

Responses of *Avicennia germinans* (Black Mangrove) and the Soil Microbial Community to Nitrogen Addition in a Hypersaline Wetland

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Abstract The responses of dwarf black mangroves (*Avicennia germinans*) and components of the microbial community to the addition of nitrate over a 2-year period were examined. The field study was conducted in the Indian River Lagoon (Florida) in a mangrove-dominated impoundment that was established for purposes of mosquito control. The responses of mangroves to the regular addition of nitrate were insignificant or relatively minor compared to responses of the components of the microbial community. Denitrification rates, measured in the field and laboratory, increased significantly in fertilized plots and nitrous oxide emission rates were almost six times higher in fertilized plots. Nitrogen fixation was significantly lower in fertilized plots. Results suggest that mangrove systems in the N-limited Indian River Lagoon are likely to be long-term sinks for any increases in N loading.

Keywords *Avicennia germinans* · Mangrove · Florida · Denitrification · Nitrogen fixation · Microbial community

Introduction

Salinity and nutrient availability have a major influence on the structure and function of mangrove ecosystems. Nitrogen (N) and phosphorus (P) availability limit mangrove productivity (Boto and Wellington 1983; Chen and Twilley 1999; Feller et al. 1999; Feller et al. 2003) and growth form (Feller 1995). Salinity also affects production and growth form as well as mangrove mortality. Dwarf (scrub) growth forms are typically associated with highly saline habitats (Castaneda-Moya et al. 2006; Nandoo 2006; Sobrado and Ewe 2006). Increasing salinity causes height and growth to decrease, especially of *Avicennia* species (Ye et al. 2005; Lara and Cohen 2006; López-Hoffman et al. 2007), and mortality to increase (Suárez and Medina 2005).

Nutrient limitations have been shown to vary in space even where no strong salinity gradient exists (Feller et al. 2002; Lovelock et al. 2006a). In Belize, the growth of tall (4–7 m) red mangroves (*Rhizophora mangle* L.—Rhizophoraceae) was N-limited near the shoreline where interstitial salinity averaged 35–37 (Lovelock et al. 2006a). At the interior end of the study transects, salinity was only two to three units higher but the red mangroves averaged ≤1.5 m tall and were strongly limited by P availability. Adding P to dwarf trees in the interior zone results in a significant increase in growth and the trees became much taller and larger (Feller 1995).

In a related study in the Indian River Lagoon in Florida, shoot growth of red and black (*Avicennia germinans* (L.) Stearn—Avicenniaceae) mangroves at all locations along a tree-height gradient was limited by N availability and the greatest response to N addition occurred in dwarf black mangroves that occurred toward the interior of the wetland (Feller et al. 2003; Lovelock et al. 2006b). In contrast to the lack of a clear salinity pattern along the transects in Belize

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(see above), there was a distinct salinity gradient at the Florida study site with salinity ranging from 39–40 in the tall red mangrove zone to 49–57 in the area dominated by dwarf mangroves (Feller et al. 2003).

The mechanisms by which nutrients and salinity interact to influence mangrove growth have not been well studied, but studies tend to focus on plant physiological processes. For example, Lovelock et al. (2006b) found that the mechanisms by which mangrove species respond to increasing salinity vary according to the prevailing limiting nutrient. In P-limited habitats, they concluded that internal processes that control the movement of water throughout the plants (e.g., hydraulic conductivity) have an important control over productivity. In contrast, in N-limited habitats where salinity varied across the study sites, patterns of allocation of leaf area and adjustments to photosynthetic rates were more important than hydraulic conductivity.

Microbial responses to nutrient and salinity gradients have the potential to indirectly affect mangrove productivity through their influence on nutrient availability. In the context of N availability, the most fundamental microbial processes that regulate the long-term N balance of ecosystems are N fixation, which adds available N to the ecosystem, and denitrification, which removes N. Nitrogen fixation can be high in N-limited mangrove systems (Lee and Joye 2006) and a significant source of available N in dwarf mangrove habitats (Hicks and Silvester 1985; Joye and Lee 2004). The presence of N-fixing bacteria and cyanobacteria, for example, improved the nitrogen economy of *Avicennia* seedlings (Toldeo et al. 1995; Bashan et al. 1998). Nitrification and denitrification are relatively low in mangrove sediments (Zuberer and Silver 1979; Rivera-Monroy et al. 1995; Pelegrá et al. 1997; Kristensen et al. 1998; Bauza et al. 2002) and microbial mats (Lee and Joye 2006), but denitrification increases significantly when N is provided as nitrate to the microbial community (Corredor et al. 1999; Muñoz-Hincapié et al. 2002; Chiu et al. 2004; Allen et al. 2007). High rates of microbially mediated nutrient sequestration (Alongi et al. 2005; Rivera-Monroy and Twilley 1996) and mineralization (Alongi et al. 2005) occur when mangroves receive nutrients from human activities.

