

Bacterial community contribution to nitrogen fixation and nitrous oxides production in the Sontecomapan Lagoon

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ABSTRACT: Nitrogen fixation and denitrification are processes poorly studied in Mexican coastal lagoons. In this study the N incorporation and loss rates of the bottom water and sediments were analyzed in six zones of Sontecomapan lagoon during “Nortes” and rainy seasons and their relation with the rivers outflows and the coastal marine waters entrance. The rate of N_2 fixation was calculated through acetylene reduction and the denitrification through N_2O production techniques. The N_2 fixation was lower than the nitrogen loss as N_2O production (denitrification). The greatest fixation rate was registered in the “Nortes” season; being favored by oxic conditions and high organic matter concentrations in the sediments. In the rainy season, the N_2 fixation was lowered by 80% compared to the “Nortes” season. Denitrification was favored by low salinities in November and was 23% higher than in February, 2005. In the rainy season the nitrous oxide production was related to the high $N-NO_3^-$ concentrations and decreased by 18% when the salinity increased from 13 to 22 PSU.

KEYWORDS: Coastal lagoon, denitrification rate, bottom water, N_2 fixation rate, sediment.

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Introduction

The coastal waters are continuously enriched with anthropogenic inorganic nutrients, from polluting sources as wastewaters generated by peripheral human settlements or by farming activities. As they are shallow ecosystems it is assumed that the processes that occur at the water-sediment interface can control the biological processes that happen at the water column, thus influencing the system productivity (Capone, 1983; Day *et al.*, 1989).

Microbial metabolic processes play an essential role in the mineralization of the organic matter that is deposited into the sediments, thus enriching the interstitial water with soluble forms of nitrogen (NH_4^+ , NO_2^- , NO_3^-), phosphorus (HPO_4^{2-}), sulphur (SH^- , SO_4^{2-} , H_2S) and iron (Fe^{2+} , Fe^{3+}); these produced ions are then transported towards supernatant waters by diffusion and by biological processes. The nitrogen (N) cycle is a key biogeochemical process in which transformations of multiple nitrogen compounds are mainly driven by bacterial activity. The cycle controls the nitrogen nutrient availability for biological productivity in aquatic systems, as well as, atmospheric CO_2 fixation and biogenic carbon dioxide export from the surface layer towards the atmosphere (Zehr & Ward, 2002).

The N enrichment of the water column may be caused by rainfalls and supply due to the superficial and underground drainage as well as by biological fixation. The loss of N may be due to the discharges of lagoon waters towards the coastline under current effects and/or the reduction of nitrate to molecular nitrogen (N_2), through denitrifying bacterial activity with the subsequent return of N to the atmosphere, and the permanent loss of organic and inorganic nitrogen compounds by deposition process (Wetzel, 1981; Andersen *et al.*, 2004; Liu & Qiu, 2007). The microbial nitrogen fixation is the most important process that turns atmospheric nitrogen to fixed nitrogen, whereas bacterial denitrification is the most significant process of molecular nitrogen regeneration.

The biological nitrogen fixation is confined to specialized groups of prokaryotes, which own the nitrogenase enzyme and can have heterotrophic or autotrophic metabolism. There is a wide variety of factors that can affect N_2 fixation, no matter the type of ecosystem: oxygen, pH, temperature, light, salinity, inorganic nitrogen and organic substrates available, all of them can govern the nitrogenase activity in specific conditions (Viner, 1982; Hebst, 1998; Evans *et al.*, 2000). Diverse methods have been used to quantify the N_2 fixation rate, one of them is the stable isotopes technique (^{15}N) however, the more widely used is acetylene reduction of (C_2H_2) to ethylene (C_2H_4) (Larkum *et al.*, 1988; Herbert, 1999; Falcón *et al.*, 2002, 2007). The C_2H_2 reduction rate, compared with

the N_2 incorporation rate to the cellular biomass, has a conversion factor of approximately 3 moles of C_2H_4 formed with respect to 1 mole of fixed N_2 (Atlas & Bartha, 2002).

In coastal environments, most of the recycled N is in the form of ammonium (NH_4^+). This NH_4^+ is formed by bacterial decomposition and deamination of organic matter, and it diffuses from the sediments towards the superficial layer of the water column where it can be assimilated by phytoplankton. In presence of oxygen, part of the regenerated NH_4^+ is oxidized to nitrate (NO_3^-), which can be used as terminal electron acceptor by the denitrifying bacteria (*Pseudomonas*, *Thiobacillus*, *Thiosphaera*) producing gaseous forms of N (N_2O , N_2) (Kemp *et al.*, 1990).

