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RESEARCH ARTICLE

Nitrogen Fixation in Preserved, Reforested, Naturally Regenerated and Impaired Mangroves as an Indicator of Functional Restoration in Mangroves in an Arid Region of Mexico

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Abstract

Although several damaged mangrove ecosystems have been restored worldwide, so far, it has not been established whether a restored mangrove system regains all the functional properties of preserved mangroves. This study measured nitrogen fixation as an indicator of whether disturbed mangroves that were reforested or naturally regenerated fully recovered from this disturbance at a functional level. Rates of nitrogen fixation were measured for one year in impaired, preserved, reforested, and naturally regenerated mangroves dominated by the black mangrove (*Avicennia germinans*). There was no significant difference in rates of nitrogen fixation among preserved and adjacent reforested and naturally regenerated mangroves, but a significant reduction occurred in an impaired mangrove. Nitrogen fixation was mainly controlled by pH, salinity, and temperature. The highest rates of nitrogen fixation occurred in summer at pH values less than 6.4, whereas the impaired mangrove had higher pH and salinity and had very low nitrogen fixation activity. These results suggest that nitrogen fixation can be used as an ecological indicator of the success of reforestation and as a sensitive measure of perturbations in mangroves.

Key words: ***Avicennia germinans*, function restoration, nitrogen fixation, reforestation.**

Introduction

Mangroves are an important type of tropical and subtropical ecosystem that form a natural barrier against tropical storms and tidal waves (Spalding et al. 1997). Additionally, mangroves are known for high productivity, essential linkage in estuarine food webs, sustaining marine fisheries, and extensive populations of birds (Lee 1995; Primavera 1998; Holguin et al. 2001). For centuries, mangroves have provided a wide range of products, from food and wood to bioactive compounds for the tanning and pharmaceutical industries (Spalding et al. 1997).

In the past few decades, mangroves have been reduced worldwide by logging, aquaculture (mainly shrimp cultivation), charcoal production, coastal engineering projects, agriculture, unregulated deposition of pollutants into the wetlands (Primavera 1998), and encroaching urban development in coastal areas (Holguin et al. 2006). Rates of mangrove deforestation have declined, over 3% of remaining mangroves worldwide were lost between 2000 and 2005, 3.8% in North and Central America, and 5.0% in Southeast Asia (FAO 2007). Over 35% of the world's mangroves have been lost since 1980, whereas more than 50% have been destroyed in recorded history (FAO 2007).

Concerns for mangrove conservation date back to 1760, when Don José, King of Portugal, issued an edict to restrict the widespread cutting of mangroves in Brazil to protect the tanning industry (FAO 2007). Increasing concerns about the consequence of loss of mangrove has led to conservation, restoration, and legislative initiatives for protection. Protection is now enforced in many areas, including Florida, Mexico, and Costa Rica (Spalding et al. 1997). Programs of mangrove reforestation and afforestation were started in China in the 1950s and in Bangladesh in the late 1960s (Ellison 2000). The primary objective of most mangrove reforestation and afforestation programs is to obtain forest products, but other objectives include sustainability of fisheries and protection of coasts against wave and storm surges and tsunamis (Ellison 2000).

Although restored mangroves might structurally resemble natural forests, even allowing reestablishment of animal species, it remains an open question whether reforested sites

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This study is dedicated to the memory of the late Dr. Gina Holguin (1957–2007), who studied bacteria communities of mangroves and tirelessly campaigned for conservation of Laguna de Balandra, the location of this study.

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can recreate the primary functions of mangroves (McKee & Faulkner 2000). This is an essential consideration before mangroves are considered fully restored (Lewis 1982; McKee & Faulkner 2000). The time lag required after initial reforestation to assess ecosystem functionality is longer than the time lag needed to assess survival of the vegetation and normal species composition, biomass, tree height, density, and dominance (McKee & Faulkner 2000). Consequently, assessing functionality has been the prevailing criterion to assess the success of reforestation programs (Ellison 2000; McKee & Faulkner 2000).

Despite high productivity, mangrove ecosystems are nutrient-limited, especially in nitrogen and phosphorus (Alongi et al. 1993, 2002; Holguin et al. 2001). Fixation of atmospheric nitrogen by diazotrophic bacteria, some associated with mangrove trees, is the major natural input of nitrogen to compensate for loss of nitrogen through denitrification (Howarth & Marino 1988; Toledo et al. 1995a). Currently in many areas, nitrogen input also comes from ammonium, nitrate, and nitrite runoff from nearby farmland. There is evidence that clear-cutting of mangroves may cause loss of microbial diversity and function (Kaly et al. 1997; Bosire et al. 2005). Specifically, nitrogen fixation was lost in deforested mangroves (Sjöling et al. 2005).