In this study, we measured the responses of individual dwarf black mangroves and the microbial communities responsible for denitrification and nitrogen fixation, two of the key microbial processes related to the N cycle. Nitrogen was applied to plots that each contained a single dwarf black mangrove over a 2-year period in a high salinity habitat in an N-limited ecosystem. Based on previous studies of N addition to mangrove systems, we anticipated that both components of the system (i.e., the microbial community and the dwarf black mangroves) would respond to the addition of N. However, we predicted that the N-fixation and denitrifier communities would respond quickly

to the addition of N, intercepting it and limiting the response of fertilization on the dwarf mangroves.

Study Site

The study was conducted in a mangrove ecosystem in a mosquito control impoundment (SLC-24), which is located at N27°33', W80°33' on the Atlantic Ocean side of the Indian River Lagoon (IRL) on North Hutchinson Island in St. Lucie County between Ft. Pierce and Vero Beach, Florida. The Ft. Pierce area has a subtropical climate with average annual, maximum, and minimum temperatures of approximately 23, 28, and 18°C. Precipitation has a strong seasonal component. The annual average (approximately 1,340 mm) is distributed over an extended relatively dry period (November–May long-term mean is 523 mm) and a shorter rainy season (June–October long-term mean 814 mm). December is the driest month (mean=51 mm) and September the wettest (mean=202 mm).

SLC-24 was developed in 1970 by building a dike that isolated an existing mangrove-dominated wetland from the IRL (Rey et al. 1990). SLC-24 was isolated from the IRL between 1970 and 1985. A culvert was installed in the dike in 1985 to remove excess water that had accumulated during two tropical storms. The culvert was closed and SLC-24 remained isolated until 1987 when the original culvert was reopened and four additional culverts were added. Vegetation cover in SLC-24 decreased from 75% to near 30% between 1970 and 1985 (Rey et al. 1990) but began to recover following the installation of the culverts that allowed a tidal connection between SLC-24 and the IRL. Tidal exchange is, however, limited because of the small number and size of the culverts. Tidal fluctuations are almost always less than 50 cm. The sandy soils have a low organic matter content (typically less than 5%; Feller et al. 2003).

Black mangrove is the dominant species in SLC-24 but red and white (*Laguncularia racemosa* L.—Combretaceae) mangrove and buttonbush (*Conocarpus erecta* L.—Combretaceae) are also present and, in a few areas, the dominant species (D. Whigham, personal observation). The dominant understory plant throughout SLC-24 is *Batis maritima* L. and other common understory species are *Salicornia virginica* L. (Chenopodiaceae), *Salicornia bigelovii* Torr. (Chenopodiaceae), and *Borrhchia frutescens* (L.) DC. (Asteraceae).

Earlier research in impoundment SLC-23, immediately adjacent to SLC-24, had clearly shown that nitrogen limited the growth of dwarf, intermediate, and tall black and red mangroves at the creek margin, the transition zone between the creek and the wetland interior and the wetland interior (Feller et al. 2003; Lovelock and Feller 2003; Lovelock et al. 2006b).

Table 1 Characteristics of Fertilized and Control plots at the beginning of the two year experiment

Variable	Control plots	Fertilized plots
Leaf N (%)	1.57±0.06	1.59±0.03
Soil temp (C)	22.7±0.05	22.8±0.09
pH	7.57±0.10	7.51±0.05
Salinity (ppt)	58.3±1.56	56.4±1.86
Eh (mV)	93.8±10.6	131.4±13.3
Sulfide (mM)	nd ^a	nd
Nitrate (mg/l)	2.74±1.13	5.48±1.03
Ammonium (mg/l)	1.03±0.29	1.21±0.48
Number <i>Batis maritima</i>	29.7±6.7	29.9±8.3
Cover of <i>Salicornia</i> spp. (%)	19.2±6.6	16.4±3.3