On the other hand, in the sediment, the vertical distribution and abundance of N species are governed by the redox state of the different substrates thus, in the sediment oxygenated superficial layer, environmental ammonification and nitrification processes will be more important than those of denitrification. In the sediments, temperature, NH_4^+ concentration, pH, dissolved CO_2 , salinity, macrofauna activity and macrophytes presence are also important for regulating N_2 fixation, nitrification and denitrification processes.

The denitrification is especially active in shallow waters that present anaerobic zones rich in organic matter (Thamdrup & Dalsgaard, 2002; Madigan *et al.*, 2004). It is considered as one of the most important mechanisms in coastal waters biogeochemistry because it is energetically the most favorable form of anaerobic metabolism and also removes significant amounts of NO_3^- therefore, denitrification can influence primary productivity and seems to act as a “buffer” system, preventing the excessive NO_3^- increase in water bodies (Groffman, 1994). Generally, the zone of denitrification is typically located at few millimeters below the oxic superficial sediment layer, where there are limited oxygen concentrations (Ferrara & Bianchi 1990; Revsbech *et al.*, 1980). Some of the current techniques for evaluating denitrification rates are based on the quantification of N_2O formation in anaerobic incubations; this can be obtained by inhibiting the passage from N_2O to N_2 by non-competitive N_2O -reductase enzyme inhibition using acetylene (C_2H_2) (Taylor, 1983; Seitzinger, 1988).

The importance of N as a primary regulator for productivity has induced to the examination of the N-metabolism nature and importance in the coastal benthic communities and the establishment of these activities connection with the pelagic ecosystem (Herrera-Martinez *et al.*, 2000).

The denitrification rate is controlled by the nitrification one, which supplies NO_3^- as a substrate. Thus, the connection process between nitrification and denitrification represents a way to turn nitrogen outside of the recycling routes due to the loss of N in gaseous form towards the atmosphere. This connection is quantitatively important for N cycle in the coastal continental and estuarine sediments, where the N lost by denitrifica-

tion could represent around half of the entries for continental contributions (Seitzinger, 1988).

In addition to the nitrification process, there are two other main sources for NO_3^- : diffusion towards the sediments from the water column, and its transport through underground waters (Capone & Bautista, 1985; Seitzinger, 1988; Day *et al.*, 1989).

The study of the N biogeochemical cycle in coastal lagoons is fundamental to elucidate the role of these ecosystems within the global carbon cycle and the global climate change, since it has a relevant role in the water column productivity and eutrophication. In the same way, the biological N_2 fixation helps to recover the N lost by denitrification in the coastal ecosystems and providing new nitrogen.

Currently in Mexico, there are few scientific reports on N_2 fixation and denitrification processes in aquatic ecosystems. Although both processes are major components for nitrogen cycling in coastal lagoons, only some of these studies consider nitrogen fixation and denitrification processes together (Valenzuela-Siu *et al.*, 2007; Hakspiel-Segura, 2014).

Because of the importance of N species within Mexican lagoon ecosystems, the aim of this investigation was to analyze the incorporation and loss processes of N species from the bottom water as well as from superficial sediments, in order to contribute to a better understanding of the nitrogen cycle functioning in Sontecomapan Lagoon.

Materials and Methods

Study Site and sampling

Six sampling sites were selected according to the salt and fresh water interchanges from the coastline and the rivers flowing into the lagoon: Arroyo La Boya (near the pass of the lagoon to the sea). La Palma and Sábalo (both near the wouhs of small rivers). El Cocal and Punta Levisa (on the navigation canal) and Costa Norte in the interior of the lagoon and a zone of submerged vegetation (*Ruppia maritima*) (Figure 1). Samplings were performed at three dates (November, 2004, and February and June, 2005) corresponding to two seasons of the year: “Nortes” winds (November and February) and rains (June).

The bottom water samples were obtained with a horizontal one-liter Van Dorn bottle. The oxygen concentration was determined by the Winkler technique (Aminot & Chaussepied, 1983). The salinity was measured with an ATAGOS MILL-E refractometer ($\pm 1\text{‰}$) and its pH with an Orion potentiometer. The water samples for the determination of nitrogen inorganic species (N-NH_4^+ , N-NO_2^- and N-NO_3^-) were filtered through 45 mm diameter Whatman GF/F membranes and deep frozen immediately until their

processing, following the colorimetric methods recommended by Aminot & Chaussepied (1983).

