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N_2-fixation along a gradient of long-term disturbance in tropical mangroves bordering the gulf of Mexico

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Abstract Microbial processes are key elements in determining the productivity of mangroves, and reductions in these processes reflect the loss of microbial biodiversity and function due to fabricated disturbances. Because nitrogen is a major limiting nutrient for the productivity of these ecosystems, the goal of this study was to determine profiles of inorganic nitrogen combined with several environmental parameters, all in relation to the degree of long-term hydraulic impairment of a tropical, monospecific black mangrove (*Avicennia germinans*) forest that showed degradation ranging from total loss of mangrove cover to no disturbance. N_2-fixation, oxygen levels, and nitrite contents decreased significantly with the severity of the disturbance, and almost null levels were reached in the completely degraded zone, whereas salinity achieved very high values. Concomitantly, total N, ammonium, and P contents and ammonia volatilization increased significantly. Pore-water temperature and pH increased moderately. Other soil physical properties (sand, silt, clay, organic matter, and total C), which varied among the sampling sites, were not correlated with the level of disturbance. Principal component analyses, including environmental and biological parameters, suggested that the most significant finding was the considerable loss of N_2-fixation with increasing impairment, which was concomitant with significant increases in volatilization of ammonia and salinity. The results show that microbial N-cycling processes are highly sensitive to salinity and to man-made disturbances that modify the water level and flow.

Keywords Disturbance · Impairment · Mangroves · N_2-fixation · Salinity

Dedication This study is dedicated to the memory of the Mexican mangrove researcher Dr. Gina Holguin (1957–2007) of CIBNOR, Mexico.

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Introduction

Mangroves are well recognized for their extremely high productivity, along with coral reefs and rain forests and for being the breeding, nursery, growth, refuge, and feeding zones for a wide variety of organisms (Lee 1995; Primavera 1998; Holguin et al. 2001). These marine ecosystems help sustain coastal fisheries in the tropics (Lee 1995; Primavera 1998; Aburto-Oropeza et al. 2008) and provide several environmental services to humans, such as shoreline stabilization, protection against tsunamis, and protection for corals reefs by acting as giant filters for terrestrial sediment and man-made pollutants (Sjöling et al. 2005; Spalding et al. 2010). Nonetheless, considerable areas of mangrove forest have been lost worldwide and destruction

continues to occur. Although the causes of loss are well documented (Kaly et al. 1997; Sjöling et al. 2005; Holguin et al. 2006; Bosire et al. 2008; Strangmann et al. 2008), the consequences for sediment fertility, the microflora profile and activity, nutrient properties, element recycling, and the possible functional recovery of the ecosystem after reforestation efforts are not well understood (Kaly et al. 1997; Holguin et al. 2001).

Tide cycles, water level variability, salinity, and geomorphology are the main factors that regulate the functions of mangrove ecosystems (Lee et al. 2006; Twilley and Rivera-Monroy 2009). Within this framework, microbial processes are the specific cornerstone variables of the mangrove ecosystem and are central to their productivity (Alongi 1996; Holguin et al. 2001). In particular, the diazotrophic, free, and associative nitrogen-fixing bacterial communities are a critical element in mangrove growth and productivity (Toledo et al. 1995a; Bashan et al. 1998; Holguin et al. 2001; Ravikumar et al. 2004) because mangrove ecosystems are N-limited (Holguin et al. 1992). Moreover, the loss of microbial diversity and function is a consequence of deforestation (Sjöling et al. 2005).

Bosire et al. (2008) suggested that mangrove forests might recover without active restoration efforts. They recommended that restoration planning should first search for the potential existence of environmental stress in the ecosystem, such as a blockage of tidal inundation channels that may prevent the occurrence of secondary succession and then plan the removal of the stress before attempting restoration. The present study was designed in this context. For a better assessment of restoration and reforestation projects, there is a need to understand the underlying processes of soil transformations and the functional degradation of mangrove forests due to human activities (Kaly et al. 1997; Sjöling et al. 2005; Bosire et al. 2008; Strangmann et al. 2008). Such an essential function for mangroves is N_2-fixation. Nitrogen is a major limiting nutrient that affects mangrove ecosystem productivity (Holguin et al. 2001) and has been proposed as a quantitative indicator to evaluate the impairment and restoration of mangroves in arid zones (Vovides et al. 2011).

