direct denitrification (An and Joye, 2001; Fennel, et al., 2009). According to Fennel et al. (2009), sediment oxygen consumption (SOC) should be an effective predictor of sediment denitrification. The depth of the aerobic zone in aquatic sediments is directly affected by SOC, which is associated with organic matter decomposition and nitrification (Seitzinger, et al., 2006; Small, et al., 2014).

Coastal aquatic environments are among aquatic systems with the highest degree of eutrophication, derived from increased nutrient inputs (Vidal et al., 1999) and these environments present some of the highest reported denitrification values (Herbert, 1999). Boesch (2002) reported that 67% of the combined surface area of US estuaries exhibited moderate to high degrees of eutrophication and similar results have emerged in Europe and other continents (Duarte et al., 2009).

Freshwater systems are assumed to account for approximately 20 % of the total global denitrification (Seitzinger et al. 2006) because sediment denitrification is the major pathway of fixed N loss from natural and human-altered aquatic systems. However, Burgin & Hamilton (2007) question if the relative role of denitrification as a removal pathway has been overestimated over the years. It has to be noted that denitrification in lake sediments has been widely studied in temperate and boreal environments, but studies in tropical lake sediments are scarce and very little data is available (Pina-Ochoa and Alvarez-Cobelas, 2006; Solomon, et al., 2009). This is even more evident for denitrification measurements in coastal lagoons

The aim of this study was to evaluate the relative importance of denitrification rates and its relation with oxygen consumption and other regulatory factors in two contrasting tropical shallow lagoons with different trophic states.

STUDY AREA

Imboassica (22° 25' S e 42° 56' W) and Cabiúnas Lagoons (22° 00' S e 42° 00' W) are situated in the north coast of Rio de Janeiro state (southeast of Brazil, Fig. 1). This area has minimum and maximum monthly mean temperatures ranging from 20.7 °C in July to 26.2 °C in February, respectively (Inmet, 1992). Both Imboassica and Cabiúnas Lagoons are elongated perpendicularly to the coastline, are shallow and relatively close to each other (30 km), and have a similar watershed size (50 and 45 Km², respectively).

Imboassica Lagoon, with an area of 3.26 Km², is located in a Quaternary Fluvio-Marine Plain where the tropical forest was logged (Cprm, 2000). The predominant land use in the watershed of this lake is extensive pasture, but the lake receives important nutrient inputs from urban sources. Sewage discharges contributed to eutrophication of

Imboassica Lagoon mainly after 1995 (Esteves, 1998). Imboassica was classified as mesotrophic to eutrophic during the study period (Salas & Martino, 2001). Cabiúnas Lake is smaller (0.34 Km²) and less impacted by human activities because it is located within a conservation area (Restinga de Jurubatiba National Park). This ecosystem is located in a Quaternary Marine Sandy Plain where the original terrestrial vegetation (named Restinga) is preserved (Cprm, 2000). Cabiunas was classified as oligotrophic during the study period (Salas & Martino 2001). Some limnological characteristics for Imboassica and Cabiunas lagoons during the studied period are presented in Table 1. Both lagoons present carbonate sediments with some level of bioirrigation. They present submerged aquatic macrophytes, but only in the margins. More information about both lagoons can be find at Marotta et al. (2010).

MATERIALS AND METHODS

Denitrification, Sediment Oxygen Consumption (SOC) and nitrate and ammonium fluxes were measured monthly from February to October 2001 according to Dalsgaard et al. (2000). During each month, water (van D'orn bottle) and big sediment cores (Kajak core sampler) were collected from the central region of each lagoon, without the presence of submerged macrophytes. Smaller sediment cores (Plexiglas cores, 15.8 × 3.6 cm) were carefully subsampled from the big cores in the field and immediately transported to the field laboratory.

In the field lab, the small cores were fixed in two big containers (ca. 50L), one for each lagoon, with a stirring system specially designed to start-end incubations. In each lagoon, we incubated eight cores with stirring systems (5 with sediment and water and 3 only with water). In each container, the cores were completely covered with lagoon water, that was kept circulating with aquarium pumps to maintain oxygen

concentration at saturation level. All cores were kept under these aerated conditions at lagoon *in situ* temperature during 2 to 4 hours for stabilization. Temperature in the cores did not changed more than 2°C during the whole stabilization and incubation period. All incubations were performed in the dark. Sediment oxygen consumption was used as a proxy of site aerobic mineralization (Dalsgaard et al, 2000).