Plots were sampled the first day that 5 l of estuarine water from the Indian River (with or without fertilizer added) was added (February 7, 2001)

nd none detected

^a During the same period of time that soil samples were collected from the 12 plots, we collected groundwater from three shallow wells near the plots. Sulfide concentrations were nd, 0.05, and 0.85 mM

Experimental Design

The fertilization study was conducted in an area dominated by dwarf black mangroves. Table 1 provides initial conditions in the twelve 1 × 1 m study plots that were each centered on one dwarf black mangrove. Treatments were randomly assigned to the plots (*N*=six fertilized and six controls) and the experiment was started in February, 2001. Plots were watered on a monthly basis, except during a month or two each year when there was standing water in the impoundments, with 5 l of IRL water (controls) or 5 l of IRL water in which 6 g of NaNO₃ was dissolved. Total N addition to each fertilized plots was 10 gN/m²/year. Treatments ended in February 2003.

Methods

Mangrove Measurements

Prior to the start of the experiment, three shoots on each black mangrove (hereafter referred to as *Avicennia*) were tagged for annual measurements of leaf production (number of new leaves produced/year), branch production (number of new branches produced/year) and shoot growth (increase in shoot length in cm/year) using procedures described in Feller et al. (2003). Shoots were re-measured in February 2002 and 2003. In February of 2001 and 2003, we collected ten senesced leaves from each plant for N determination (no senesced leaves were present during the February 2002 sampling period). The composite leaf samples were dried at 60°C and

analyzed for N with a Perkin-Elmer 2400 CHN Analyzer after they were ground in a Wiley Mill (0.38 mm mesh).

At the beginning of the experiment, we also surveyed other vascular plants in the plots. The number of individually rooted *B. maritima* shoots in each plot was counted and the percent cover for species of *Salicornia* was visually estimated.

Soil Measurements (Laboratory)

During each February sampling period, two soil cores (3.9 cm diameter and 10 cm long) were removed from each plot using an aluminum sampling tube that was sharpened at one end. The cores were immediately sealed at both ends with rubber stoppers and placed on ice and returned to the laboratory for removal of interstitial water that was analyzed for salinity, NO₃-N, NH₄-N, pH, and sulfide (details below). Following the same sampling schedule, two additional cores were removed from each plot and the cores used for measurements of N-denitrification (details below). Each year, field-based denitrification measurements using cores were made several times (typically three to five) over a 2-week sampling period. Soil cores removed from the plots were replaced by cores collected within 1 m of each plot.

Soil samples were collected in February 2002, dried (105°C, 48 h), ground and analyzed for Total N, Total P, and Total K after digestion with a modified Kjeldahl approach (Bremner and Mulvaney 1982).

In the laboratory, interstitial water was collected from cores by extruding the soil from the sampling tubes into plastic sandwich bags that were flushed with N and sealed. A ceramic soil sipper (Rhizon soil moisture samplers: Model 12.01.SA, Eijkelkamp, Giesbeek, The Netherlands) was inserted into each core through the plastic bag. Sippers were connected to 50 ml syringes and suction was placed onto each syringe by pulling the plunger and fixing it into position with a stopper. Interstitial water collected from one core from each plot was analyzed for salinity (AO Spencer hand-held refractometer) and pH (Oakton pH 510 Series pH/mV/°C meter). We measured sulfide in 2001 following procedures described in McKee et al. (1988) but sulfide was not detected in any of the samples so we did not measure sulfide in 2002 or 2003. For comparative purposes we also sampled deeper interstitial water (@ 50 cm) in 2001 and measured sulfide levels of 0.05 and 0.85 mM.

Interstitial water from the second core from each site was filtered through a 45-µm filter, frozen, returned to SERC, and analyzed for NO₃-N and NH₄-N using standard APHI methods 303-A173 and 303-S021, respectively (APHA 1995). Samples were analyzed with an Astoria-Pacific International Model 300 automated colorimetric analysis system.