Our working hypothesis was that the degree of impairment of a monospecific black mangrove (*Avicennia germinans* L.) forest determines changes in N profiles and in soil environmental parameters. The objectives of this study were as follows: (1) to evaluate the functional degradation of mangrove ecosystems by monitoring N_2-fixation and environmental parameters along a gradient of forest degradation; and (2) to evaluate whether N_2-fixation, which is a possible indicator of mangrove function and health in arid zones (Vovides et al. 2011), can be used in other environments, such as tropical zones, where the structure and cover of mangroves are much more developed. To accomplish these objectives, we selected a gradient of degrading tropical mangrove forest (from undisturbed to complete tree death) on the north coast of the state of Veracruz, Mexico. This forest has been impaired since the 1980s by the construction of three long levees, ranging from 500 to 700 m that support power line towers. The levees blocked north–south water flow to the mangrove ecosystems that grow between and around the levees. Because of the physical barriers, the mangrove trees displayed "top dying", which is an unexplained phenomenon that slowly kills trees by stripping them of leaves, branches, and twigs to such an extent that the effects can be detected by satellite images (Blasco et al. 2001; Spalding et al. 2010). Since the construction of the levees (~30 years ago), 6 ha of the mangrove ecosystem have lost their tree cover and there has been a severe loss of trees in another 14 of the 4,864 ha ecosystem (J. López-Portillo, unpublished data).

Materials and methods

Study area and sampling sites

This study area was at the Laguna de Tampamachoco, Tuxpan, Veracruz, Mexico (21° 0.9′ N, 97° 47′ W) along a gradient of forest degradation ranging from a full monospecific forest coverage of an *A. germinans* mangrove ecosystem to total tree loss. This gradient occurred most notably toward the slightly higher grounds, which are less affected by tides and it might also have been caused by poor water circulation and evaporation of the water between the constructed levees. This process resulted in a hypersaline environment ranging from 35‰ to 65‰ up to 140‰ in soil where the mangrove cover has been lost. Between the non-affected area (close to the edges of the lagoon) and the area with complete forest loss (Fig. 1), the trees display dying of the crown, commonly called top dying. Six sampling stations were established following a gradual degree of impairment in a transect perpendicular to the lagoon margin (Fig. 1c). The first site (S1) was located in the non-degraded area near the margin and the last site (S6) was located in the completely degraded mangrove area, in which all of the trees had been lost at the highest topographic point along the transect. Each site was separated from the next by 66 m, which resulted in a total distance of 330 m to cover the entire gradient of forest degradation. Another non-degraded site (S0), which served as a positive control, was selected at the same tide influence line at ~1.3 km north of the completely degraded mangrove of station S6 (Fig. 1b). We assumed that sites S6 and S0 were subject to a similar tide influence (Fig. 1a).

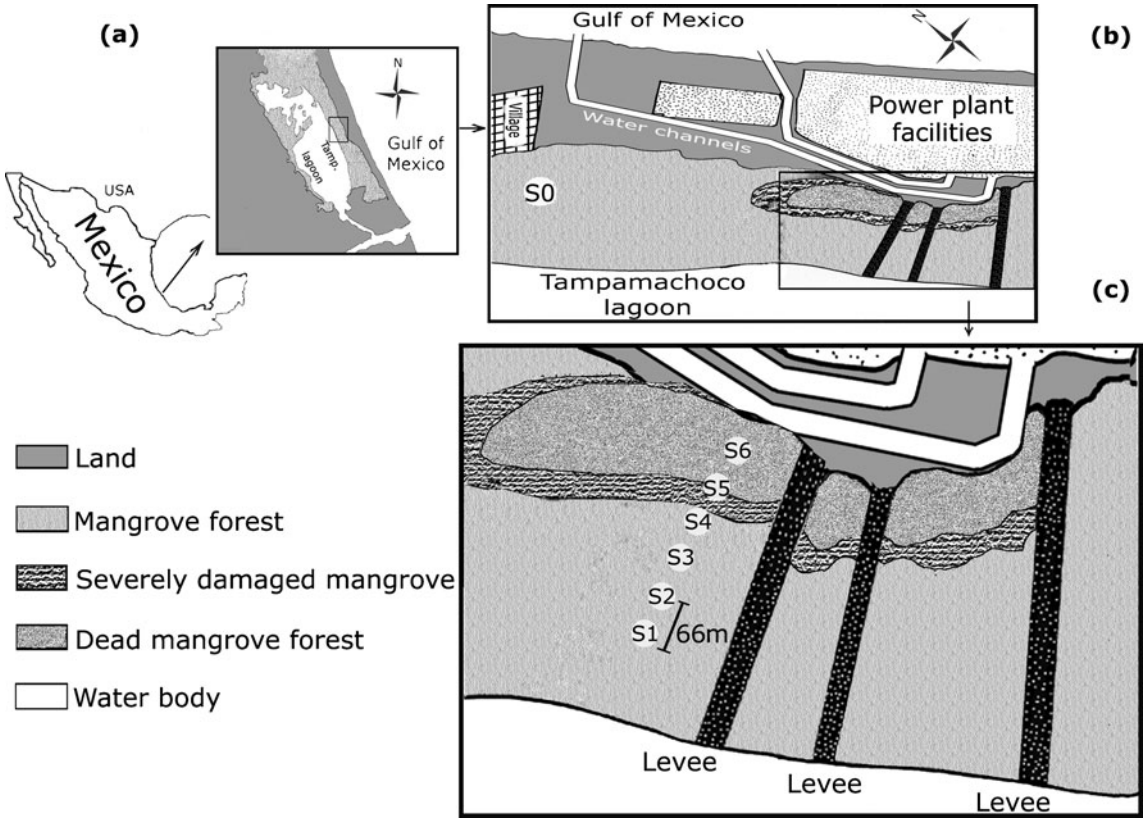

Fig. 1 Location of the study site at Laguna de Tampamachoco, Veracruz, Mexico and of the sampling sites (S1–S6) along a disturbance gradient. Site S0 is the well-preserved reference site for the most impaired site (S6)