Our working hypothesis is that nitrogen fixation is a variable that depends on the degree of ecosystem impairment; hence, measurement of nitrogen fixation can serve as an indicator of whether an impaired ecosystem that was reforested or naturally regenerated can recover from disturbance and serve as a tool to evaluate restoration projects at the ecosystem functional level. This was done by measuring, over a one-year period, nitrogen-fixing activity and related, possibly controlling, environmental variables in preserved, reforested, naturally regenerated, and impaired mangroves dominated by black mangrove (*Avicennia germinans* L.) in an arid, subtropical region of northwestern Mexico.

Methods

Study Sites

We selected mangroves at Laguna de Balandra and Laguna de Enfermeria, located north of the city of La Paz in Baja California Sur, Mexico The mangroves have similar geological and geomorphologic conditions (Holguin et al. 1992). The mangrove at Balandra (24°19′45″N, 110°19′45″W) covers 22.5 ha. The mangrove at Enfermeria (24°13′49.87″N, 110°18′21.6″W) covers 5 ha. They are strongly influenced by tides; the supply of freshwater is restricted to a few flash floods that come from the hilly terrain of the upper drainage basin during summer tropical storms. Average annual rainfall is 175 mm (Comision Nacional del Agua 2009). Three species grow at Balandra: red mangrove (*Rhizophora mangle* L.), black mangrove (*Avicennia germinans* L.), and white mangrove (*Laguncularia racemosa* [L.] Gareth); two species grow at Enfermeria: *A. germinans* and *R. mangle*.

Five sites with different ecological histories are present within the two lagoons. Two sites had very limited human impact; these are the preserved sites (sites A and B). Because of superb preservation, these two locations were included in the RAMSAR international listing of protected wetlands in 2007 and, later in 2008, declared natural protected areas by the municipality of La Paz. Two sites were restored in 1995 after illegal clear-cutting in 1991 (sites C and D). At site C (0.19 ha), seedlings of *A. germinans* were successfully planted (Toledo et al. 2001) and are now trees, approximately 1.8 m tall. As a consequence of restoration at site C, the nearby, surrounding cleared area (site D) underwent natural regeneration (Bashan & Toledo 2008). The mangroves at site C and site D are visibly indistinguishable in plant cover from the preserved mangroves at site A and site B. *R. mangle* and *L. racemosa* were established by natural succession at site C, in addition to the planted *A. germinans*. Site D is exclusively covered by *A. germinans*. These four sites are located at Balandra; site E is an impaired mangrove that was damaged by a temporary block of the feeder channel at Enfermeria by temporary road construction in 1995. One year later, the channel was reopened, but the original 5-m-wide channel was reduced to less than 3.5 m. This limited water flow within the mangrove. Consequently, most of the trees died and almost no mangrove seedlings were recruited since then (Strangmann et al. 2008). This reduced the extent of the mangrove from 3 ha to less than 1.4 ha. This impaired mangrove site was selected for comparison with the conditions at Balandra without impairment. A complementary site (site F, at the outskirts of the city of La Paz (24°07′07.59″N, 110°20′52.40″W) included both preserved mangrove and mangrove that was clear-cut in 2006. This site was sampled once in May 2009 to provide supplementary data on the effect of clear-cutting on nitrogen fixation in the general area. Site F is part of a mangrove ecosystem that was described by Holguin et al. (2006). Consequently, it was assumed to have similar floral and environmental characteristics. All sites described in this study are in the general geographical area of the Bahía de La Paz, and therefore comparable.

Soil and Pore Water Properties

Four 2-kg soil samples from the top 10 cm were collected at each site in August and December 2006 and January, March, April, and June 2007. Each sample was divided into two equal portions. From each sample, 1 kg of soil was dried at 60°C and passed through a 2-mm sieve to determine organic matter (OM) content (Walkley & Black 1934). From the other 1-kg portion of each sample, pore water was extracted by centrifuging soil samples at 1,717 *g* for 10 min. Pore water, total nitrogen, and total phosphorus content were measured using standard procedures (Strickland and Parsons 1972). Additionally, four soil samples collected at each site in August 2006 were used to determine soil texture (Folk 1966) and bulk density (Tisdall 1951).

Forest Structure

To characterize forest structure, we counted trees within two 5-m^2 plots at each site. In high-density stands we counted

four 1-m^2 plots (a total of 14 plots). Species composition, tree density, tree height, and species frequency were recorded. Because mangroves at these sites are shrub-like and branch profusely below 1.3 m (breast height), we substituted diameter at breast height for basal diameter at 5 cm above the surface. For trees with basal branching, basal areas of every branch were added. Dead trees at the impaired site were not included in the calculations of forest structure.

Relative frequency (number of plots in which a species occurs, expressed as a percentage of all the examined plots) was calculated according to Curtis and McIntosh (1950) to determine importance value (I_v). The I_v is a relative value of the ecological contribution of a species and is calculated by summing the species' relative density (%), relative frequency, and relative dominance (proportion of basal area occupied by each species relative to the area occupied by all species). The result is a maximum value of 300 for all the species in a stand. The magnitude of the I_v is a reliable indicator of importance of a species within a stand (Curtis & McIntosh 1951).