The sediment fluxes of nitrate and ammonium and oxygen were determined with a total incubation from 1 to 2 hours at Imboassica and 2 to 4 hours at Cabiunas lagoons.s. Oxygen consumption was only measured when oxygen final concentration was never lower than 80% saturation (Dalsgaard et al, 2000). After measuring the nitrate, ammonium and oxygen fluxes, the cores were kept open under the water inside the big containers for another period of stabilization of 3 hours before addition of $^{15}\text{NO}_3^-$, indicating the beginning of denitrification incubation. Denitrification rates were obtained through the isotopic pairing technique (IPT) proposed by Nielsen (1992) and after addition of 1 ml $^{15}\text{NO}_3^-$ (5 mM) to each sediment core yielding a concentration of approximately 100 μM in the water column. This relatively high concentration of added $^{15}\text{NO}_3^-$, allows the determination of very low denitrification rates, because the mass spectrometer is sensitive enough to measure small increases of $^{29}\text{N}_2$ concentrations. In this study, the detection limit was 0.01 $\mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$.

We waited a period of 5 to 10 minutes for $^{15}\text{NO}_3^-$ equilibration. After the incubation of 1 to 2 and 2 to 4, respectively in Imboassica and Cabiunas lagoons, we ended the denitrification incubation by adding some drops of ZnCl_2 (1mM) and mixing the sediment and the water in the core. After one minute, we sample 20 ml of the slurry and transferred to 12ml exetainers with 100 μl ZnCl_2 (1mM) for preservation. Within a week, 3ml of the water inside the exetainers were replaced with He and the exetainers were kept at 4°C until analysis at the University of Aarhus (Denmark).

Assuming random mixture of the added $^{15}\text{NO}_3^-$ with $^{14}\text{NO}_3^-$ in the water and produced in the sediment by nitrification, the formation of $^{29}\text{N}_2$ ($^{14}\text{N}^{15}\text{N}$) and $^{30}\text{N}_2$ ($^{15}\text{N}^{15}\text{N}$) by the end of the incubation were used to calculate actual denitrification rates and Dn (coupled nitrification–denitrification) and Dw (denitrification from nitrate from the water column) fractions (Nielsen, 1992). because the mass spectrometer can measure as long the lower measurements performed at this study.

Oxygen, temperature and salinity concentrations were measured in the field and in the laboratory with an oxymeter (YSI-55). Nitrate, nitrite, phosphate and chlorophyll-*a* were measured according to Golterman, et al., (1978) and ammonium according to Bower and Holmhansen (1980). Nitrogen isotope species were analyzed in a mass spectrometer (Europa Scientific) at the University of Aarhus (Denmark).

The sediment oxygen consumption values from both lagoons presented a Gaussian distribution (normality test, $p < 0.05$) and were compared using the unpaired t-test at the 5 % level. The denitrification rates and nitrate and ammonium fluxes presented no Gaussian distribution (normality test, $p < 0.05$). We compared the denitrification rates with the Mann-Whitney test ($p < 0.05$). The Dn and Dw fractions were compared through the nonparametric Kruskal-Wallis test followed by Dunn's post test, which were both evaluated at the 5 % level.

RESULTS

The denitrification rates were very low in both environments and never reached values higher than $5.0 \mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$. Imboassica and Cabiunas denitrification rates varied from 0.21 to $1.7 \mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ and 0.01 to $4.3 \mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$ (Fig. 2 A and B), respectively. The denitrification rates were slightly higher in Cabiúnas Lagoon in

the first three months of the study, but as a whole, there was not a significant difference (Mann-Whitney, $p < 0.05$) between the lagoons.

Sediment oxygen consumption was significantly higher in Imboassica than in Cabiunas Lagoon (t-test, $p < 0.05$). Imboassica and Cabiunas SOC rates varied from 1,838 to 4,248 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ and from 426 to 1,294 $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ (Fig. 2 C and D), respectively.