Soil properties

Four 2-kg soil cores at each site from the top 25 cm were collected (in July 2008 and August 2009). Core pairs from each sampling day were mixed to form a composite sample of the site and transported to the laboratory on ice for physical and chemical analysis. Samples were dried at 65°C for 96 h, ground up, and passed through a 2-mm sieve. Organic matter, total C and total N content were determined using a C and N analyzer (TruSpec CN, LECO, St. Joseph, MI). The soil texture was determined as described by Folk (1966). The electric conductivity was measured in situ using a multi-parametric field device (YSI 55, YSI, Yellow Springs, OH).

Interstitial water sampling and analysis

Three samples of interstitial water were carefully collected at a depth of 10 cm with a customized water extractor consisting of acrylic tubing (0.5-cm internal diameter; 50-cm long) connected to a 60-mL plastic syringe that was adapted at its base with a three-way stopcock (McKee et al. 1988) to purge the water into 50-mL centrifuge tubes with minimum oxygen alterations. This water collection technique permits the extraction of interstitial water at low and high tides with no surface water contamination.

The extracted interstitial water was used to determine pH, water temperature, salinity, and dissolved oxygen in situ (YSI 55, YSI, Yellow Springs OH). To determine the nutrient content (NH_4^+, NO_3^-, NO_2^-, and P), samples of the extracted interstitial water were maintained in 250-mL sealed bottles that had been previously washed with a 10% HCl solution in de-ionized water and rinsed thoroughly with de-ionized water. The samples were maintained on ice and transported to the laboratory, where all of the samples were passed through GF/F micro-fiber filters (GF/F 47 μm, Whatman International, Maidstone, England) and stored at −80°C for up to 2 weeks before the analyses.

The ammonium (NH_4^+) concentrations in pore water were analyzed using the phenol and sodium nitroprusside technique (Solorzano 1969). Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were assayed by the sulfanilamide method, with NO_3^- previously reduced to NO_2^- in a cadmium column (Strickland and Parsons 1972). Analysis of soluble P used the ammonium and molybdate technique (Strickland & Parsons 1972). All analyses were conducted using a spectrophotometer (DR/2010 Hach, Loveland, CO).

N_2-fixation

N_2-fixation was assessed indirectly using the acetylene reduction assay (ARA, Holguin et al. 1992). Soil slurries were collected at 5 cm below the surface using 5-mL sterile syringes with the top end of the syringe removed; this technique provided an effective vacuum barrel. From each sampling site, three samples were collected in June 2008 and three in August 2009 (n=42). Duplicate slurries were placed in 60-mL sterile serum vials, hermetically sealed with serum stoppers and enforced with aluminum rings. The duplicate slurries were collected from sites that were located close to one another to minimize variation due to micro-environmental and soil heterogeneity. The samples were kept on ice and transported to the laboratory within 4 h of collection, where ARA was analyzed according to Holguin et al. (1992) as modified by Vovides et al. (2011).

Volatilization of ammonia from sediment

Ammonia (NH_3) volatilization from the sediment was measured in situ using the chamber method technique (Hutchinson and Livingston 1993). Briefly, three 20-L flat buckets (113 cm in diameter and 20-cm high) were placed upside-down at each sampling site, and their rim was buried to a depth of 5 cm in the sediment to avoid the escape of gas from the buckets. Each bucket covered 1 m^2 of the soil surface and thus stored a total volume of 15 L of atmosphere. A valve was adapted to the top of each bucket and connected to a flow pump system made of a portable aquarium pump enclosed in an air-tight box. The enclosed atmosphere was pumped out at a rate of 5 mL s^{-1} into three vials containing 5 mL of an ammonia-trapping solution comprising 1% sulfuric acid. Because of the very large ratio of entrapped atmosphere to the size of the samples, no measurable vacuum occurred in the chambers. Samples were collected at the moment of chamber installation in the field (time 0) and 24 h after placement. Solution-trapping vials were kept on ice until their arrival at the laboratory and analyzed for NH_4^+ according to Solorzano (1969). Ammonia volatilization was estimated by subtracting the quantity of ammonia at the time of chamber placement from the final quantity of ammonia obtained after 24 h, as suggested by Hutchinson and Livingston (1993).