Nitrogen Fixation (Acetylene Reduction Assay)

For sites A–E, nitrogen fixation was assessed every month for one year (September 2006 through August 2007), using the indirect method of acetylene reduction assay (Holguin et al. 1992). Soil slurries were collected at 5 cm below the surface with 5-mL sterile syringes with the top end of the syringe removed. This provided an effective vacuum barrel. Slurries were collected near the base of a total of four randomly selected black mangrove trees at each site. Duplicate slurries were placed in 60-mL sterile serum vials and hermetically sealed with serum stoppers enforced with aluminum rings. Slurries were taken from close distances to minimize variation from micro-environmental and soil heterogeneity. From each sampling location, 20 samples were collected (100 samples per month). For sampling at site F, 30 samples were collected once in May 2009, 15 from the preserved area, and 15 from the nearby clear-cut area. Slurries were kept on ice and transported to the laboratory within 4 hours of collection. Then, 10% of the atmosphere of each vial containing 10 mL soil slurry were substituted with gaseous acetylene and incubated at 28°C. Ethylene production in each vial was quantified, using a gas chromatograph (HP 5890 Series II, Palo Alto, CA, U.S.A.) equipped with a flame ionization detector and a capillary column (HP-PLOT/AL203 S, 50-m long, 0.032-mm internal diameter, with a 0.8-m-thick film for affinity separation of elements). The inlet temperature was 180°C with an inlet pressure of 10 psi. The injector and detector temperatures were 250°C. The column temperature was 100°C at the time of injection and increased at the rate of 15°C/min until it reached 200°C. The chromatograms were used to integrate the areas of the curves of C_2H_2 and ethylene (C_2H_4) to estimate C_2H_4 production.

Environmental Parameters and Nutrient Content in Interstitial Water

At the same sampling points where slurries were obtained, the pH of interstitial water was measured with a pH meter (Oakton 11, Oakton Instruments, Vernon Hills, IL, U.S.A.) and salinity, water temperature, and concentration of dissolved oxygen were measured (YSI 55, YSI, Yellow Springs, OH, U.S.A.). Interstitial water was collected with a customized water extractor consisting of acrylic tubing (0.5-cm internal diameter; 50-cm long) connected to a 60-mL plastic syringe adapted at its base with a three-way stopcock (McKee et al. 1988) to purge water into 50-mL centrifuge tubes with minimum oxygen alteration. This water collection technique extracts interstitial water at low and high tides with no contamination with surface water.

For determining nutrient content, samples of interstitial water were kept in 50-mL sealed centrifuge tubes that were previously washed with a 10% HCl solution in de-ionized water and rinsed thoroughly with de-ionized water. The samples were kept on ice until transported to the laboratory. There, all samples were filtered with GF/F micro-fiber filters (GF/F 47 mm, Whatman International, Maidstone, England) and stored at −80°C for up to two weeks before analyses.

Ammonium (N from NH_4^+) concentrations were analyzed by the phenol and sodium nitroprussiate technique (Solorzano 1969) using a microplate reader (Multiskan Ascent, Thermo Scientific, Waltham, MA, U.S.A.). Nitrite (NO_2^-) and nitrate (NO_3^-) were evaluated with an auto-analyzer (Quikchem 8000 equipped with an auto-sampler XYZ-500, Lachat Instruments, Loveland, CO, U.S.A.), according to the method described in Strickland and Parsons (1972). Soluble, reactive phosphorus analysis was run with the same auto-analyzer as nitrogenous compounds, using the ammonium molybdate technique (Strickland & Parsons 1972).

Statistical Analysis and Choice of Models for Analysis

(1) Differences in the content of OM, nitrogen fixation rate, pore water temperature, pH, salinity, and ammonium production over the course of a year were measured with a mixed-effects model. Fixed factors include study sites, and the covariate time: the sine of months coded evenly spaced between 2 and 8, to model the cyclical behavior of yearly data. In the random part of the model we specified the repeated measurements done at each site. Dependent variables were transformed as follows: Neperian logarithm of OM content and nitrogen fixation, Neperian base antilogarithm of pH, and square root of ammonium production. Pore water temperature, salinity, and dissolved oxygen were not transformed. All transformations were performed to allow an adequate distribution of residuals in the models. Further, we modeled variance (power function, varPower) and the temporal correlation structure (continuous autoregressive, CAR1) of the data (Pinheiro & Bates 2002).

(2) Regression-based tree models (De'Ath & Fabricius 2000) were used to explore the relationship between environmental parameters and nitrogen fixation, using the programming language R, a language and environment for statistical computing (R Development Core Team 2007). The ad hoc function randomly selected half of the observations, fitted a regression-based tree model, and kept track of

splitting variables and critical values. This process was iterated 500 times and the most likely tree was generated. This method overcomes the possibility that a single tree model is an artifact given by the data; by taking random samples of the database, we ensured that only legitimate signals were reported. General regression tree models split the database in a binary fashion, based on two criteria: (1) the difference in the mean values of the response variable and (2) the deviance within each group (Crawley 2002).