Ammonium and nitrate fluxes were very variable in Imboassica and Cabiunas lagoons being more intense and with higher fluxes at the first one. NH_4^+ fluxes varied from a release of 1,212.9 $\mu\text{mol NH}_4^+ \text{ m}^{-2} \text{ h}^{-1}$ in March 2001 to an assimilation of 347.5 $\mu\text{mol NH}_4^+ \text{ m}^{-2} \text{ h}^{-1}$ in May 2001 in Imboassica and from a release of 54.3 $\mu\text{mol NH}_4^+ \text{ m}^{-2} \text{ h}^{-1}$ in March 2001 to assimilation of 51.7 $\mu\text{mol NH}_4^+ \text{ m}^{-2} \text{ h}^{-1}$ in August 2001 in Cabiúnas lagoon (Figure 2 E and F). NO_3^- fluxes also varied from sediment release to assimilation in both lagoons, but this variation was in a lower range than the NH_4^+ fluxes (Figure 2 G and H). There was not a clear pattern of assimilation or release of NH_4^+ and NO_3^- , neither a correlation of these fluxes with denitrification or SOC rates. The relative dominance of the Dn over the Dw fraction was observed in both lagoons (Fig. 2 A and B). Both environments presented a significant relationship (F test, $p < 0.05$) between coupled nitrification-denitrification rates (Dn) and SOC (Fig. 3). However, the slope of this relationship and the R value were higher in Cabiúnas than in Imboassica Lagoon.

The water nitrate concentrations were below 2.0 μM at all sampling months in both lagoons. Ammonium concentrations were also lower than 2.0 μM during all sampling months in Cabiúnas lagoon. However, ammonium concentrations varied from $< 2.0 \mu\text{M}$ to 46.8 μM in Imboassica Lagoon. Although solar radiation did reached the

bottom of the studied lagoons, there was no colonization of microalgae over the sediment.

DISCUSSION

The present study shows rates of sediment denitrification and water nitrate concentrations up to two orders of magnitude lower than the average estimated for coastal lagoons (Herbert, 1999; Piña-Ochoa & Álvarez-Cobelas 2006; Fennel et al., 2009). The absence of a temporal variability over the studied months and between both lagoons were quite unexpected and different from the usually observed in the literature. The metabolism of both lagoons changes during the year (Marotta et al, 2010) and these changes apparently did not have any influence on the denitrification rates. The studied lagoons are quite different in terms of geological formation, some limnological characteristics (Table 1) and anthropogenic impacts (more details in Marotta et al, 2010). Even with all these differences, denitrification rates were very low and similar in both lagoons.

Previous experiments in these lagoons showed that all measurements performed with $^{15}\text{NO}_3^-$ addition above 15 μM (final concentration) resulted in constant Dn values (data not shown) and we used 100 μM $^{15}\text{NO}_3^-$ in our incubations, so one of the assumptions of the IPT method was fulfilled (Nielsen, 1992). The same methodology was also used for measuring sediment denitrification rates in several lakes from the Amazon Region. The majority of the measured rates were also low, but in few environments they were one order of magnitude higher (manuscript in preparation). This is a strong indication that the isotope pairing technique method works in the

studied lagoons and that the denitrification rates were accurately measured and represent real denitrification rates. Ferguson and Eyre (2007) suggested that the IPT method could underestimate denitrification rates up to 85%. Even assuming this level of underestimation and correcting the measured rates in our study, the majority of the measurements would still be below $5.0 \mu\text{mol N}_2 \text{ m}^{-2} \text{ h}^{-1}$, a surprisingly low rate.

The low nitrate concentrations in the water column could be a reasonable explanation for the low denitrification rates reported in this study. Nitrate concentrations were always lower than $2.0 \mu\text{M}$ at all sampling months, a much lower value than reported for environments with higher denitrification rates (Herbert, 1999). The total denitrification rates were low, but we observed an important contribution of coupled nitrification–denitrification in both the oligotrophic and the meso-eutrophic tropical lagoons.