Forest structure

To characterize the forest structure, we measured all of the trees within one 100-m^2 plot at each site (n=7). Because the forest was a monospecific stand of *A. germinans*, no species composition analysis was conducted. The tree density, tree height, diameter at breast height (DBH), and basal area were assessed following (Cintron and Novelli 1984). The DBH of main trunks were measured at 130 cm following Brokaw and Thompson (2000). Considering that some of the trees displayed canopy death, the height measurements considered the highest branches that showed green leaves.

Statistical analyses

To compare the forest structure, soil properties, N_2-fixation, and environmental parameters among the sampling sites, one-way and two-way nested analyses of variance ANOVA were performed followed by Tukey's honestly significant differences (HSD) post-hoc test where appropriate, using the programming language R (R Development Core Team 2007). Percentage values related to soil texture (Table 1) were arcsine-transformed prior to the analysis. All of the statistical tests were evaluated at $P<0.05$ or at the P levels specifically indicated. Two principal component analyses (PCAs) were performed to evaluate the relationships of environmental parameters (salinity, pH, temperature, oxygen, and P content) and N profiles (NO_2^-, NO_3^-, NH_4^+, and N_2-fixation) among the sites using software for ecological analyses (primer-E, version 6.1.6, Plymouth UK)(Clarke and Gorley 2006).

Results

Soil properties

Table 1 presents a summary of the physical and chemical properties of the sediment along the gradient of disturbance; among the sites, site S1 is the best preserved, site S6 is the most deteriorated, and site S0 is the undamaged control. A one-way ANOVA revealed variability in the sand content among sites ($F_{6,14}$=112, $P<0.001$). Non-significant differences were found when comparing site S5 vs. S2 and site S6 vs. S0 and S1.

The silt content ranged from 22% to 88%; the lowest content was found at site S1 and the highest at site S3. The sites differed significantly from each other with respect to silt content ($F_{6,14}$=165, $P<0.001$). Non-significant differences were obtained only in comparisons of sites S2 and S4 and sites S2 and S5. The amount of clay was significantly higher at site S1 (the most preserved) compared to all other sites ($F_{6,14}$=26, $P<0.001$). The clay content did not differ significantly among sites S2, S3, S4, S5, and S6 ($P>0.05$). More statistically significant clay content was present at site S0 only when compared to sites S5 and S6 (Table 1).

The content of organic matter (OM) in the sediments ranged from 23.4% to 35.3% (Table 1). The lowest OM was detected at the best-preserved site (S1) and the highest was at site S5. Site S1 differed significantly from all of the

Table 1 Soil physicochemical profile and forest structure of the sampling sites along a gradient of disturbance

Site Disturbance level	S0 Least impaired	S1	S2	S3	S4	S5	S6 Severely damaged
Sediments							
Sand (%)	39.4±0.9d	44.0±1.5d	14.7±0.6b	1.0±1.2a	23.7±0.6c	15.7±0.6b	38.7±0.6d
Silt (%)	46.8±0.6b	21.8±1.2a	74.9±2.6de	88.5±1.2f	68.5±2.3d	77.8±1.1e	57.5±1.2c
Clay (%)	13.9±0.3b	34.2±1.2c	10.3±2.4b	9.9±2.0ab	7.7±1.9ab	6.5±0.6ab	3.6±1.4a
OM (%)	33±1.0cd	23.4±1.3 a	30.2±0.5bc	26±1.2ab	28±1.3abc	35.3±1.0d	31.2±1.0cd
C (%)	19.1±0.6de	13.6±1.0a	17.5±0.2bcd	15.1±1.0ab	16.3±1.0abc	20.5±0.5e	18.1±0.6cde
N (%)	1.2±0.04c	0.7±0.5a	0.9±0.01b	0.9±0.02b	1.0±0.02b	1.2±0.09c	1.1±0.01
C:N	16±0.1a	18.6±0.2a	18.2±0.6a	17±0.5a	16.7±0.5a	17.5±1.2a	17.2±0.1a
Eh (mV)	16.5±1.0d	35±1.0e	18.5±0.5d	−6.5±0.7c	−67.5±3.0b	−69±2.6ab	−71±0.6a
Forest structure							
Height (m)	4.8±0.2ab	5.8±0.1a	4.7±0.3ab	4.4±0.3ab	4.0±0.6b	3.5±0.4b	NP
DBH (cm)	10.6±0.6a	12.5±1.2a	7.4±0.9a	9.2±2.1a	10.4±2.7a	10.6±2.0a	NP
Density (trees/100 m^2)	15	13	31	13	10	9	NP
Basal area (m^2/100 m^2)	0.17	0.26	0.27	0.22	0.18	0.16	NP

Values are mean±standard error, except for density and basal area, which are absolute values per plot. The *same letters in a row* indicate no significant differences between the plots. Site S0 is the well-preserved reference site for the most impaired site (S6)