Results

Soil and Pore Water Properties

Sand content ranged between 23 and 80%. Analysis of variance (ANOVA) combined with Tukey's test showed nonsignificant differences of sand content between sites B, D, and E. The sand content of site A (preserved) was significantly higher than that of the other sites. Sand content was similar for site C (reforested) and site E, but site C differed from the rest of the sites (Table 1). Silt content was significantly highest at site E but no differences were found between sites B, C, and D. Clay content showed significant differences among all sampling sites. Significant differences between sites for the N:P ratio are observed only for site E. For OM content, no measurable variation throughout the year was detected by the mixed-effect model but sites differed significantly. Because we detected no measurable variation throughout the year in OM content, we presented only the differences among sites. Site E had significantly lower OM than the other sites ($t_{[15]} > 2.77$, $p < 0.014$, $df = 15$; Table 1). Only sites B and C were similar, whereas all other sites differed statistically from each other ($t_{[15]} > 2.88$, $p < 0.011$, $df = 15$; Table 1).

Structure of the Mangroves

Site C was planted with *Avicennia germinans* seedlings; natural recruitment of *Laguncularia racemosa* and *Rhizophora mangle* occurred; site C now resembles a natural, mixed mangrove, except that the reforested sites had significantly lower tree density ($F_{[4,\ 9]} = 10.78$, $p < 0.01$) compared with preserved sites A and B and the impaired site E (Table 2).

Table 1. Characterization of soil conditions at the study sites: preserved sites A and B, reforested site C, reforested by natural regeneration site D, and impaired forest site E.

	Soil				*Porewater*
Site	*OM (%)*	*Sand (%)*	*Silt (%)*	*Clay (%)*	*N:P*
Preserved (A)	18 ± 0.5a	80 ± 5a	14 ± 4a	6 ± 3a	14 ± 2a
Preserved (B)	13 ± 0.6b	23 ± 8b	30 ± 6b	48 ± 4b	13 ± 2a
Reforested (C)	19 ± 0.5b	51 ± 8c	30 ± 6b	18 ± 4c	15 ± 0.8a
Naturally regenerated (D)	10 ± 1.3c	24 ± 8b	23 ± 6ab	53 ± 4d	15 ± 0.8a
Impaired (E)	3 ± 0.2d	40 ± 8cb	48 ± 6c	13 ± 4e	45 ± 7b

Values of soil and pore water properties are means ± standard error ($n = 4$). N:P denotes ratio of moles of nitrogen to moles of phosphorus. Values in each column denoted with a different letter differ significantly at $p < 0.05$. OM, organic matter.

Table 2. Summary of structural characteristics of mangrove species at five sites.

Species/Site	*Stand Density (trees/m^2)*	*Basal Diameter (cm)*	*Basal Area (cm^2/m^2)*	*Frequency (%)*	*Height (m)*	I_v
Avicennia germinans						
A	7.2 ± 2.13a	2	61 ± 28a	67	1.2 ± 0.08b	246
B	3 ± 0.33b	5	40 ± 13a	67	1.3 ± 0.90b	229
C	0.68 ± 0.12b	7	14 ± 9b	33	1.8 ± 0.35a	134
D	1.5 ± 0.28b	5	29 ± 10a	100	1.2 ± 0.20b	300
E	3.1 ± 1.10b	5	31 ± 11a	80	0.9 ± 0.10b	254
Laguncularia racemosa						
A	0.5 ± 0.20a	2	4 ± 0.46b	17	0.9 ± 0.20b	29
B	1.5 ± 0.50a	9	8 ± 1.20b	33	1.3 ± 0.02a	71
C	0.56 ± 0.40a	4	11 ± 7a	33	1.5 ± 0.60b	114
D	n.p.					
E	n.p.					
Rhizophora mangle						
A	0.2 ± 0.05a	3	3 ± 1.30a	17	1.1 ± 0.02a	25
B	n.p.					
C	0.14 ± 0.1a	3	2 ± 1a	33	1.3 ± 0.05a	52
D	n.p.					
E	0.2 ± 0.06a	3	3 ± 1a	17	1 ± 0.03a	35

Sites A and B (preserved), site C (reforested), site D (naturally regenerated), and site E (impaired). For each site, two 5-m^2 plots were studied. Because of high plant density at sites A and B, four 1-m^2 plots were studied (14 plots in total were sampled). Values represent means + standard error. Different letters for each plant species and for each parameter indicate significant difference at $p < 0.05$, using two-way ANOVA. n.p., not present.