Denitrification was measured on cores using the acetylene block method as described in Hefting et al. (2004). Briefly, the procedure involved incubating soil cores in gas-

tight jars that were flushed with nitrogen gas prior to adding acetone-free acetylene. Jars were incubated at 20°C and gas samples from the head space collected after 20 h. The gas samples were analyzed at Utrecht University in a GC Varian 3300 gas chromatograph equipped with an electron capture detector and Poropak Q columns. In February 2003, we also measured denitrification using cores collected from a separate set of 1 × 1 m plots. Six plots served as controls and received 5 l of IRL water. The other six received 5 l of IRL water in which 1 g N m⁻² had been dissolved. The purpose of this effort was to determine if there were any temporal effects of N addition by comparing plots that had been fertilized for 2 years with plots that received a one-time fertilization.

Soil Measurements (Field)

In 2001, we measured Eh and soil temperatures in each plot at a depth of 10 cm. Eh was measured with calibrated platinum electrodes as described by Faulkner et al. (1989) and soil temperatures were recorded with a Eijkelkemp soil thermometer. Eh measurements in the field were adjusted (McKee et al. 1988) by adding +244 mV (the potential of a calomel reference electrode) to each field measurement.

In all 3 years, nitrous oxide emission rates from each plot were measured using closed flux chambers with an inner diameter of 24.5 cm and a height of 20 cm. Each chamber had a hole in the top into which a two-hole rubber stopper was placed. Tubing was used to connect the chamber, through the stopper, to an INNOVA Model 1312 photoacoustic multi-gas monitor (TGA, INNOVA, Denmark). Prior to measurements, the flux chambers (one per plot) were inserted into the soil by placing the chamber in the plot and cutting a shallow trench around the chamber with a sharp knife. The chamber was then pushed into the shallow trench to exclude exchange with the atmosphere. The height of the headspace above the soil was measured to determine the headspace volume of each flux chamber. Annually, measurements were made over a period of approximately 2 weeks. The chambers were rotated back and forth between plots (six plots were measured one day and the second set of six plots was made the following day) so that multiple measurements were made on each plot during a 2-week period. The number of measurements per plot over a period of approximately 2 weeks was eight in 2001 and three in 2003. In 2002, we were only able to make one set of measurements on each plot because the impoundment was flooded for most of the 2-week period and measurements could only be made when the water level was at or below the soil surface.

Once the chambers were connected to the photoacoustic multi-gas monitor, they were covered with aluminum foil and nitrous oxide flux was measured. The duration of the

measurement period for each chamber was determined by how long it took for the N₂O concentration to stabilize. Nitrous oxide emissions were calculated from the increase in N₂O concentration over time from linear regression analysis. Ambus and Robertson (1998) demonstrated that TGA-based measurements of soil N₂O flux were statistically identical to conventional ECD gas chromatography method.

Nitrogen fixation was measured in each plot in February and July 2002 and February 2003 by exposing duplicate, 10-g soil samples from each plot to 10% acetylene and measuring ethylene production (Hardy et al. 1968). Each soil core was taken from 0–5-cm depth and immediately placed intact into a 125-ml canning jar fitted with a rubber septum. The jar was injected with acetylene and placed into the hole left by the soil core to incubate at in situ temperatures. The headspace was sampled three times over the next 24 h and stored in pre-evacuated Hungate tubes. Headspace gas samples were returned to SERC and analyzed for ethylene with flame ionization detection on a Shimadzu GC-14A gas chromatograph (Shimadzu Instruments, Inc) and a Poropak N (mesh 80-100) column. Fluxes were calculated from the slope of a least-squares regression of ethylene concentration versus time in each jar and expressed as a function of soil mass determined by drying at 110°C. Fluxes from replicate jars in a plot were averaged so that the true replication was $n=6$. In order to facilitate comparisons with the literature, ethylene flux was converted to N flux assuming a theoretical ratio of 3 mol ethylene produced per 1 mol of N₂ fixed, and expressed on an area basis using soil bulk density data from the study site (1.21 g cm⁻³) to a depth of 5 cm.

Statistical Analyses

Analysis of variance (ANOVA) was used to compare time (year) and treatment (fertilized versus control) as main effects and interactions between time and treatment (SAS Institute 2002-2004). Variables used in the ANOVA were: rate of denitrification (separately for core and chamber studies), rate of nitrogen fixation, growth of tagged *Avicennia* shoots, number of leaves produced per year on tagged *Avicennia* shoots, number of branches produced per year on tagged *Avicennia* shoots, and the nitrogen content of senesced *Avicennia* leaves. Analyses were performed on transformed data. Means were compared using Tukey's test and the LM Means procedure was used to compare means when there was a significant interaction term in the ANOVA. ANOVA was also used to compare denitrification data for plots that had been fertilized in 2001, 2002, and 2003 with plots that were only fertilized in 2003. One-way ANOVA was used to compare Total N, Total P, Total K and N:P of soils collected from control and treatment plots in February 2002.