NP not present (all trees were dead at site S6)

other sampling sites ($F_{6,14}$=15.9, P<0.001). Similar trends were detected for the total C (%C, g/100 g soil) ($F_{6,14}$= 15.9, P<0.001), which varied from 13.6% (at site S1) to 20.5% (at site S5). Similar to the results obtained for organic matter, significant differences in C content were found when comparing site S1 to all of the other sites ($F_{6,14}$=15.9, P<0.0001), which were statistically similar to one another. No differences were found among sites S2, S3, and S4, whereas sites S0, S5, and S6 did not differ significantly from one another but were significantly higher than the rest. Despite the variations in C and N content, non-significant differences in the C:N ratio were found among all of the sites ($F_{6,14}$=1.9, P=0.14; Table 1). Finally, the redox potential (Eh) varied significantly among the sites ($F_{6,19}$=38.35, P<0.001), and there was a remarkable shift from positive values of Eh in the less disturbed sites to more negative values in the most disturbed sites (Table 1).

Forest structure

The highest tree stand density was observed at sites S1 and S2 (calculated as equivalent to 1,300 and 3,100 trees ha^{-1}) and the lowest was determined at sites S5 and S6 (calculated as equivalent to 1,000 and 900 trees ha^{-1}). The DBH and basal area were reduced by 15% in comparisons of the least and the most affected sites (S1 vs. S5), but the ANOVA on DBH values indicated that there were no significant differences among sites ($F_{5,85}$= 0.94, P=0.46; Table 1), suggesting a relatively homogeneous tree cover. Finally, the tallest trees were found at site S1 (5.8±0.1 m) and the shortest at site S5 (3.5±0.4 m; ~40% reduction), and our field notes indicate that this was positively due to top death; no trees were alive at site S6.

N_2-fixation, environmental parameters, and interstitial water properties

A significant loss of N_2-fixation capacity occurred along the gradient of disturbance ($P_{1,34}$< 0.0001, F=74.11, n=36). The N_2-fixation capacity was highest at the least disturbed site (S1) and was non-detectable at the most disturbed sites (S5 and S6) (Fig. 2a). One-way ANOVA indicated differences among the sites with respect to N_2-fixation ($F_{6,38}$=47, P<0.001, Fig. 2a); N_2-fixation at site S1 was significantly higher than that determined at the control site S0 (P<0.001) and at the degraded sites S5 and S6 (P<0.001). Non-significant differences in N_2-fixation were detected among sites S4, S5, and S6 (P> 0.05, Fig. 2a).

The soluble reactive P (PO_4^{3-}) increased significantly along the gradient of disturbance ($F_{1,34}$=80, P<0.001) and was highest at the most disturbed site ($F_{6,38}$=30, P<0.001). Non-significant differences were observed among the less disturbed sites S0, S1, S2, and S3 (Fig. 2a). In contrast, the oxygen content decreased significantly from site S1 to site S6 ($P_{1,31}$< 0.001, R^2=0.68) and a significant variation was observed among the sites ($P_{6,38}$<0.001, F=61). The highest variation was found at site S0 (Fig. 2b). Non-significant differences in oxygen content were observed between sites

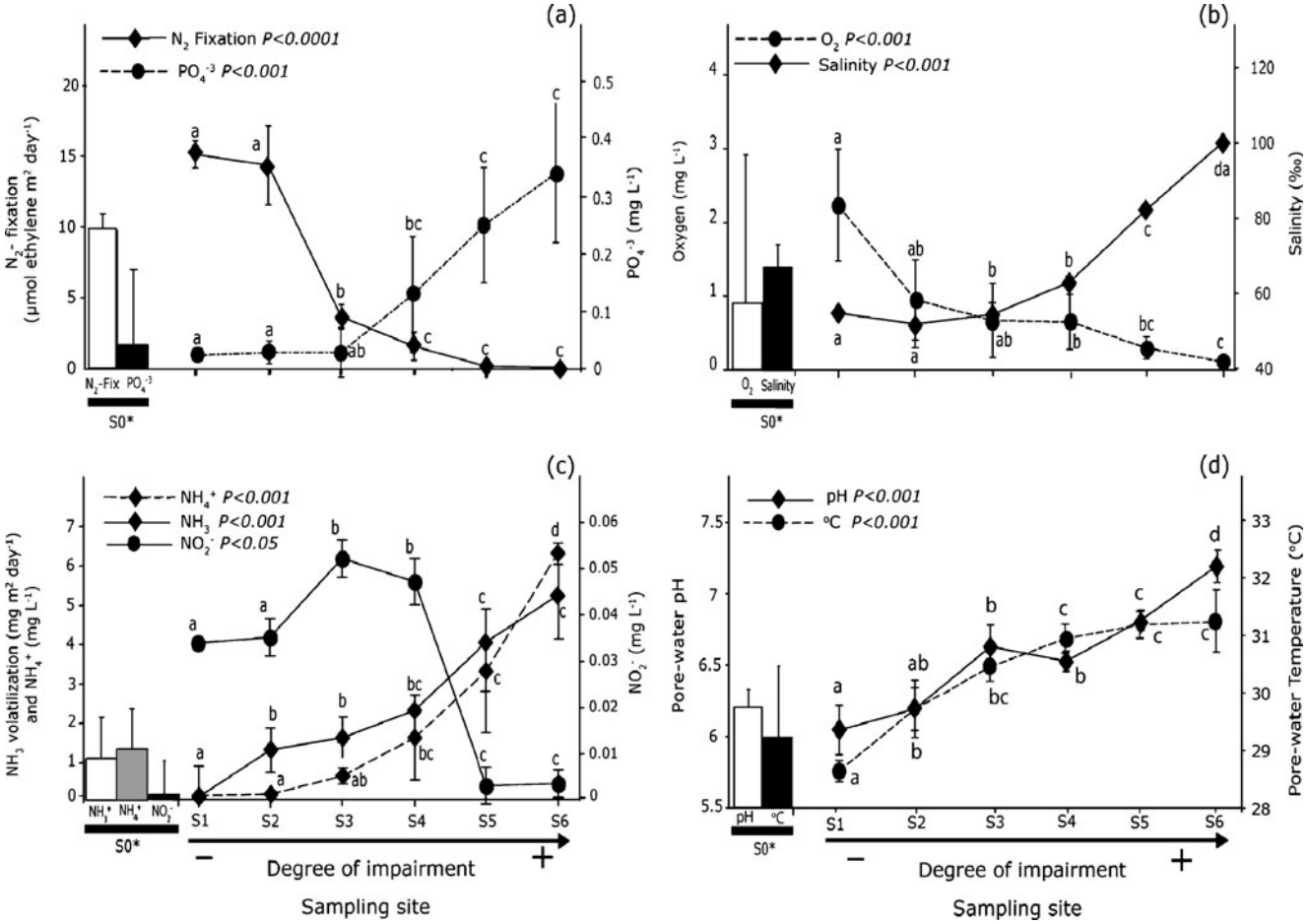

Fig. 2 **a**–**d** N_2-fixation and other environmental parameters along the gradient of disturbance. Values denoted with a *different lowercase letter* along each curve differ significantly based on one-way ANOVA followed by Tukey's HSD. *Vertical bars* represent one SE

S0 and S1 ($P>0.05$), but site S1 was significantly different from site S6 ($P<0.001$). The salinity increased significantly with the level of disturbance from site S1 to site S6 ($P_{1,34}<0.001$, $F=24.62$, Fig. 2b), where it reached extreme values (~100‰); significant differences were found among all of the sites ($P_{6,38}<0.001$, $F=33$). No significant differences in salinity were detected among sites S1, S2, and S3; site S4 was statistically similar to site S3. By contrast, significant differences were observed between sites S5 and S6, S1, and S6, and S6 and S0. There were no significant differences between sites S1 and S0 (Fig. 2b).

Ammonium in the pore water and NH_3 volatilization increased throughout the gradient of disturbance ($P_{1,32}<0.01$, $F=65.84$, and $P_{1,49}<0.01$, respectively). The control site S0 was significantly different from site S6 ($P_{6,38}<0.001$, $F=31$) but not from S1 ($P>0.05$) with respect to either NH_4^+ or NH_3 ($P_{6,38}<0.001$, $F=54$). Nitrite decreased markedly from site S4 to sites S5 and S6 and demonstrated similarity between the following site pairs: S1–S2, S3–S4, and S5–S6. There were significant differences between sites S1 and S2 with the impaired sites S5 and S6 ($P<0.001$) and site S1 with S0 ($P<0.001$). There were non-significant differences in NO_2^- between sites S0 and S6; very low levels were present (Fig. 2c).

A significant increase in pH was registered along the gradient of disturbance ($y=5.8+0.21x$, $R^2=0.69$, $F_{1,34}=75.44$). One-way ANOVA revealed a variation of pH between the sites ($F_{6,28}=17.6$, $P<0.001$). Significant differences were observed in comparisons of sites S1, S4, S5, and S6. No differences were detected between sites S0, S1, and S2 (Fig. 2d). The water temperature increased significantly (~3°C) along the gradient of disturbance ($y=28.6+0.51x$, $R^2=0.88$, $F_{1,34}=42$). A significant variation in temperature was observed among the sites ($F_{1,34}=10.4$, $P<0.001$) and the greatest differences were detected between the least disturbed site S1 and the most disturbed sites S5 and S6. No differences were detected between sites S0, S1, S2, and S3, among the intermediate sites S3, S4, and S5 or among the most disturbed sites S4 to S6 (Fig. 2d).

Two PCAs were performed using all of the obtained data. The first PCA explained 97.7% of the variation. The first component (PC1) accounted for 92.6% of the total

variability in the data and it is best explained by salinity at the sites. The second component (PC2) accounted for 5.1% of the variation in the data and was highly correlated with N_2-fixation. The PCA separated the data into two main groups according to salinity and N_2-fixation (Fig. 3a). Sites S1, S2, S3, S4 (in areas that were less affected along the gradient of disturbance), and site S0 demonstrated a low level of salinity and higher N_2-fixation rates in relation to the most disturbed sites (S5 and S6), which had the highest salinity concentrations and the lowest N_2-fixation rates. The correlation of PO_4^{-3}, temperature, NO_2^-, NH_3, NH_4^+, pH, and oxygen content to both principal components was much lower than salinity and the rate of N_2-fixation.

Due to the marked influence of salinity on the dispersion of data in the first PCA analysis, a second PCA was performed with exclusion of the salinity factor from the model (Fig. 3b). In this analysis, the first component (PC1) accounted for 82.4% of the data variation and was highly correlated with the N_2-fixation, NH_4^+, and NH_3. The second component accounted for 10.5% of the variation and was highly correlated with NH_4^+, NH_3, and pH (Fig. 3b). Three groups can be distinguished in Fig. 3b: the best-preserved sites (S0, S1, and S2) with the highest N_2-fixation rates (Fig. 3b, right oval); the group formed by intermediate sites along the disturbance gradient (S3 and S4) with lower N_2-fixation rates and higher concentrations of NH_4^+ in the pore water and NH_3 volatilization (Fig. 3b, central oval); and the group formed by the most disturbed sites (S5 and S6) with the lowest N_2-fixation rates and the most reduced N forms in the pore water (Fig. 3b, left oval). In this second PCA, the correlation of NO_2^-, PO_4^{3-}, temperature, and oxygen content to both principal components was much lower than the rate of N_2-fixation, NH_4^+, and NH_3.

Discussion

N_2-fixation is paramount for the well being of mangrove ecosystems and is the best-known microbial process in the ecosystem (Sengupta and Chaudhuri 1991; Holguin et al. 1992; 2001; Toledo et al. 1995b; Woitchik et al. 1997; Lee and Joye 2006; Vovides et al. 2011). It may serve as a practical tool to evaluate restoration projects, deterioration

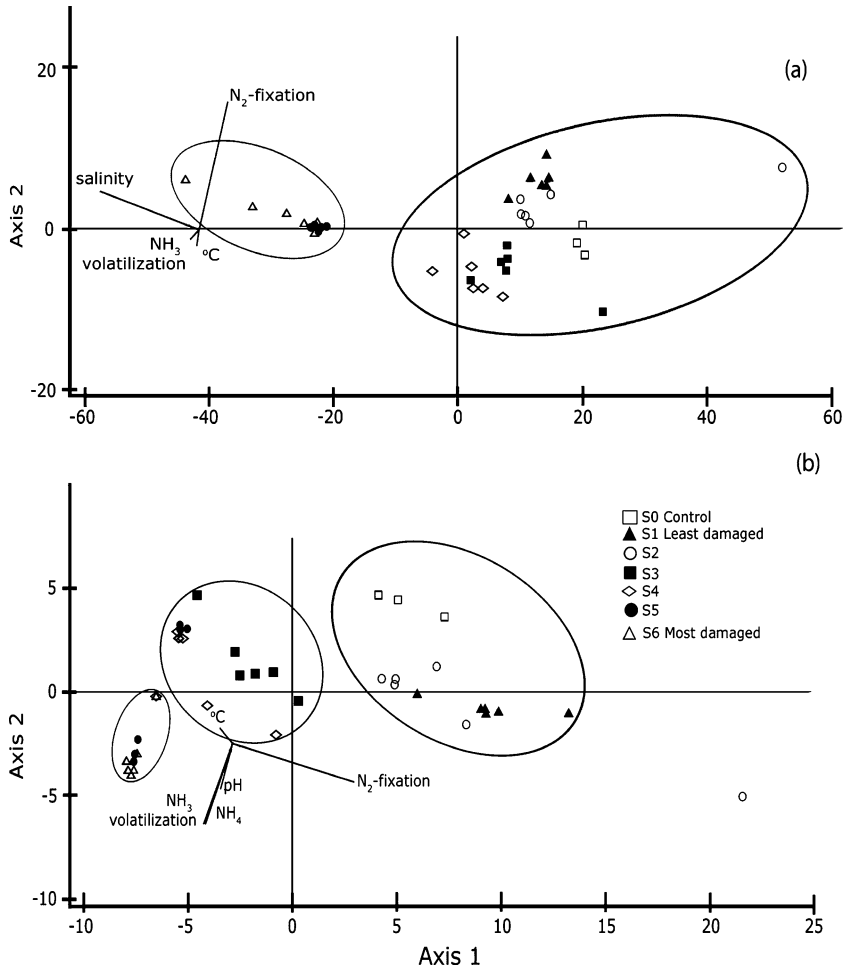

Fig. 3 **a** Principal component analysis (PCA) of all environmental parameters along the gradient of disturbance. **b** PCA of the same parameters but excluding the salinity parameter

of existing ecosystems, or the risk of loss of mangrove by encroaching urbanization, as reported by Holguin et al. (2006) and Vovides et al. (2011) in arid regions. Our study expanded the usefulness of N_2-fixation as a tool to assess mangrove health and document loss of N_2-fixation and the shifts in nutrient profiles in mangrove sediments from disturbances of the water regime in tropical mangroves, which represent the vast majority of mangrove area worldwide (Spalding et al. 2010).

A shift in nutrient profiles started at site S3, with a marked increase of PO_4^{3-} that is probably related to greater solubility in increasingly reduced sediments. PO_4^{3-} is generally adsorbed to particles of Fe^{3+} and carbonate in oxic sediments but dissolves upon reduction of Fe^{3+} to Fe^{2+} in anoxic sediments (Sjöling et al. 2005).

One of the most frequent consequences of mangrove impairment in this tropical mangrove was the large increase in salinity in the sediments, similar to reports of arid region mangroves (Holguin et al. 2006; Strangmann et al. 2008). The loss of oxygen and rise of salinity and pH values at sites S3–S6 are mostly related to the increase in water temperature from the lack of water flux. Higher water temperature results in greater evaporation and increased salinity and pH values. The marked reduction of N_2-fixation at sites S3 and S4 is caused by increased pH values and concentration of NH_4^+, while total inhibition of N_2-fixation at sites S5 and S6 is caused by the combined effect of increased salinity, NH_4^+, and pH (Dicker and Smith 1981; Howarth and Marino 1988; Pessarakli and Zhou 1990; Zahran 1999). On the contrary, when pH values are less than 6.5, N_2-fixation occurs at high rates (Polman and Larkin 1988, Vovides et al. 2011). This occurred at the least affected sites and the control site; while N_2-fixation was significantly lower at higher pH values at the most impaired sites.

The low content of NH_4^+, the low rates of volatilization of NH_3 and the relatively high rates of N_2-fixation at the conserved mangrove sites suggest a rapid turnover of inorganic N into bacterial and plant biomass. Ammonium and NH_3 are indicators of reducing conditions, as anoxic and alkaline sediments tend to release NH_3 (Beutel 2006). Our measurements are consistent with this, demonstrating that sites with no N_2-fixation showed high concentrations of NH_4^+ in the water and high volatilization of NH_3.

Although some microbial communities function well under anoxic conditions, they contribute little to N cycling (Boto 1982; Howarth and Marino 1988; Holguin et al. 1992). The effect of the microbial pathways in overall inorganic N cycling is rather poorly understood (Buring and Hamilton 2007). A pathway that could be relevant to N cycling is anaerobic ammonium oxidation (anammox), a chemolithoautotrophic process by which ammonium is combined with NO_2^- under anaerobic conditions to produce N_2. The potential existence of this process has been described for mangrove forests (Meyer et al. 2005; Andreote et al. 2009). Anammox potential has been found to be closely related to availability of NO_2^-, which in turn depends on denitrification rates (Meyer et al. 2005; Buring and Hamilton 2007). The conditions under which anammox occurs were found at sites S3 and S4, where NO_2^-, NH_4^+, and low oxygen content were present.

Accumulation of NH_4^+ in impaired mangroves is common (Strömberg et al. 1998; Sjöling et al. 2005) and is attributed to an increase in water temperature and oxygen depletion from the system by interrupted water recirculation in the sediments (Bosire et al. 2008) and negative effects of increased salinity on diazotrophic communities (Dicker and Smith 1981; Bosire et al. 2008; Vovides et al. 2011). The increase in NH_4^+ was probably caused by bacterial decomposition, mineralization, and ammonification of dissolved organic matter from accumulated plant detritus (Kristensen et al. 1988; Sjöling et al. 2005), which in a constantly flooded zone, could lead to anoxic and reducing soil conditions. Because N_2-fixation occurs under micro-aerophilic conditions or in the absence of oxygen (Howarth and Marino 1988; Fay 1992), the anoxic condition was not the cause for inhibition of N_2-fixation.

Further evidence that the loss of N_2-fixation capability comes from increasing salinity, pH, and NH_4^+ in the sediments, thus related to the degree of mangrove impairment and not the geographical location of the sites, was from the sites themselves. Although the control site was at a level of tidal influence as the most affected site and 400 m inland from the lagoon in the center of these mangroves, it demonstrated N_2-fixation and N profiles similar to the least impaired sites (located 60 and 120 m from the lagoon, respectively).

In summary, N_2-fixation is gradually lost along a gradient of disturbance in tropical mangroves. This occurred mainly from increased salinity, ammonium content, and pH that results from to poor management of the water level and flow conditions. Our results support the hypothesis that N_2-fixing bacteria constitute a key functional group of microorganisms relevant to the function of wet tropical mangrove forests where nitrogen may be a less limiting factor and the microorganisms are sensitive to hydrological changes from man-made disturbances, as in mangroves in arid zones (Vovides et al. 2011).

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