For all species, site A had a density of 7.9 trees/m^2 and site B had 4.5 trees/m^2. The lowest density (1.38 trees/m^2 and total basal area 27 cm^2/m^2) occurred at site C. Species composition was similar at sites A, B, C, and E, and resembled the mangroves containing *A. germinans*, *L. racemosa*, and *R. mangle* that are common in this region (Holguin et al. 2006). Site D contained only *A. germinans* (Table 2). I_V analysis showed that *A. germinans* is the dominant species at all sites, having high basal area, frequency, and density. Although density of *A. germinans* at site C is lower than at the other sites, this species dominates the site, resulting in a similar common forest structure. Despite lower tree density for all species at site C, density of *L. racemosa* was similar for sites A and C, whereas densities for *R. mangle* were similar for sites A, C, and E. Basal diameter of *A. germinans* at the naturally regenerated site D is not different from the basal diameter of *A. germinans* at the natural sites B and E. Basal diameter of *R. mangle* at site C is equal to basal diameter at sites A and E (Table 2).

Nitrogen Fixation and pH Change in Preserved, Reforested, Natuarally Regenerated, and Impaired Mangroves

Nitrogen fixation rates varied greatly at each site during the year, but followed the same seasonal pattern at all sites (Fig. 1a). The model showed high nitrogen fixation in autumn (September–November) and a low nitrogen fixation in spring (March–May) for all sites. Consistently, the impaired site E showed significantly lower rates of nitrogen fixation ($t_{[15]} > 2.5$, $p < 0.012$, $df = 15$) throughout the year, compared to the other sites. The specific models of sites A–D did not differ significantly from each other throughout the year ($t_{[15]} < 1.21$, $p > 0.123$, $df = 15$; Fig. 1a).

Over the year, pH of all sites (A–E) varied from 3.4 to 8.1. At the impaired site E, the pH was significantly higher in October (>7.5) and the overall shape of the mixed-effects model of site E differed significantly from all other sites ($t_{[126]} > 3.2$, $p < 0.01$, $df = 126$). Interestingly, nitrogen fixation and pH displayed inverse temporal trends throughout the year with offset peaks (Fig. 1a,b).

Environmental Monitoring in Preserved, Reforested, Naturally Regenerated, and Impaired Mangroves

Environmental parameters, evaluated by the mixed-effect model, varied greatly during the year (Fig. 2a–d). The highest temperature of pore water occurred in September (average of 30.5°C) and the lowest in the following January (17.9°C). The peaks and patterns were similar at all sites, with no statistical differences among them (Fig. 2a).

Dissolved oxygen was highest in October and February and lowest in June and January. Dissolved oxygen in February at site E (average 4.89 mg/L) was significantly higher than at all other sites ($p < 0.01$), whereas the lowest content of dissolved oxygen occurred at site E in January (0.15 mg/L) (Fig. 2b).

Pore water salinity was significantly higher at site E than at the other sites throughout the year ($t_{[15]} > 2.67$, $p < 0.017$, $df = 15$; Fig. 2c). The highest salinity (77‰) occurred between June and August at site E (dry and very hot period); the lowest salinity (31.5‰) occurred at site A in May (Fig. 2c).

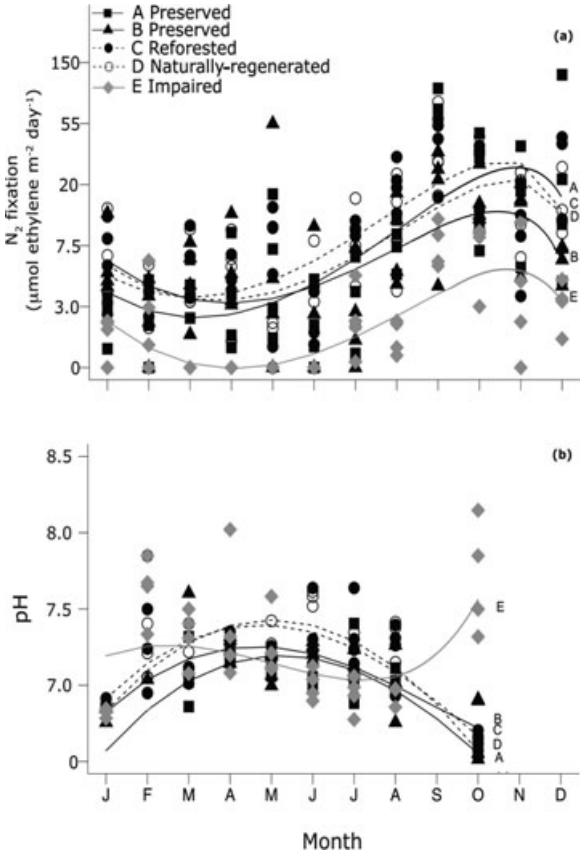

Figure 1. Mixed-effects model of: (a) monthly nitrogen-fixation variation measured in the course of one year at the five sites; (b) monthly pH variation during the year at the five sites. Each curve represents the predicted model of nitrogen fixation for each site (A–E). Points represent actual averaged data over both sites and months. For clarity, *y*-axes are represented in Neperian logarithm scale.

Nitrate and nitrite contents were not detectable by the methods used (<0.1 µmol). Ammonium was highly variable during the year and did not differ between sites A, B, C, and D (Fig. 2d); impaired site E was significantly higher than the other sites ($p < 0.001$).

With regression tree analysis, we estimated which environmental parameters split the data set at a root, until no further branches can be made and terminal nodes are shown for mean nitrogen fixation. We separately analyzed the impaired site from the reforested and preserved sites because of the clear differences shown by the statistical analysis of nitrogen fixation and environmental parameters at site E (Figs. 1a, 2a–d, and Table 1). Results of regression tree analysis for the preserved and reforested sites suggest that pH is the major control over nitrogen fixation (Fig. 3a). At pH levels less than 6.49, nitrogen fixation was the highest (22.56 µmol ethylene m^{-2} day^{-1}). At pH greater than 6.49, temperatures greater than 28.6°C promoted nitrogen fixation (9.18 µmol ethylene m^{-2} day^{-1}). At impaired site E, salinity appeared to control

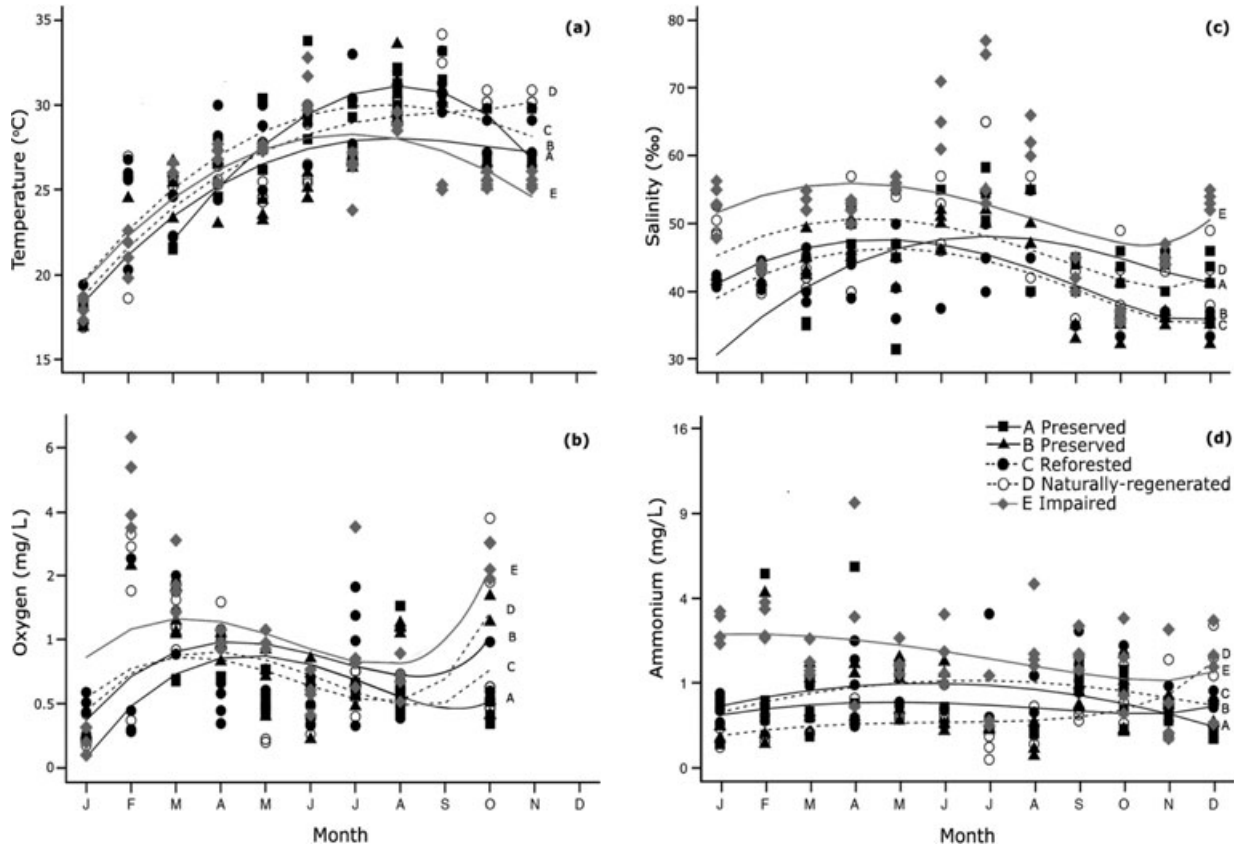

Figure 2. Mixed-effects model of environmental parameters during one year: (a) pore water temperature, (b) dissolved oxygen, (c) salinity, and (d) ammonium concentrations; *y*-axis is represented in square root scale. Each curve represents the predicted model of one environmental parameter for each site (A–E). Points represent average data of both sites and months.

activity of diazotrophic bacteria. Lower salinity (<47.6‰) was related to greater nitrogenase activity (5.61 μmol ethylene m^{-2} day^{-1}). At higher salinities, nitrogen fixation was promoted by lower pH (Fig. 3b).

Discussion

Nitrogen fixation has been the most studied microbial function in mangroves (Sengupta & Chaudhuri 1991; Woitchik et al. 1997; Lee & Joye 2006). Although nitrogen fixation in mangrove sediments is likely to be limited by insufficient energy sources (Howarth et al. 1988), atmospheric nitrogen, fixed by cyanobacteria, was assimilated by the trees (Bashan et al. 1998). For example, mangrove seedlings inoculated with the diazotrophic cyanobacterium *Microcoleus* sp. grew faster than seedlings that were not inoculated (Toledo et al. 1995*b*) and several species of mangrove seedlings inoculated with strains of *Azotobacter* isolated from mangrove soils showed a promoted growth and increased chlorophyll and carotenoid content (Ravikumar et al. 2004). We found that nitrogen fixation was greater during the summer in all the mangroves, similar to trends for culturable heterotrophic bacteria in preserved mangroves (Gonzalez-Acosta et al. 2006). Toledo et al. (1995*a*) found similar nitrogen fixation trends in preserved mangroves and a clear association between nitrogen fixation and pore water salinity.

In this arid region, approximately 90% of rainfall and most freshwater input from surface and subsurface flow occurs in summer (July–September), which lowers the pH. This promotes activity of the heterotrophic bacterial community in the lagoon (Gonzalez-Acosta et al. 2006). During Hurricane "John" in September 2006, rainfall was 122 mm, whereas the average precipitation for September was 55 mm (1941–2005; Comision Nacional del Agua 2009). Immediately after this tropical storm, we found atypically higher rates of nitrogen fixation (6.5–18.7% increase) at all sites, rates much higher than those found in October, only one month later and with a slightly lower temperature.

Our most significant findings were that: (1) there was a significant reduction of nitrogen fixation in the impaired mangrove and in recently clear-cut mangroves (site F), compared to the reforested and preserved mangroves. (2) Reforested, naturally regenerated, and preserved mangroves did not differ significantly in nitrogen fixation activity.

(1) Low nitrogen fixation in the impaired mangroves are most likely caused by greater salt concentrations (>47.6‰) and relatively high pH, which are major suppressors of

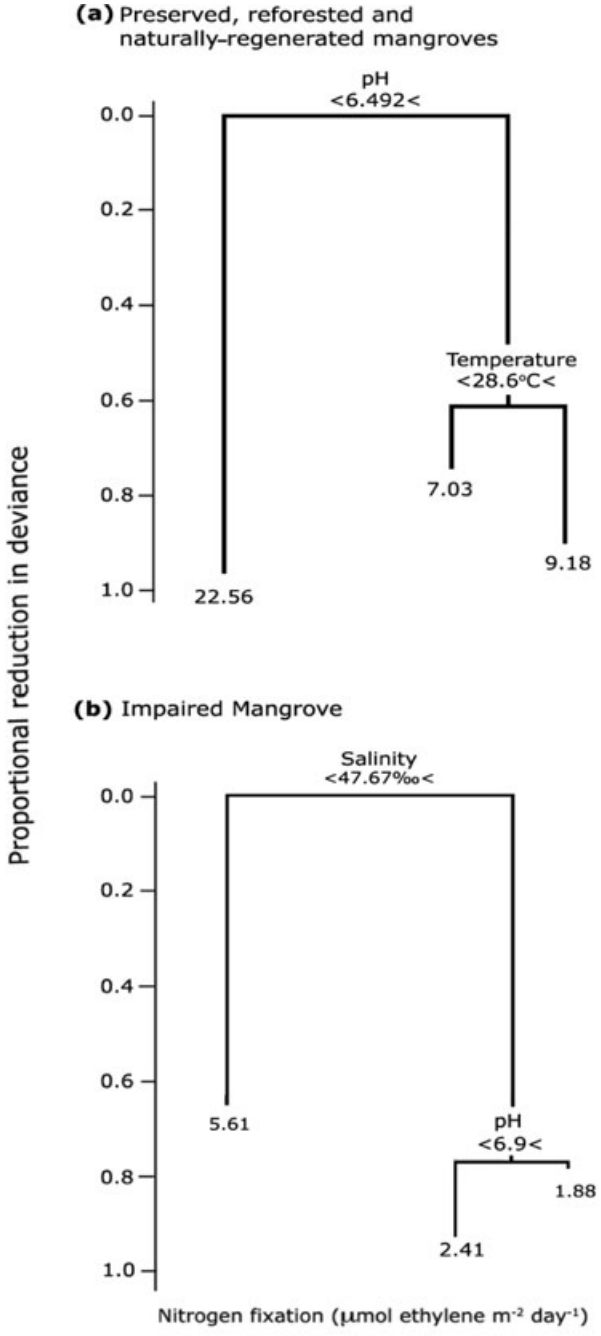

Figure 3. Tree regression analysis of the main environmental factors affecting nitrogen fixation in preserved and reforested mangroves (a) and in the impaired mangrove (b). Greater-than and less-than signs (>,<) denote lower and higher values of the value indicated between the symbols.

nitrogenase activity, as is the case for *Rhizobium* (Zahran 1999). It is known that several environmental parameters, such as ammonium, salinity, oxygen, and sulfides, inhibit nitrogen fixation in wetlands (Howarth & Marino 1988). Where organically rich sediments are present, molybdenum and iron compounds promote nitrogen fixation (Paerl et al. 1987). Hence, low nitrogen fixation in impaired mangroves can be further explained in three ways that are not mutually exclusive.

(a) Nitrogenase activity is suppressed because OM is too limited to compensate for salinity stress on the diazotrophic community (Howarth et al. 1988). A probable loss of OM at the impaired mangrove, after a road was constructed, must have occurred with changes in the hydrology of the lagoon, including higher evaporation and accumulation of salt (Strangmann et al. 2008). In turn, net primary productivity of the forest declined, leading to decreasing deposition of OM and alteration of the nitrogen flux (Fulweiler et al. 2007).

(b) The highest rates of nitrogen fixation occur at pH values less than 6.5 (Polman & Larkin 1988). Our regression tree analysis indicated that pH is the main controller of nitrogen fixation and, in turn, pH is affected by water temperature, dissolved oxygen, and organic acids derived from detritus (Wetzel 1983). Low OM at the impaired site would mean that there is less organic acid to regulate the soil redox potential. Also, higher salt concentrations can lead to higher pH, as was the case at site E. Even though oxygen is a common inhibitor of nitrogenase activity (Howarth & Marino 1988), our data did not reveal oxygen as a regulator of nitrogen fixation at this site, as determined by the tree analysis. Yet, oxygen is a variable that influences pH (Wetzel 1983). High pH values at site E can be influenced not only by salinity, humic acids, and temperature, but also by high concentrations of oxygen in the lagoon.

(c) Nitrogen fixation is inversely proportional to the N:P ratio (Howarth et al. 1988), a condition that occurred at our sites. The N:P ratio of impaired site E was approximately 45:1, whereas those of the preserved, reforested, and naturally regenerated sites were 14:1 to 15:1, close to the optimum for nitrogen fixation (16:1), as suggested by Howarth and Marino (1988). Some authors have stated that microbial communities play a major role in nutrient recycling in mangroves and that disturbance of mangroves affects the functioning of their soil microbial community, including diazotrophs (Alongi et al. 1993; Holguin et al. 2001). We did not expect the N:P ratio to be so high in the impaired mangrove because the OM in the sediments was low and the water channel was blocked by the road. Very likely, this mangrove received high loadings of nitrogen from the drainage water from two shrimp farms located several hundred meters from the lagoon. High tides can transport nitrogen and phosphorus wastewater from the shrimp ponds, which negatively affected the already altered hydrology of the lagoon. With high salinity, the pH, N:P ratio, and low OM, nitrogen fixation is minimal. These results further support our goal to measure and use nitrogen fixation as a tool to assess and monitor mangrove restoration projects.

(2) Rates of nitrogen fixation at the preserved, reforested, and naturally regenerated sites ranged from 39 to 47 mg N m^{-2} $year^{-1}$, about seven to eight times higher than at the impaired site. These values are far lower than values (>2 g N m^{-2} $year^{-1}$) reported for tropical, tidally dominated arid-zone mangroves in Australia (Howarth et al. 1988; Boto & Robertson 1990). However, approximately 40 mg N m^{-2} $year^{-1}$ could represent an important input of nitrogen for mangroves in arid climatic zones, because this mangrove receives very little fresh water from runoff, less than 180 mm/year on average and close to none in some years.

This study demonstrated that for functional recovery of an arid region mangrove, a time period of 12 years after reforestation or the start of natural regeneration was sufficient to establish the community of functioning diazotrophic bacteria at the same levels as the nearby preserved sites, as was hypothesized by Holguin et al. (2001). Holguin et al. (2001) proposed that restoration of mangrove ecosystems should involve the rehabilitation of the microbial community of the sediments. Only an initial characterization of the nitrogen-cycle bacterial communities of mangrove roots at Laguna de Balandra with molecular methods has been done so far (Flores-Mireles et al. 2007). The rehabilitation of this mangrove, as assessed by our data on sediment characteristics, indicated that there was sufficient nitrogen input in reforested or naturally regenerated mangroves to sustain the mangrove ecosystem in an arid region where nitrogen is normally very limited (Holguin et al. 2001, 2006).

In summary, our results showed that measuring nitrogen fixation can be proposed as a practical tool to evaluate the success of mangrove restoration.

Implications for Practice

- Functional recovery of restored arid region mangroves can be assessed by the amount of nitrogen fixation occurring in sediments in the restored mangroves.
- For the restoration of ecosystem functions related to nitrogen requirements of mangrove trees in arid areas, 12 years after start of reforestation or natural regeneration are sufficient.
- Measurements of nitrogen fixation can be used to determine the degree of impairment of an arid region mangrove.

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