The sediment samples were collected by diving, using 22 x 5cm inner diameter (i. d.) polycarbonate corers, avoiding disturbance of the sediment column. The pH and Eh were measured using ORION (2mm and 3mm i. d. respectively) needle-type mini-electrodes, in order to avoid disturbing the sediment during penetration they were inserted in a stepped way each 5 mm of depth. The interstitial water for measuring the nutrients was obtained from the first centimeter of sediment, using a polycarbonate capillary tube (10 cm length and 5 mm i.d.) sealed in its lower bound and pierced with a series of 1 mm i.d. holes throughout their last centimeter. The interstitial water from the first centimeter of depth was obtained by suction using a 50 mL syringe connected to the capillary tube by a latex hose. The water samples were filtered with a 25 mm diameter Whatman GF/F membrane and stored in 60 ml hermetic bottles previously gasified with N₂ to prevent oxidation from the samples' air, and then deep frozen to -18 °C until their processing.

The remaining sediment from the cores was used for determining the percentage of organic matter (%OM) and sediment texture. Replicated cores were sliced into 1 cm thick layers and treated separately for %OM, using the potassium dichromate excess titration method, with a 0.5N solution of ferrous sulphate for organic matter oxidation (Gaudette *et al.*, 1974) and the sediment texture by Bouyoucos' hydrometer method (Holme & McIntyre, 1984).

Incubations for N₂ fixation and denitrification rates

The bottom water samples were obtained with a horizontal Van Dorn bottle and 300 mL from the sample were incubated in 500 mL hermetic glass jars. The first centimeter of the sediment was placed in another series of jars containing 300 mL of bottom water previously filtered through Whatman GF/F membranes. The incubations were performed *in situ* (two clear bottles and one dark) for four hours, one series by test. At the beginning of the incubations, 10% of the air volume from each jar was extracted through a cork with turnover septum stoppers —folding rubber apron— (Fisher Scientific) placed in jar cover (Moran-Villa *et al.*, 2009), and the same volume of acetylene was injected into the jar (C₂H₂). The jars stayed within a water bath to keep them at room temperature.

N₂ fixation measurements

After the four-hour incubation period, a sub-sample from the gaseous phase was taken and stored in a hermetically closed vial. The N₂ fixation rate was estimated by acetylene reduction technique (ARA) (Capone, 1993; Morán-Villa *et al.*, 2009). The ethylene analy-

ses (C_2H_4) were performed with a Perkin Elmer Auto Analyzer gas chromatograph with a flame ionization detector (FID) equipped with a 15 x 0.55 mm i.d. and 1 mm thick REST-EK Corporation capillary column. The temperature of the oven, injector and detector was 165 °C and 230 °C respectively. As a gas carrier, a 1 mL min⁻¹ He flow was used. A 100 μl aliquot from C_2H_2/C_2H_4 gas sample was injected. The gas chromatograph was calibrated with a standard 100 ppm C_2H_4 , and the reduction rate of C_2H_2 was converted to N-fixed using the conventional molar conversion factor 3:1 proposed by Postgate (1982) and Seitzinger & Garber (1987), and the Bunsen coefficient of solubility (Flett *et al.*, 1976). The total fixation of N_2 rate was calculated by adding the phototrophic fixation rate (in the light) to the heterotrophic one (in darkness).

N₂O measurements

The analysis of N_2O present in the gaseous phase was made with a VARIAN chromatograph with a flame ionization electron capture detector ⁶³Ni. As a gas carrier, a 30 mL min⁻¹ He flow was used. The temperatures of the column and the detector were 50 °C and 300 °C respectively. A 1 ml aliquot was injected and the peak area was measured with a peak integrator. A standard of 100 ppm N_2O (Alltech) and the Bunsen coefficient of solubility (Flett *et al.*, 1976) were used.

$$\mu mol (N_2O \text{ l}^{-1} \text{ t}^{-1}) = \left(\frac{\text{peak area sample}}{\text{peak area standard}} \right) \times \left(\frac{C.S. \text{ injected} (V_{\text{gas}} \times V_{H_2O} \times 0.1)}{\text{Vol. injected}} \right) / \Delta t$$

where:

C.S. = Coefficient of N_2O standard

V_{gas} = Volume of the gas phase in the incubation bottle

V_{H_2O} = Volume of incubated water (or sediment, as the case might be)

0.1 = Bunsen coefficient for nitrous oxide at the appropriate salinity and temperature

Δt = Incubation time

The volume of the gas phase injected into the gas chromatograph was 100 μl

Statistical analysis

In order to determine the physical and chemical factors that influence the N_2 fixation (FixN) and denitrification (N_2O) rates during the “Nortes” and the rainy seasons, a Canonic Correspondence Analysis (CCA) using Multi-Variate Statistical Package (MVSP) 3.22 ® program was performed on the log_e standardized data, using the centered data options and the Kaiser rule for the axes extraction (Kovach, 1999).

Results

Physical and chemical parameters

Bottom water

Table 1 shows the mean values of oxygen, temperature, pH and salinity at the bottom water of the six stations. The bottom water was found in suboxic conditions but with acceptable values for most of the pelagic organisms. Oxygen varied between 2.6 and 10.1 mg/l⁻¹ in the zones with greater boat traffic. The pH was around neutrality all year long (7.23 ± 0.55). Temperatures varied between 20 and 30 °C. Salinity varied from 0.2 to 37 PSU (mean value = 19.6 ± 11.4 PSU), with the lowest values measured in November (from 0.2 to 28 PSU). The Eh values were highly variable but generally positive (Table 1).

N-NH₄⁺ concentration had mean values of 3.98 ± 3.21 μmol l⁻¹, with the lowest values registered at La Boya and Costa Norte (0.01 and 0.39 μmol l⁻¹ respectively) and the highest at Punta Levisa and La Palma (10.6 and 7.9 μmol l⁻¹ respectively) during “Nortes” season (February). In general, the ammonium concentration was 6 times higher in February than in November (6.08 ± 3.3 and 1.04 ± 0.65 μmol l⁻¹ respectively). During the three surveys, the N-NO₂⁻ concentrations fluctuated between 0.15 and 9.0 μmol l⁻¹ (La Boya and El Cocal, respectively); the low concentrations of this nutrient in the bottom water could be due to an intense ammonium oxidation to nitrate. The high concentration of N-NO₂⁻ found in El Cocal was due to the effect of the sediment re-suspension provoked by the constant boat transit in the navigation canal, and it is in this same sampling area where the smallest N-NO₃⁻ concentration was obtained (0.8 μmol l⁻¹) in February. In June (rain season), high concentrations of N-NO₃⁻ in all the sampling areas (81 ± 35.5 μmol l⁻¹) were generally registered.

Sediments

The sediments were of the silty-sandy type, moderately rich in organic matter ($1.73 \pm 1.5\%$), with the highest values in the sampling sites near the two river mouths (Table 2). The Eh varied from -240.9 to 291 mV.

The highest concentrations of ammonium were recorded during the “Nortes” period (November 2004, February 2005) at Punta Levisa (26.5 μmol l⁻¹ N-NH₄⁺), La Palma (22.6 μmol l⁻¹ N-NH₄⁺) and La Boya (25.2 μmol l⁻¹ N-NH₄⁺). Generally, ammonium concentrations in the sediment were higher than at the bottom water (Tables 1 and 2). Nevertheless, the diffusive flow of the sediment towards the water column sometimes allows leads to higher concentration in the bottom water than in the sediment (Tables 1 and 2).

Table 2. Variation of physical and chemical parameters in sediments (1-cm depth). Values obtained in the three samplings seasons. “Nortes” (November = N and February = F, and rains (June = J). nd = undetermined

Study Sites	Org. C (% dry weight)		Clays (%)		Silt (%)		Sand (%)		Salinity (PSU)		pH		Eh (mV)		N-NH ₄ ⁺ (μ mol L ⁻¹)		N-NO ₂ ⁻ (μ mol L ⁻¹)		N-NO ₃ ⁻ (μ mol L ⁻¹)											
	N	F	J	N	F	J	N	F	J	N	F	J	N	F	J	N	F	J	N	F	J									
Costa Norte	1.2	0.7	2.2	4	1	5	6	6	14	90	94	81	15	30	8	8.5	6.9	6.2	-67	100	-40	8	14	0.06	14	7	0.2	2	2	121
Punta Levisa	2.1	1.0	0.6	6	15	2	2	2	1	88	65	96	11	32	19	8.7	7.4	2.3	-192	291	-38	27	17	0.7	17	1	0.1	0.3	10	129
Sábalo River	2.1	5.9	3.4	nd	5	4	8	8	9	84	53	88	13	32	18	8.5	6.9	6.7	32	96	33	8	2	0.9	2	0.7	0.1	4	7	64
El Cocal	0.3	0.8	1.3	10	11	3	30	30	10	50	61	88	18	33	2	8.6	7.4	6.6	151	45	-26	8	5	0.01	5	0.4	0.7	3	0.8	57
La Palma River	3.6	0.5	1.4	2	15	14	10	10	4	84	63	94	4	32	0.2	7.1	6.3	6.9	-17	125	49	0.6	23	0.6	23	110	2	2	6	43
La Boya	2.6	0.5	3.0	6	9	3	10	10	7	82	69	91	23	32	33	7.7	8.4	7.4	-54	73	-241	25	2	4	2	1	1	2	9	71
Mean ± Sd	1.8 ± 1.4			6.8 ± 4.7	9.8 ± 8.1	78.9 ± 14.8	19.7 ± 11.6	7.1 ± 1.5	17.8 ± 122.4	8.1 ± 9.2	10.4 ± 25.7	29.6 ± 42.1																		

The highest concentrations of N-NO_2^- were registered in November at La Palma ($22.6 \mu\text{mol l}^{-1}$), Punta Levisa ($16.5 \mu\text{mol l}^{-1}$) and Costa Norte ($13.9 \mu\text{mol l}^{-1}$) and were related to negative Eh, pH between 7.1 and 8.7 and high percentages of organic carbon (3.6, 2.1 and 1.2 % org C respectively). The highest concentrations of N-NO_3^- in sediment were registered in June 2005, at Costa Norte and Punta Levisa (121 and $129 \mu\text{mol l}^{-1}$ respectively). No significant difference in the concentrations of N-NO_3^- between the bottom water and the interstitial water of the sediments were found.

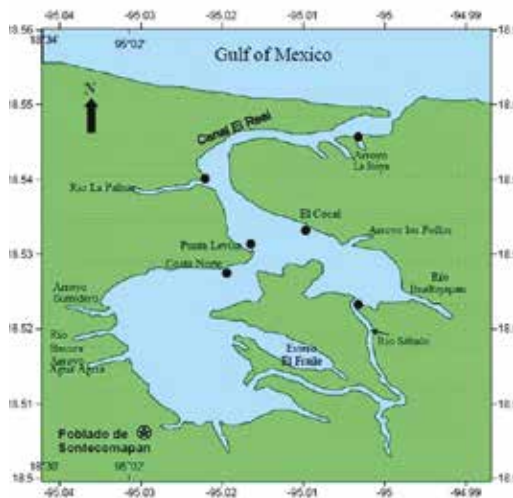


Figure 1. Sampling sites at the Sontecomapan Lagoon, Veracruz.

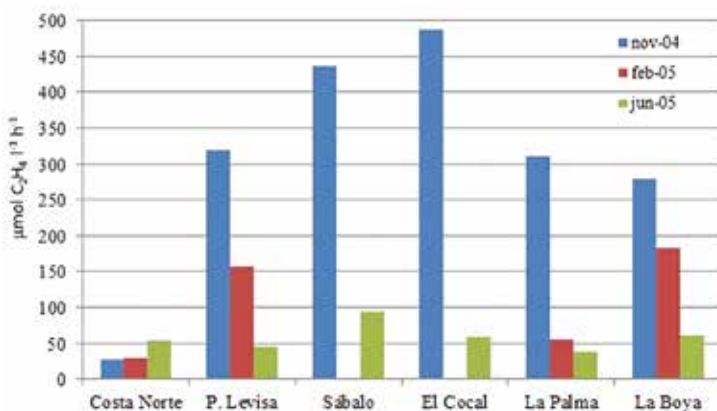


Figure 2. Variation of N_2 fixation rate of the bottom water during the two studied seasons, November, 2004 to February, 2005 (“Nortes” season) and June, 2005 (rainy season) at the Sontecomapan Lagoon.

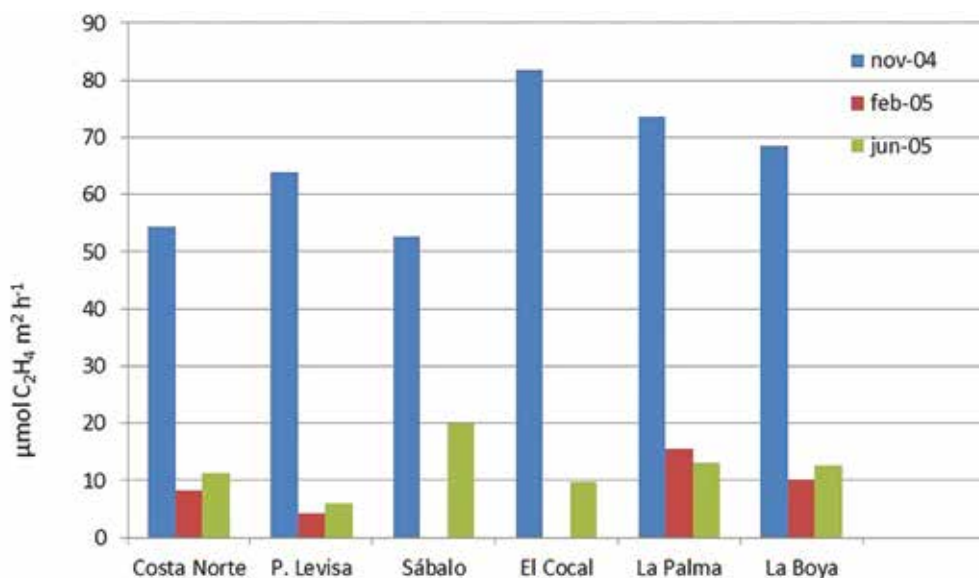


Figure 3. Variation of N₂ fixation rate in the sediment (1 cm deep) during the two seasons studied, November, 2004 to February, 2005 (“Nortes” season) and June, 2005 (rainy season) in the Sontecomapan Lagoon.

Nitrogen fixation

In all the sampling stations, nitrogenase activity was detected. In the bottom water, the highest N₂ fixation rates ($309.98 \pm 160.35 \mu\text{mol C}_2\text{H}_4 \text{ m}^2 \text{ h}^{-1}$) were observed in November (“Nortes” season). A drastic decrease (81%) was observed in rainy season ($58.72 \pm 19.5 \mu\text{mol C}_2\text{H}_4 \text{ m}^2 \text{ h}^{-1}$), when the highest salinity was noted at the bottom water (19.6 ± 12.8 PSU). In the sediments the fixation rate of N₂ was 10 times lower (31.62 ± 28.4) than in the bottom water (Fig. 3), and the lowest values were recorded in February and June, 2005 in Punta Levisa (4.17 and $5.98 \mu\text{mol C}_2\text{H}_4 \text{ m}^2 \text{ h}^{-1}$ respectively).

Denitrification rate

Generally, it was observed, as well as for the N₂ fixation, that the highest N₂O production occurred in November ($764 \pm 114.3 \mu\text{mol N}_2\text{O m}^2 \text{ h}^{-1}$), this being the reason why this process was favored by the low salinities (19.6 ± 11.06 PSU) and the highest oxygen concentrations registered ($6.6 \pm 1.53 \text{ mg L}^{-1}$) at this season. The lowest rates were observed in February ($593.9 \pm 107.4 \mu\text{mol N}_2\text{O m}^2 \text{ h}^{-1}$), being 23% lower than those from November. During the rainy season, the denitrification rate varied between 465.5 and 1094 $\mu\text{mol N}_2\text{O h}^{-1} \text{ m}^2$. At this season the highest values were obtained at La Boya (Fig. 4).

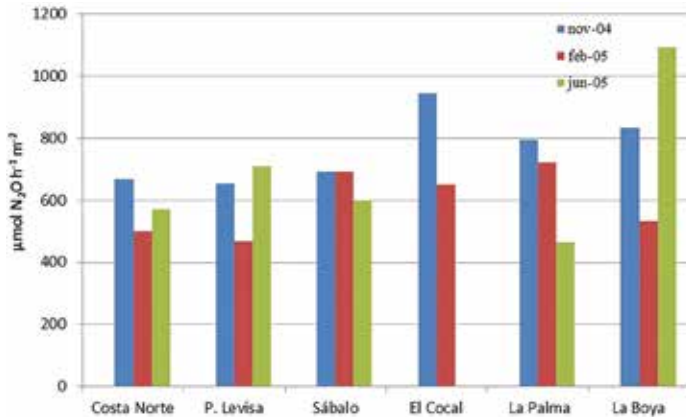


Figure 4. Denitrification rate in sediments (1 cm deep) measured by N₂O production during the two seasons studied, November, 2004 to February, 2005 (“Nortes” season) and June 2005 (rainy season) at the Sontecomapan Lagoon.

CCA analysis

The CCA analysis showed that in November the high N₂ fixation rate recorded at the bottom water had an inverse relationship with the slightly electronegative Eh ($r=-0.663$), and a positive relationship with N-NO₂⁻ ($r=0.92$). Within the same period, the high N₂ fixation rate in the sediments was also correlated to slightly electronegative Eh ($r=-0.680$), but also to high sand concentrations ($r=-0.667$), low ammonium concentrations ($r=0.696$) low nitrates ($r=-0.782$) and high O₂ concentrations in bottom water (Fig. 5).

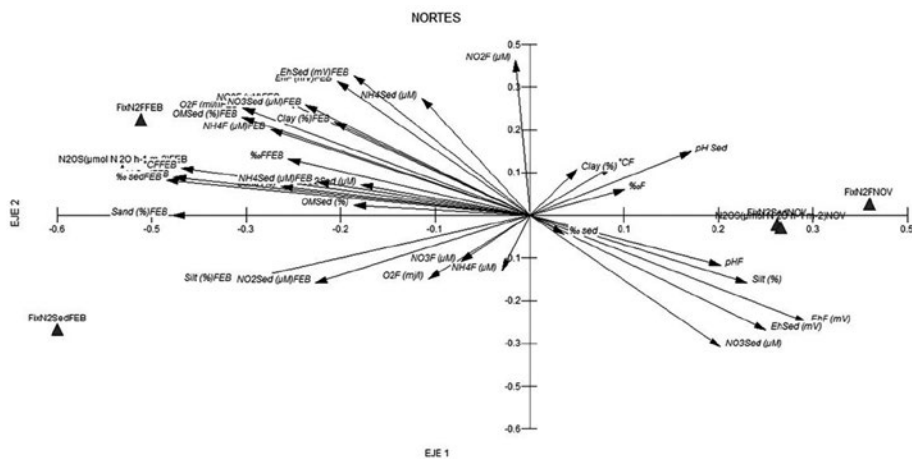


Figure 5. Multivariate CCA analysis performed for the “Nortes” season (November to February) from the 18 physical and chemical variables, which gives a contribution to the total variance of 98.46% in relation to the values of N₂ fixation (FixN₂) and denitrification (N₂O) rates, in bottom water and sediment.

In February, the low N_2 fixation rate in bottom water was correlated positively with electropositive values of Eh ($r=0.795$), high O_2 ($r=0.636$), and negatively with high $N-NH_4^+$ concentrations ($r= -0.696$), low $N-NO_2^-$ ($r= -0.659$) and low OM percentage ($r= -0.77$) in the sediment. Within the same period, the low N_2 fixation rates in the sediment were positively linked to the greatest percentage of silt ($r=-0.788$), and negatively to the lowest percentage of sands ($r= -0.958$), slightly basic pHs ($r= -0.955$) and low $N-NO_3^-$ concentrations ($r= -0.602$) in the interstitial water. In November, the high N_2 fixation rate in the sediments (higher than in February) was correlated to slightly electronegative Eh ($r=-0.680$), high sand concentrations ($r=-0.667$), low ammonium concentrations ($r=0.696$), low nitrates ($r=-0.782$) and high O_2 concentrations in the bottom water (Fig. 5).

As for the N_2 fixation, the denitrification rate in the “Nortes” season, was correlated to slightly electronegative Eh values ($r=-0.680$), low $N-NO_3^-$ concentrations in the interstitial water ($r=0.782$) and low salinities ($r=0.98$). The rainy season (June) was related to high $N-NO_3^-$ concentrations in the interstitial water (Fig. 5 and 6).

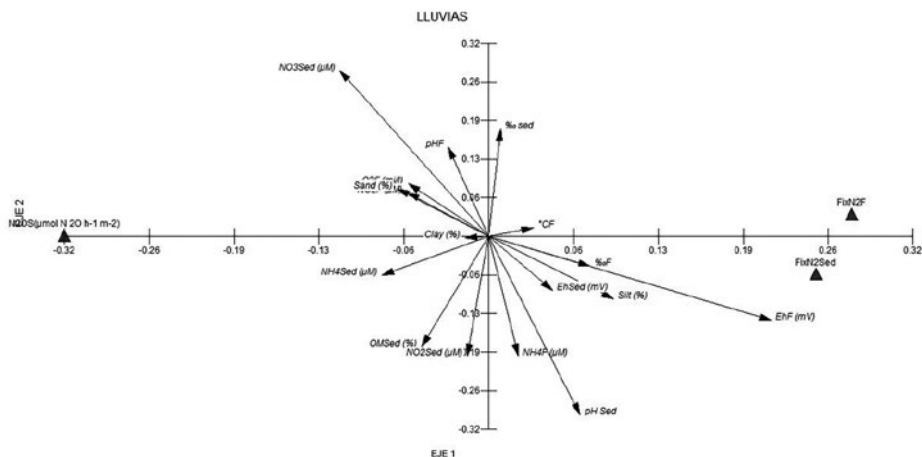


Figure 6. Multivariate CCA analysis performed for the rainy season (June) from the 18 physical and chemical variables, which gives a contribution to the total variance of 100% in relation to the values of N_2 fixation ($FixN_2$) and denitrification (N_2O) rates, in bottom water and sediment.

Discussion

Although it is considered that the N_2 fixation is a O_2 -sensitive process, the highest rates were recorded during the “Nortes” season (November, 2004 and February, 2005), when the oxygen concentrations in the bottom water ($7 \pm 1.5 \text{ mg L}^{-1}$) were the highest. This can be partly due to the development of diazotrophic microorganisms of conformational

protection mechanisms against nitrogenase inactivation by high O_2 concentrations, as reported by Soto-Urzúa & Baca (2001). A second reason should be linked to wind re-suspension of sediment particles driven by Nortes winds at this season. Indeed, re-suspended particles may constitute a microhabitat (within a few microns deep into the particle's interior) with aerobic and anaerobic spaces favoring the bacterial processes of N_2 fixation and denitrification (Ferrara-Guerrero *et al.*, 2007). In the sediments the deposit of labile organic matter is also related to these hydrodynamic processes and bio-perturbation from benthic organisms, which will induce environmental changes in each sampling station (Brune *et al.*, 2000).

The oxygen sediment concentrations in the two seasons studied were high enough to allow recent organic matter mineralization and to provide enough ammonium to the sediment, which would explain the lower rates of N_2 fixation compared to bottom water.

The fact that the highest ammonium concentrations of the bottom water were registered in February, 2005 is due to strong winds and tidal effects that provoked sediment re-suspension thus allowing NH_4^+ diffusion towards the bottom water, as has been already demonstrated for this type of environment by Kemp *et al.* (1990). In such shallow ecosystems, the interchange of dissolved substances through the water-sediment interface is a process that affects the chemical composition of the water column since it regenerates the ammonium and nitrate required for the primary biomass production in the superficial layers (Maksymowska-Brossard & Piekarek-Jankowska, 2001). Generally, the relatively low concentrations of $N-NH_4^+$ in bottom water favored N_2 fixation in both seasons.

The high salinities registered at the bottom water in June inhibited the N_2 fixation, so it was about 80% lower than in November and February, this coincide with Herbst (1998), Serraj & Drevon (1998) who reported that salinity is a N_2 fixation inhibitor (Figs. 2 and 5). Generally, planktonic nitrogen fixation is greater in freshwater than in marine ecosystems (Nielsen *et al.*, 2003) however, it appears to be no difference in nitrogen fixation rate in the sediments in relation to different salinities from bottom water (Figs. 3 and 5).

The denitrification rates measured in our study match with those reported for eutrophic estuarine ecosystems impacted by waste water inputs (Seitzinger, 1988). The relationship between the high denitrification rates and the high $N-NO_2^-$ concentrations in reduced sediments was not too clear, as high denitrification rates were also recorded in reduced sediments with low $N-NO_2^-$ concentration during the rainy season. According to Codispoti *et al.* (2001) nitrites tend to accumulate in the water column only when the oxygen concentrations are $<2.5 \mu M$, however, this trend is hardly observed in the sediments due to the interment activity of the benthic meiofauna provoking the formation of patches in the superficial sediments (Hendricks, 1993; Sheibley *et al.*, 2003) and thus the

formation of slightly oxidized microhabitats with low N-NO_2^- concentrations. This was observed in the “Nortes” season at El Cocal station where high denitrification rates, low NO_2^- concentrations and slightly oxidized sediments were observed.

The denitrification rate was also affected by salinity and sediment organic matter in agreement with Richardson *et al.* (2004) and Rysgaard *et al.* (1999) who reported, in estuarine ecosystems, 50% decrease of denitrification with an increase of salinity from 0 to 10 PSU. In our study we found a 18% decrease of denitrification rate when the salinity changed from 13 to 32 PSU.

The high N_2O concentrations produced during the denitrification depend mainly on electronegative Eh values, nitrate concentration and sediments texture. Nevertheless, the denitrification process was extremely variable temporarily and spatially, because when the N-NO_3^- concentration increases, it is not fully used for denitrification process since a proportion can be reduced to NH_4^+ (Ullah & Zinati, 2006).

On the other hand, given that the mineralization of the organic matter that has been recently deposited in the superficial sediments produces the greatest quantity of N-NH_4^+ and N-NO_3^- used for the denitrification; it can be expected that the sites with highest deposition of labile OM present a high N_2O production.

Conclusions

The exchange of ammonium and nitrate ions between the water-sediment interface and the bottom water is a key factor for nitrogen regeneration that strongly varies between the different seasons of the year (“Nortes” and rains).

The results suggest that increased salinity, from 24 to 29 PSU, may limit nitrogen fixation in bottom water.

The greatest loss of nitrogen as N_2O through denitrification happened in the reduced sediments with greatest silt percentage and high NO_3^- concentrations, located at the deepest zones of the navigation canal.

The strong variations of N_2 fixation and denitrification rates registered in the “Nortes” and rainy seasons can be linked to the runoff of terrestrial nitrogen within the lagoon, originating from the bordering zones that have been deforested for farming purposes.

For future investigations on the global N cycle it will be necessary to extend the measurements of N_2 fixation, denitrification and nitrification rates as well as their connection with the regeneration and loss of N, to other Mexican coastal lagoons that are still poorly studied.

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