Low or absent denitrification rates were previously reported in other tropical lagoon sediments (Biesboer, Esteves *et al.*, 1998) and floodplain lake sediments in the Pantanal (Figueiredo Souza, *et al.*, 2012) and clearwater Amazonian lakes (Kern, *et al.*, 1996; Esteves, 1998; Esteves *et al.*, 2001; Nielsen *et al.*, 2004). Denitrification rates were also absent in periphytic communities associated with Amazonian aquatic macrophytes (Enrich-Prast & Esteves, 1998, Enrich-Prast *et al.*, 1999) and stream tropical sediments (Solomon *et al.*, 2009).

SOC rates observed in Cabiunas Lagoon are comparable to rates of a temperate meso-eutrophic lake (Sweerts *et al.*, 1991), while Imboassica Lagoon rates were higher than those observed at a temperate eutrophic shallow lagoon (Dedieu *et al.*, 2007). These differences may be mainly attributed to higher temperatures in the tropics (Cardoso *et al.*, 2014). The slope of the linear regression between Dn and SOC of the studied tropical environments (<0.001) is much lower than temperate slopes (ca. 0.10:

Seitzinger et al., 2006; Fennel et al., 2009 and 0.06: Piehler and Smyth, 2011). Not only the slope, but also denitrification rates are much lower than those observed by these temperate studies. Between the tropical environments, the slope of the meso-eutrophic studied lagoon (Imboassica) was one order of magnitude lower than the oligotrophic one (Cabiunas) (Fig. 3).

Sediment oxygen consumption rates regulate aerobic layer depth in sediments and consequently can regulate denitrification in different ways; directly, by creating anoxic conditions, or indirectly, by controlling the extent of nitrification (Jensen, et al., 1994; Nizzoli, et al., 2010, Piehler and Smyth, 2011).

The intense heterotrophic metabolism can eventually cause extinction in the sediment aerobic layer, limiting nitrification and maintaining very low nitrate concentrations. In tropical environments with low nitrate concentrations, high temperatures and intense SOC throughout the year, nitrification and coupled nitrification–denitrification can be continuously limited by oxygen limitation.

SOC direct regulation of denitrification occurred in the sediment of Cabiunas and Imboassica lagoons. This system showed lower rates of SOC and this was the most correlated factor with denitrification (data not shown). However, denitrification in sediments containing high amounts of labile organic matter is often limited by the availability of nitrate or nitrite (Fennel, et al., 2009). The extremely high SOC of Imboassica Lagoon seems to indirectly regulate denitrification by shortening the nitrification activity, and nitrate availability was the main and most important regulation factor for denitrification.

Dedieu et al. (2007) observed a negative seasonal correlation between SOC and coupled nitrification–denitrification rates in surface sediments of a temperate eutrophic shallow lagoon. Piehler and Smyth (2011) found a strong correlation between SOC and

denitrification rates in a range of temperate environments and habitats and suggested that SOC should be used to predict denitrification in environments with low nitrate availability. Our results somehow corroborate the conclusions from Piehler and Smyth. However, even though we also observed a significant correlation, every system presents its own correlation and a general correlation cannot be applied indiscriminately to predict denitrification.

ACKNOWLEDGMENTS

This research is a contribution to projects financed from several Brazilian research agencies (FAPERJ, CAPES and CNPq) and also from projects financed by Petrobras. A. Enrich-Prast and FA Esteves are research fellows from CNPq. We thank the Coordinate Research Network - CRN3 - Nnet Project. Interamerican Institute for Global Change Research.

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Figure captions:

Fig. 1: Map of the region. The studied lagoons are indicated by their names.

Fig. 2: Denitrification (A and B) and Dn (white bars) and Dw (shaded bars) rates; Sediment oxygen consumption (C and D) rates (SOC). Positive and negative ammonium (NH_4^+) (E and F) and nitrate (NO_3^-) fluxes (G and H) indicate respectively sediment release and consumption. For Data obtained for Imboassica (A, C, E and G) and Cabiúnas (B, D, F and H) lagoons. Symbols represent mean and SEM. Note differences in scales.

Fig. 3: Linear regression between Dn and sediment oxygen consumption (SOC) rates in Imboassica (black circle, N = 47) and Cabiúnas (white circle, N = 31) sediment. Both relationships are significant at the 5 % level (F test).

Figure 1:

Figure 2:

Figure 3: