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Nitrogen cycling in Sabine Lake

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1. Introduction.

As a part of continuing efforts to investigate nitrogen cycling in Texas estuaries, denitrification, nitrogen fixation, dissimilatory nitrate reduction to ammonium (DNRA), and sediment oxygen demand were measured in Sabine Lake, Texas. Estuarine phytoplankton production can be limited by N availability, and sediments often are an important source of dissolved inorganic N (DIN = $NH_4^+ + NO_2^- + NO_3^-$). Denitrification transforms combined N to gaseous forms (N_2 or N_2O; Seitzinger 1988, 1990). These end-products represent unavailable nutrient sources to most estuarine producers (e.g., phytoplankton and bacteria; Howarth et al. 1988). Thus, denitrification may drive systems toward N limitation (Seitzinger 1990). The purposes of this study were to evaluate the importance of denitrification in nitrogen cycling and examine environmental conditions influencing nitrogen cycling in Sabine Lake. A membrane inlet mass spectrometer (MIMS) for dissolved gas measurement and high performance liquid chromatography (HPLC) system for ammonium isotope measurements were used to quantify denitrification, N-fixation, DNRA, and sediment oxygen demand. Four sampling trips to Sabine Lake were made in June, September, December 2000 and March 2001. Here, we report water column measurements and incubation experiment results.

2. Study area and method

Two stations in Sabine Lake were selected to measure water column characteristics (temperature, salinity, and dissolved oxygen using a Hydrolab® multiprobe) and conduct sediment core incubation experiments. Bottom water at each station was collected for nutrient analysis and sediment core incubation. Station 1 is located in the south part of the lake and represents a higher salinity area, and Station 2 is located in the north part of the lake near the river input. The overlying water salinity at Station 2 was lower than Station 1. During the September sampling, Station 2 was not sampled and another site near Station 1 (station 1-A) was visited (Table 1). Sediments at both stations consisted of fine-grained particles, and sediment types were mud and sandy mud. Station 1 was visited in June-00, Sept-00, Dec-00 and Mar-01. Station 2 was visited June-00, Dec-00 and Mar-01.

Undisturbed sediment cores (12 cm diameter, 30 cm length; 4 per station) with bottom water were collected from a boat using a coring device equipped with a plastic pipe handle. Within 8 hours of collection, cores were transported to the laboratory, and a flow-through plunger with Teflon inlet and outlet tubes was installed over each sediment core (Lavrentyev et al. 2000). Flow-through chambers consisted of an aerated intake water vessel, Teflon flow tubes, peristaltic pump, temperature-controlled incubation bath, and sample collection vessels. Sediment cores were placed in the incubation bath at *in situ* temperature, and bottom water from the site was passed continuously over the core surface at 1.2 ml min^{-1}. To maintain consistency with previous studies in Laguna Madre and Baffin Bay, half of the cores were incubated under dim light (~ 30 $\mu E\ m^{-2}\ sec^{-1}$) whereas the others were covered with aluminum foil. Like the previous studies, dim light conditions did not cause significant light effects, so data from the two treatments were combined. Water column depth over the sediment was maintained at about 5 cm to give a water volume of ca. 570 ml in each core. After one day of incubation to allow steady-state conditions to develop, triplicate samples of feed and outlet water were collected at intervals for dissolved gas analysis. Water samples also were collected for analysis of dissolved inorganic nitrogen compounds (NH_4^+, NO_3^-, and NO_2^-).

Addition experiments with $^{15}NO_3^-$ were conducted to provide insights about the fate of nitrate at the sediment-water interface. After the first or second day of sampling, feed water was enriched with $^{15}NO_3^-$ and concentrations of $^{28}N_2$, $^{29}N_2$, $^{30}N_2$, and $^{15}NH_4^+$ were measured in inflow and outflow waters. Three different masses of nitrogen gas were produced by denitrification ($^{28}N_2$ from $^{14}NO_3^-$, $^{30}N_2$ from $^{15}NO_3^-$, and $^{29}N_2$ from $^{14}NO_3^-$ and $^{15}NO_3^-$; Nielson 1992). Dissolved N_2, O_2, and Ar were measured with MIMS using methods modified from Kana et al. 1994 (An et al. 2001). Concentration and atom % ^{15}N for NH_4^+ were determined by HPLC (Gardner et al. 1995). Sediment flux of each compound was calculated based on the concentration difference between feed and outflow water, flow rate, and cross-sectional area (Lavrentyev et al. 2000).

3. Results and Discussion

3-1. Environmental characteristics

Table 1 shows the results of Hydrolab measurements. Average salinity (9 ppt) in Sabine Lake was lower than Galveston Bay (~15 ppt) and Laguna Madre/Baffin Bay (~30 ppt). Due to its small area and volume (187 km^2 and 310 x10^6 m^3) relative to freshwater input, hydraulic residence time was short (~7 day), and average salinity differences between lower and upper parts of the lake were low (~6 ppt; http://hyper20.twdb.state.tx.us/Sabine/losabsal.html).

Monthly averaged salinity is low in winter/spring and high in summer/fall (Fig. 1). During 2000/2001, salinity was lower than average but followed this seasonal trend. In Mar-01, freshwater input was very high and lake salinity was near zero. Freshwater discharge increased rapidly after Nov-00 with maximum values in Mar-01 (Fig. 2). Turbidity was high in Dec-00 due to terrestrial debris. Surface water dissolved oxygen (DO) concentration was at saturation for the existing temperature and salinity. Bottom water was oxygenated at most stations due to wind-driven mixing and shallow water depth. Lower bottom water DO was observed at Station 2 in Jun-00. Temperature differences between bottom and surface water was minimal (Table 1).

3-2. Seasonal variations of sediment oxygen demand and denitrification rates

Denitrification rates were low in Sabine Lake, and nitrogen fixation exceeded denitrification in Jun-00, Dec-00 and Mar-01 (Table 2). N_2 gas production was observed only in Sep-00. Mean denitrification rates were higher at Station 1-A versus Station 1 in Sept-00, but the difference was not significant. Denitrification rates in Sept-00 are comparable to those reported in other Texas estuaries (Laguna Madre/Baffin Bay (0-530 μg atom N m^{-2} h^{-1}) and Galveston Bay (170 μg atom N m^{-2} h^{-1})). In Jun-00 and Mar-01, N_2 flux into the sediment from N-fixation was higher at Station 2 than Station 1.

Tables 3 shows sediment oxygen demand (SOD) in Sabine Lake. Denitrification and SOD rates in Tables 2 and 3 were measured before $^{15}NO_3^-$ addition. Negative numbers suggest that nitrogen fixation rates exceeded denitrification rates in some samples (Table 2). Both SOD and denitrification rates showed maximum values in Sept-00 (890 μmole O_2 m^{-2} h^{-1} and 110 μg atom N m^{-2} h^{-1}, respectively; Table 2, 3). Minimum SOD (229 μmole O_2 m^{-2} h^{-1}) was observed in Dec-00 at Station 1.

Seasonal variations of denitrification and SOD rates did not follow water temperature fluctuations in Sabine Lake (Fig. 3). In Jun-00, SOD and denitrification rates were low despite high water temperature. Denitrification rates showed a linear relationship with SOD when SOD was over 500 μmole m^{-2} h^{-1} (Fig. 4). When SOD was less than 500 μmole m^{-2} h^{-1}, N-fixation exceeded denitrification.

Low SOD may have resulted from organic matter limitation. Low organic carbon concentrations can limit heterotrophic activity, even under favorable temperature conditions. Since denitrification is a heterotrophic process, low organic matter availability also could limit denitrification rates (Koike and Sørensen 1988, Cornwell et al 1999). The linear relationship between denitrification and SOD also suggests organic matter limitation (Fig. 4). Organic matter delivery for sediment remineralization in Sabine Lake, however, does not appear to be correlated with freshwater input (Fig. 5a). At Station 1, SOD decreased as freshwater input increased. At Station 2, SOD increased slightly with large freshwater input increases.

One possible explanation for weak or negative correlations between freshwater input and organic matter remineralization or denitrification in Sabine Lake is the short residence time. Water column organic matter content in Sabine Lake was presented in low and high flow seasons (Fig. 6; Baskaran et al. 1997). Total organic matter (DOM + POM) doubled during the high flow season even though freshwater input increased by a factor of 10. DOM was lower in the high versus low flow season. In the low flow season, primary production may be higher due to decreased turbidity and a stable water column. Benthic remineralization uses labile organic matter produced on-site. The larger portion of POM in this season may result from on-site production rather than river input. Higher POM in lower versus upper Sabine Lake in the low flow season can be explained by higher on-site primary production (Fig. 6a). Higher SOD at Station 1 (lower) in the low flow season also supports this hypothesis (Fig. 5a).

During high flow, POM in upper Sabine Lake was high and suggests that POM is riverine. Due to the short residence time and high turbidity, on-site primary production may be low, and benthic remineralization would depend on organic matter from the river. SOD at Station 1 was lower than Station 2 during high flow (Figure 5a).

When freshwater input is low, longer residence time would allow on-site primary production, and SOD and denitrification would benefit from labile organic matter. During high flow, however, on-site production may be low, and organic matter may consist of a less labile component even though total organic matter concentration is high. This may explain observed low SOD and denitrification rates.

3-3. The relationship between SOD and denitrification

The net N_2 flux increase with SOD observed in this study was compared to the linear model suggested by Seitzinger and Giblin (1996; Fig. 7). Denitrification and SOD data measured on the North Atlantic shelf was compiled and showed a linear relationship (coupled denitrification (mmol N m^{-2} d^{-1}) = 0.116*SOD (mmol O_2 m^{-2} d^{-1}); r = 0.8). SOD and net N_2 flux observed in this study are near the low end of the data ranges used in the model (Seitzinger and Giblin 1996). In Laguna Madre/Baffin Bay and Sabine Lake, predicted denitrification rates matched with measured rates when SOD was high (Fig. 7a). However, when SOD was low, negative N_2 gas flux was observed suggesting low denitrification and high N-fixation, which was not accounted for in the Seitzinger and Giblin (1996) model. The rates at which denitrification increased with SOD (0.263 for Sabine Lake and 0.283 for Laguna Madre/Baffin Bay) were higher than predicted by the model (0.116). Although these rates were similar in Laguna Madre/Baffin Bay and Sabine Lake, Sabine Lake had higher denitrification for a given SOD (Fig. 7a).

Denitrification may be inhibited by high sulfide concentrations produced during sulfate reduction in Laguna Madre/Baffin Bay. Sulfate reduction may be low in Sabine Lake due to low salinity. In Galveston Bay, the Seitzinger and Giblin model predicted the SOD vs. denitrification relationship measured by Zimmerman and Benner (1994; Fig 7b). However, denitrification rates measured by An and Joye (2001) were higher than rates predicted by the model. An and Joye (2001) found that benthic primary production plays an important role in Galveston Bay, and SOD is a "net O_2 change" rather than a characteristic of remineralization activity.

3-4. Nitrate ($^{15}NO_3^-$) addition experiments

Inflow water was enriched with ~100 μM of $^{15}NO_3^-$, and the production of $^{29+30}N_2$ and $^{15}NH_4^+$ in each sediment core was monitored to examine the potential fate of nitrate. The effect of NO_3^- addition on other processes (SOD and denitrification) was evaluated by comparing rates before and after the addition. The production rates of $^{29+30}N_2$ and $^{15}NH_4^+$ were compared with total NO_3^- flux to clarify the partitioning of two NO_3^- reduction processes (denitrification and DNRA).

After the addition, $^{28}N_2$ flux decreased in Jun-00 and Sept-00 (Table 4). In Mar-01, $^{28}N_2$ flux increased after the NO_3^- addition. In Dec-00, $^{28}N_2$ flux increased at Station 1 and decreased at Station 2. In Sept-00, $^{28}N_2$ production was observed at Stations 1 and 1-A before the addition. After the addition, sediment N_2 consumption was up to 50.8 μg atom N m^{-2} h^{-1} (Table 4). Ideally, $^{28}N_2$ flux should not change during incubation since added NO_3^- was ^{15}N-labelled. It is not clear whether the change is due to added NO_3^- or a temporal change in remineralization activity. Assuming that temporal remineralization does not change with added $^{15}NO_3$ and denitrification is limited by organic matter availability, $^{28}N_2$ flux can decrease when $^{14}NO_3^-$ is replaced by $^{15}NO_3^-$ as a substrate for denitrification. Increased N-fixation also can reduce $^{28}N_2$ flux after the addition. Considering the uncertainty, $^{28}N_2$ flux before the addition was considered representative net N_2 flux in this study.

Unlike Laguna Madre/Baffin Bay, $^{15}NO_3$ removal was low in Sabine Lake. In June-00 at Station 1, NO_3^- flux increased from 5.44 μg atom N m^{-2} h^{-1} before to 22.1 μg atom N m^{-2} h^{-1} after the addition. NO_3^- removal was observed in other experiments. Fluxes of $^{29+30}N_2$ were high in Sept-00 and Mar-01 and accounted for 20-68% of the total NO_3^- flux. ^{15}N-labelled NH_4^+ production was not different from zero. In Laguna Madre/Baffin Bay, DNRA consumed large portions of added $^{15}NO_3^-$. In Sabine Lake, however, DNRA seems to be absent or very small. Recovery of consumed $^{15}NO_3^-$ as $^{29+30}N_2$ or $^{15}NH_4^+$ was 20-85 %. Larger portions of added $^{15}NO_3^-$ were converted to N_2 gas rather than NH_4^+, which is different from Laguna Madre/Baffin Bay. Enhancement effects of NO_3^- addition on denitrification were low in Laguna Madre/ Baffin Bay and Sabine Lake.

3-5. Nitrogen fixation after $^{15}NO_3^-$ addition

Nitrogen fixation was calculated after $^{15}NO_3$ addition (An et al. 2001). N-fixation rates were high (96 μmole N m^{-2} h^{-1}) in Jun-00 and decreased throughout the study to 10.9 μmole N m^{-2} h^{-1} in Mar-01 (Table 4). N-fixation rates were higher at

Station 1 than Station 2 in Jun-00 and Sept-00. N-fixation rates were slightly higher at Station 2 versus Station 1 in Dec-00 and Mar-01 (Table 4). Since $^{28}N_2$ flux changed before and after $^{15}NO_3$ addition in all incubation experiments, direct comparison between net N_2 flux before the addition and calculated N-fixation after the addition was difficult. However, calculated N-fixation values show that N-fixation is a significant process in Sabine Lake. N-fixation rates decreased with freshwater input (Fig. 8c). Temperature and SOD had no relationship with N-fixation (Fig. 8a & 8b). Favorable light conditions during low flow may have increased N-fixation in June-00 and Sept-00.

4. Conclusions

1. N-fixation often exceeded denitrification in Sabine Lake and net N_2 fluxes ranged from –56 to 90 μmole N m^{-2} h^{-1} during Jun-00 to Mar-01.
2. Net N_2 flux and sediment oxygen demand did not follow water temperature and showed an inverse relationship with freshwater input.
3. Potential rates of dissimilatory nitrate reduction to ammonium were absent or very low in Sabine Lake.
4. Nitrogen fixation rates showed an inverse relationship with freshwater input.

5. Acknowledgements

This project was supported by the Texas Water Development Board (TWDB; Contract #2000-483-337. The authors wish to thank Dr. David Brock, TWDB, for field assistance and project management. Dr. David Hicks, Lamar University, provided boats and assistance with field operations.

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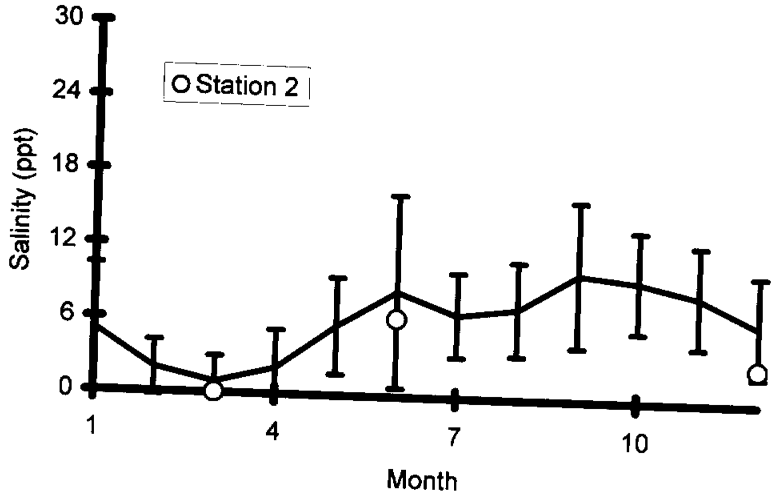
Table 1. Environmental variables in Sabine Lake

	Station 1				Station 2			Station 1-A
	Jun-00	Sep-00	Dec-00	Mar-01	Jun-00	Dec-00	Mar-01	Sep-00
Location								
Longitude	93.51.41.2				93.47.40.3			93.51.40.4
Latitude	29.49.57.0				29.58.11.2			29.50.28.4
Water depth (m)	2.2				1.8			
Sand content (%; > 64 μm)	30.5				89.0			
Salinity (ppt)								
Surface	7.85	15.17	4.76	0.53	6.31	2.91	0.05	15.8
Bottom	7.85	15.17	4.82	0.53	6.31	4.82	0.05	15.8
Temperature (°C)								
Surface	29.45	28.52	11.21	16.37	29.44	12.3	15.8	28.59
Bottom	29.45	28.52	10.57	16.37	29.42	10.57	15.75	28.59
Dissolved oxygen (mg/L)								
Surface	5.66	5.6	7.54	6.07	5.33	6.66	6.41	5.36
Bottom	5.44	5.3	7.63	5.95	3.01	7.63	6.41	5.29

Table 2. Denitrification rates (µg atom N m^{-2} h^{-1}) measured using intact core flowthrough systems. Denitrification rates were estimated from the N_2:Ar ratio changes between inflow and outflow water samples.

Station	Station 1	Station 2	Station 1-A
Samples			
Jun-00			
Light 1	-53.8	-94.7	
Light 1	-12.6	-73.9	
Light Mean	-33.2	-84.3	
Dark 1	-12.3	-86.3	
Dark 1	-43.0	-54.6	
Dark Mean	-27.7	-70.4	
Mean (SE)	**-30.4 (10.8)**	**-77.4 (8.6)**	
Sep-00			
Light 1	20.7		230.7
Light 1	78.2		65.8
Light Mean	49.5		148.2
Dark 1	193.0		49.8
Dark 1	67.0		84.2
Dark Mean	193.0		49.8
Mean (SE)	**89.8 (36.6)**		**107.6 (41.6)**
Dec-00			
Light 1	-10.5	-40.9	
Light 1	-28.9	-43.6	
Light Mean	-19.7	-42.2	
Dark 1	-69.5	-47.1	
Dark 1	-40.1	-27.8	
Dark Mean	-69.5	-47.1	
Mean (SE)	**-37.2 (12.4)**	**-39.8 (4.2)**	
Mar-01			
Light 1	-20.6	-27.6	
Light 1	-30.4	-109.7	
Light Mean	-25.5	-68.7	
Dark 1	-8.1	-80.6	
Dark 1	-43.7	-6.4	
Dark Mean	-25.9	-43.5	
Mean (SE)	**-25.7 (7.5)**	**-56.1 (23.7)**	

(a) Salinity in upper Sabine Lake



(b) Salinity of Lower Sabine Lake

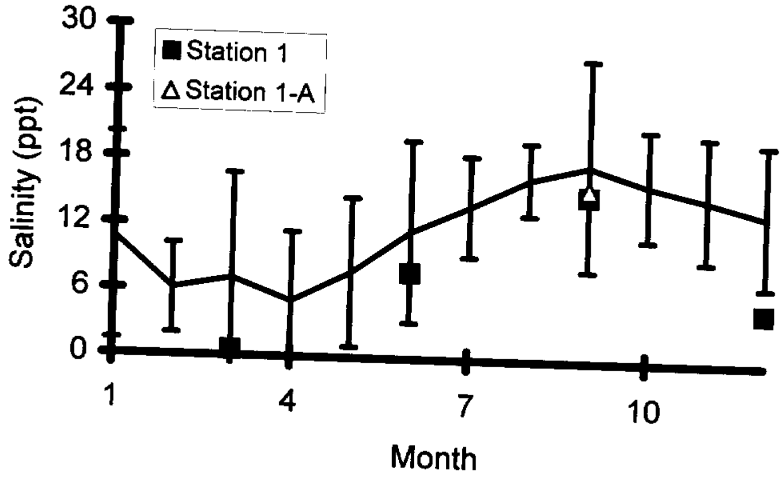


Figure 1. Salinity in upper (a) and lower (b) Sabine Lake. Line and error bar (1 STD) represents the monthly average salinity
(From: Dr Brock; http://hyper20.twdb.state.tx.us/Sabine/upsabsal.html)
and symbols represent salinity measurements during 2000 and 2001 field work.

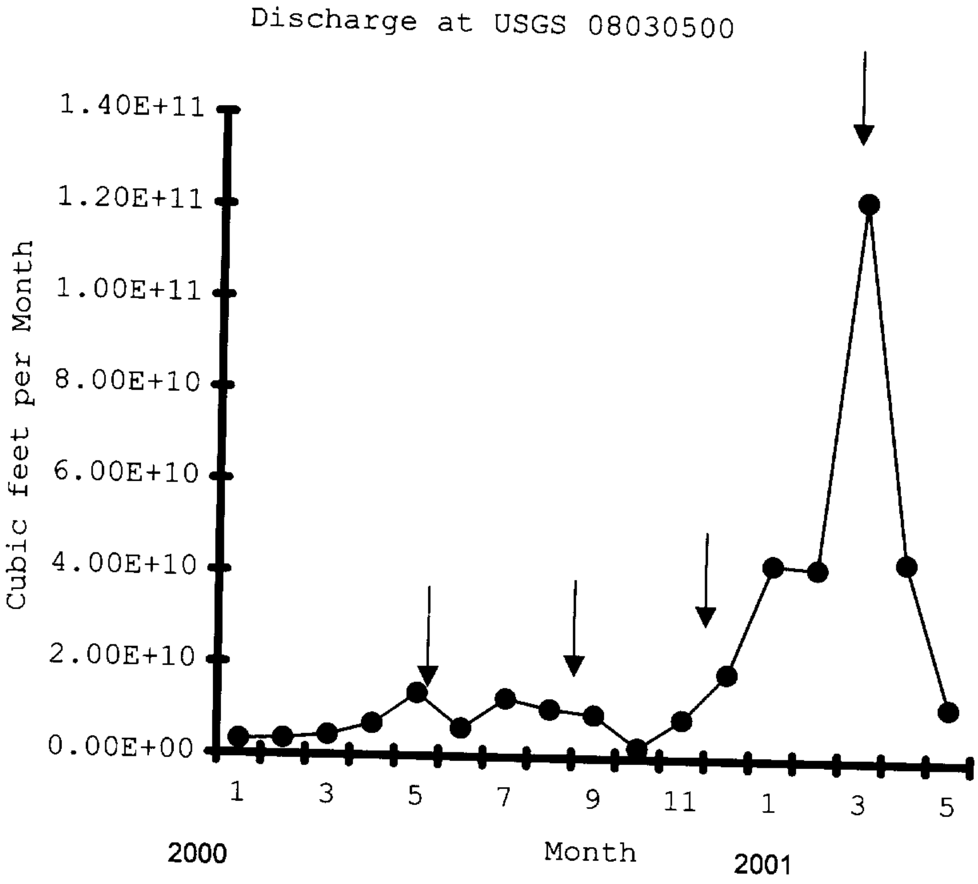
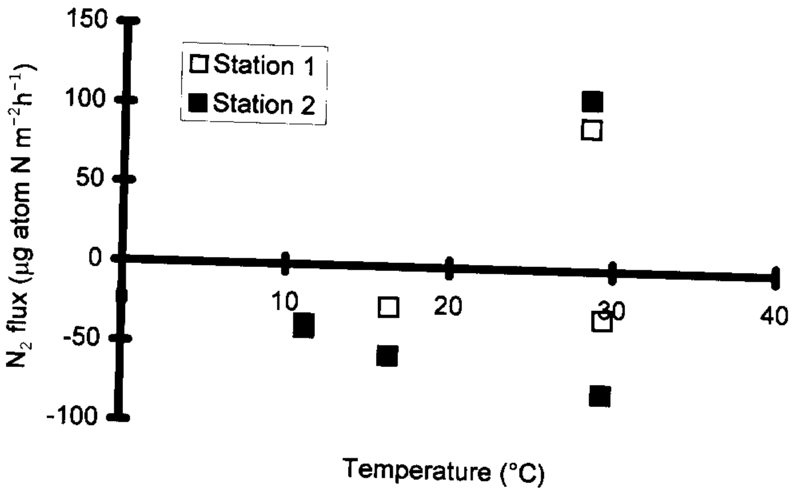


Figure 2. Monthly discharge measured at USGS station 08030500 (Sabine Rv nr Ruliff, TX). Arrows show the sampling dates.

(a) N_2 flux versus Temperature



(b) Sediment oxygen demand versus Temperature

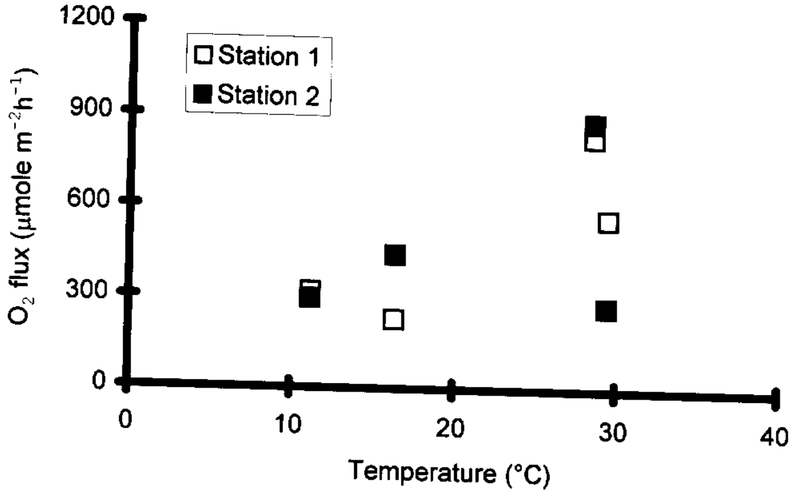


Figure 3. Denitrification rate versus temperature (a) and SOD versus temperature (b) in Sabine Lake.

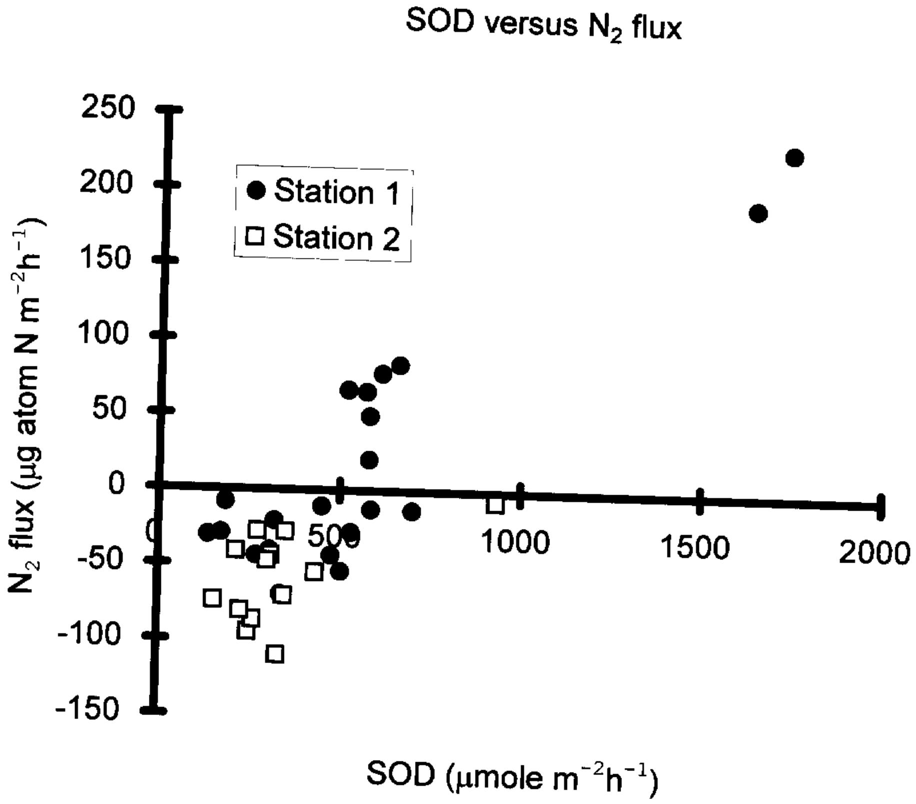
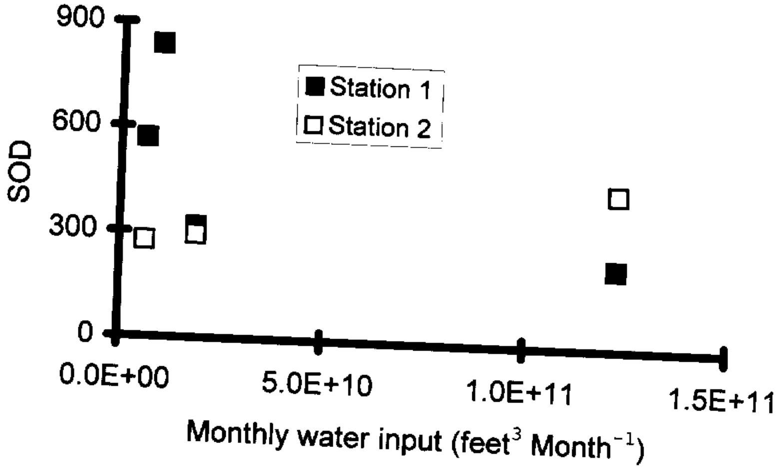


Fig 4. Relationship between sediment oxygen demand and N_2 flux in Sabine Lake.

(a) Fresh water input versus SOD



(b) Fresh water input versus N_2 flux

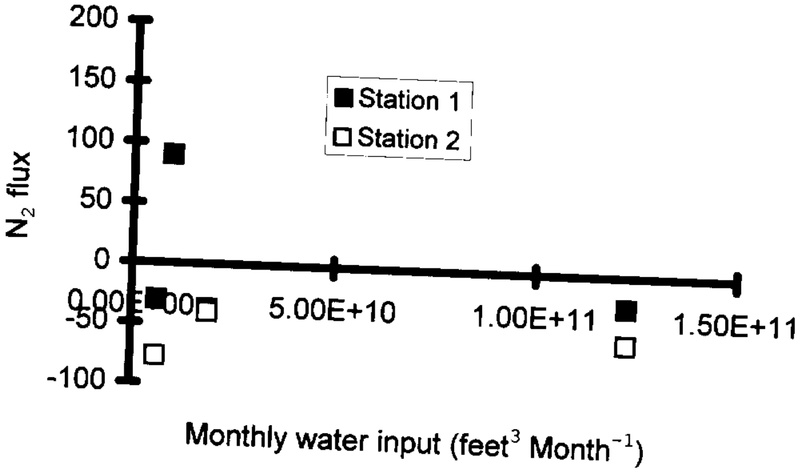
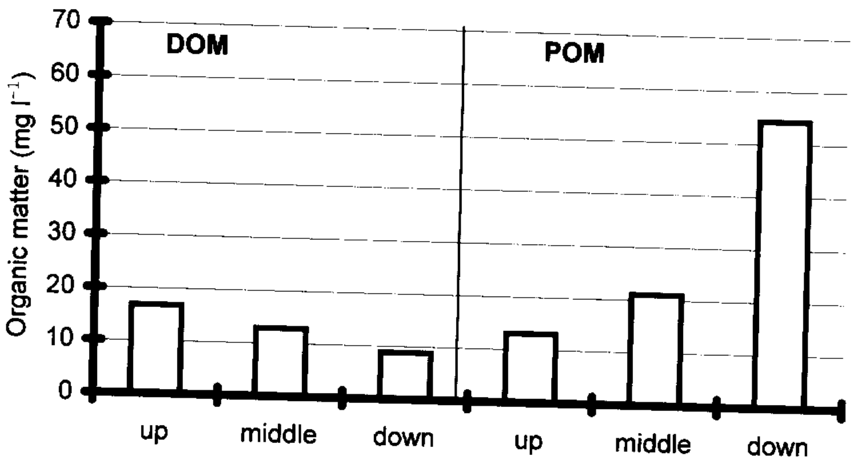


Figure 5. Relationship between freshwater input to Sabine Lake and SOD (a) and N_2 flux (b).

(a) Low flow season (Oct. 1993)



(b) High flow season (Mar. 1993)

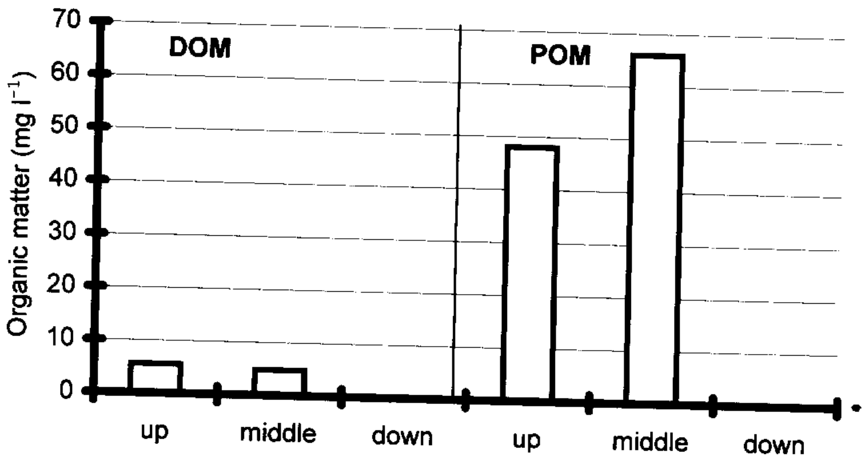
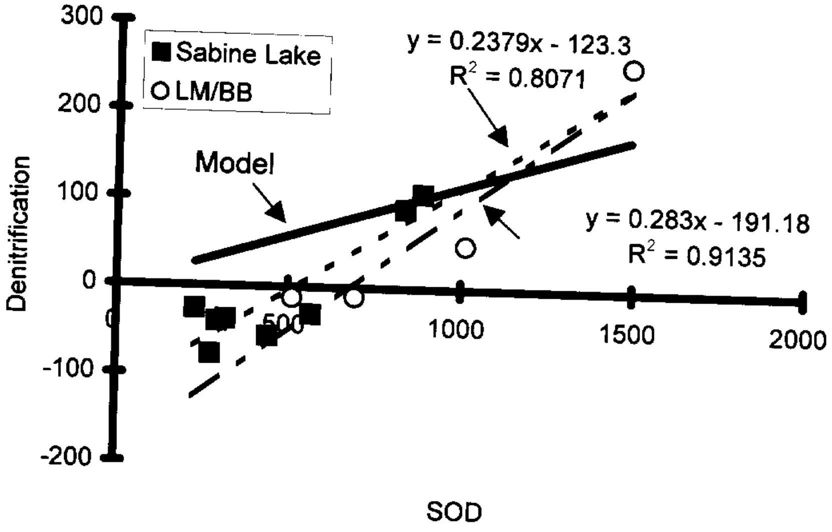


Figure 6. Dissolved organic matter (DOM) and particulated organic matter (POM) concentration in upper, middle, and lower Sabine Lake. The freshwater discharge was 1600 ft^3 sec^{-1} in low flow season and 16000 ft^3 sec^{-1} in high flow season. Data from Baskaran et al. 1997.

(a) Model versus measured denitrification



(b) Model versus measured denitrification (Galveston)

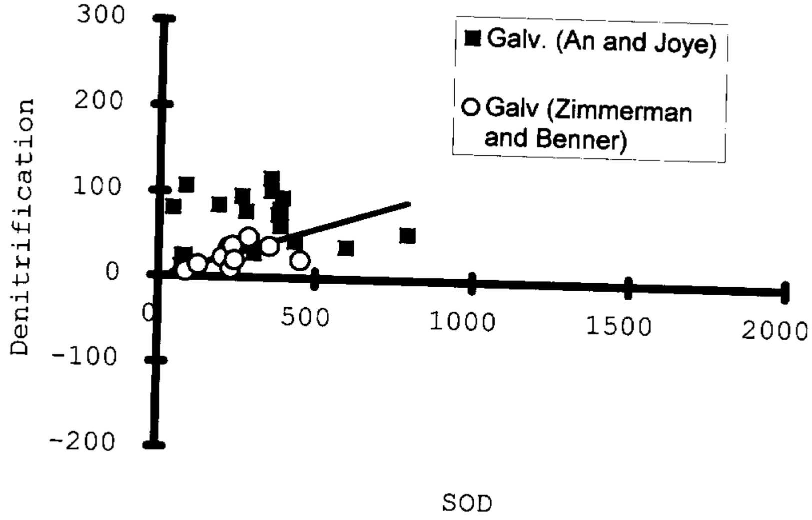
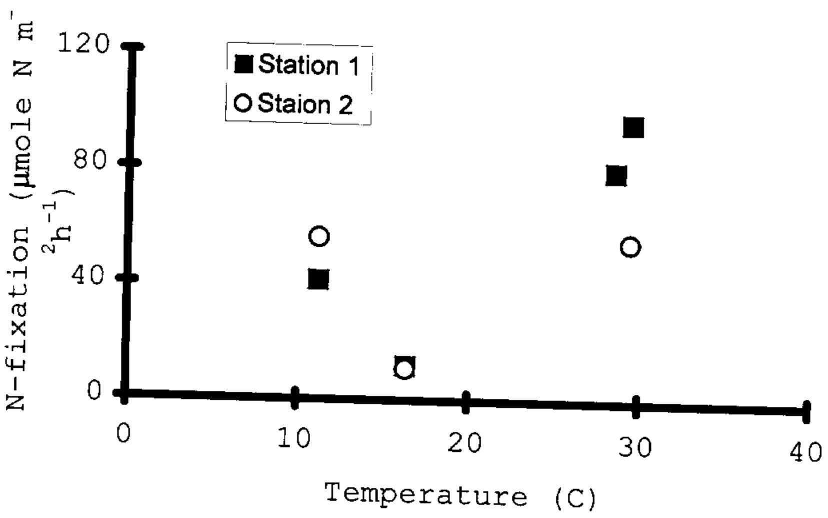
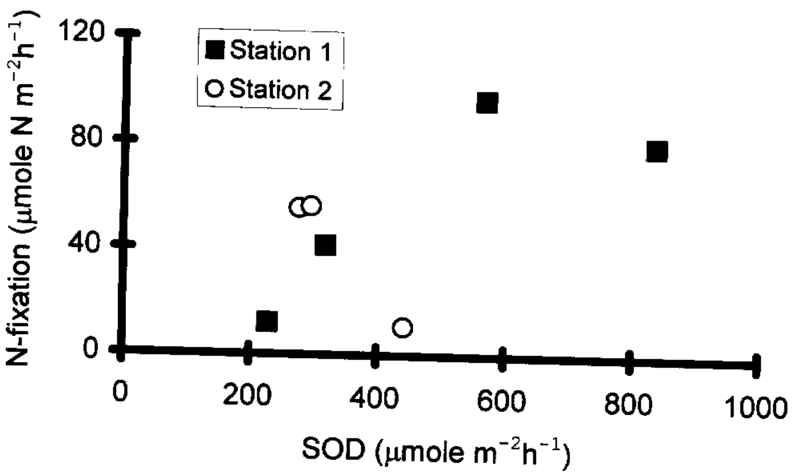


Figure 7. The relationship between sediment oxygen demand (SOD) and denitrification in Sabine Lake and Laguna Madre/Baffin Bay (a) and Galveston Bay (b).
The line shows the relationship of the two processes suggested by Seitzinger and Giblin (1998).

(a) Temperature versus N-fixation



(b) SOD versus N-fixation



(c) Fresh water input versus N-fixation

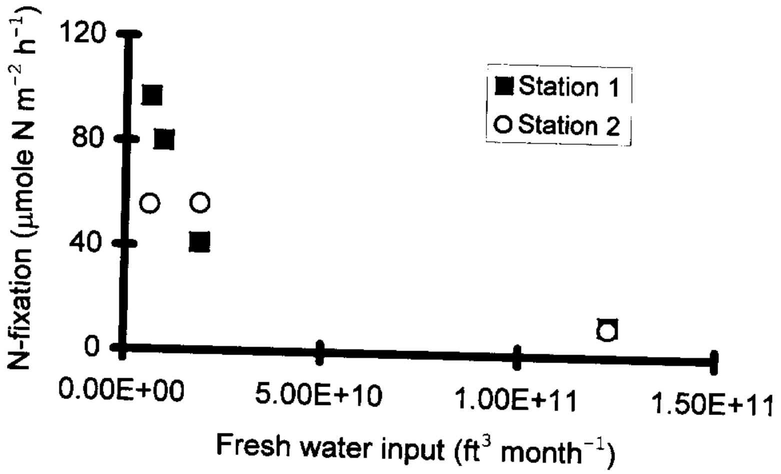


Figure 8. Relationship between nitrogen fixation and temperature (a), SOD (b) and fresh water input (c) in Sabine Lake.

Review of "Nitrogen Cycling in Sabine Lake", by An, McCarthy, and Gardner.
Contract # 2000-483-337

This is a very professional report which adds significantly to our understanding of biochemical processes in Sabine Lake and our estuaries in general. The work described, the data presented, and the analyses presented fulfill the requirements of the contract scope of work. I have a few comments and suggestions for additions or clarification to improve the final report.

On the title page, below Texas Water Development Board, please add our contract number 2000-483-337 and UTMSI report number if applicable.

In many of the figures, axes lines and labels appear light or gray in our copy. The figures should all be formatted to allow this report to be successfully photo-copied, black and white.

In the first paragraph, methods section, where sediment texture is mentioned, a bit more detail could be given. We can assume that sediments are predominently muddy at both sites, but this should be indicated. Montagna's sediment composition data might be cited.

In the Methods section, after the first sentence, you might add that raw water was collected at the same time as sediment cores were.

On page 6, since this study did not measure organic matter delivery, either data from USGS or TNRCC might be cited or the statement about the lack of correlation could be qualified: "...does not appear to be correlated".

On page 7, line 5, the residence time should be described as "longer", not "large", since even under low flow conditions, this lake has a shorter residence time than some other bays.

Page 7, 4th line from bottom, suggest insert to read "The rates at which denitrification...."

Page 8, line 3, suggest clarifying "model" to "Seitzinger and Giblin model" This paragraph is a little awkward.