Results

About 1 h after the first addition of IRL water to the plots, the fertilized and control plots were similar in all measured characteristics except nitrate concentration in interstitial water (Table 1). Other than nitrate, none of the physiochemical soil variables differed substantially between control and fertilized plots in any year.

In addition to the single dwarf black mangrove in each plot, the only other vascular plants in the plots at the beginning of the study were *B. maritima* and two species of *Salicornia* (*S. virginica* and *S. bigelowii*). Densities of *Batis* and percent cover of *Salicornia* (almost all plants were the perennial *S. virginica*) did not differ noticeably at the start of the experiment. We also made an initial sampling of senescing leaves of the black mangroves and there were no differences among the controls and treatments in the concentrations of N (Table 1).

Eh was only measured in 2001 and there was no statistical difference between control (93.8 ± 10.6 mV) and fertilized plots (131.4 ± 13.3 mV). Eh measurements indicated that the surface 10 cm of the substrate was not reduced.

Salinity varied markedly between years but not between treatments and there was no treatment \times year interaction (Table 2). Salinity in February 2003 (80.9 ± 1.7) was almost double the mean salinity in 2002 (46.0 ± 1.1), which was substantially less than the mean salinity in 2001 (58.8 ± 0.8).

Table 2 Results of ANOVA for soil chemical properties

	<i>df</i>	<i>F</i>	<i>P</i>
Interstitial water salinity	5	82.43	0.0001
Year	2	204.23	0.0001
Treatment	1	3.27	0.0736
Year \times treatment	2	0.21	0.8108
Interstitial water NO ₃	5	2.15	0.0648
Year	2	1.22	0.2984
Treatment	1	6.82	0.0103
Year \times treatment	2	0.85	0.4306
Interstitial water NH ₄	5	3.60	0.0048
Year	2	5.19	0.0071
Treatment	1	2.72	0.1020
Year \times treatment	2	2.89	0.0597
Interstitial water pH	5	0.82	0.5376
Year	2	1.01	0.3675
Treatment	1	0.82	0.3675
Year \times treatment	2	0.10	0.8948
Total soil N	1	0.000	0.954
Total soil P	1	0.02	0.8902
Total soil K	1	0.08	0.7811
Soil N:P	1	0.00	0.9696

Table 3 Results of ANOVA for core and chamber flux measurements of N₂O and for black mangrove parameters measures in control and treatment plots

	<i>df</i>	<i>F</i>	<i>P</i>
N ₂ O—cores	5	44.53	0.0001
Year	2	3.81	0.0336
Treatment	1	214.60	0.0001
Year \times treatment	2	0.21	0.8090
N ₂ O—chambers	5	12.00	0.0001
Year	2	9.32	0.0007
Treatment	1	36.53	0.0001
Year \times treatment	2	2.43	0.1056
N fixation	5	16.10	0.001
Year	2	7.45	0.0012
Treatment	1	63.88	0.0001
Year \times treatment	2	0.88	0.4216
N ₂ O—cores (compare year 1 with year 2)	3	15.20	0.0001
Time (1 versus 2 years)	1	7.02	0.0154
Treatment	1	37.13	0.0001
Time \times treatment	1	1.44	0.2443
<i>Avicennia</i> shoot growth	3	1.09	0.3608
Year	1	0.58	0.4500
Treatment	1	1.64	0.2045
Year \times treatment	1	1.67	0.1999
Number of new <i>Avicennia</i> leaves	3	2.36	0.0783
Year	1	5.03	0.0280
Treatment	1	2.23	0.1398
Year \times treatment	1	0.00	0.9669
Number of new <i>Avicennia</i> branches	3	4.73	0.0045
Year	1	8.06	0.0059
Treatment	1	6.63	0.0121
Year \times treatment	1	0.02	0.8957
N in senesced <i>Avicennia</i> leaves	3	0.42	0.7432
Year	1	1.14	0.3005
Treatment	1	0.07	0.7972
Year \times treatment	1	0.01	0.9255

There were no pronounced effects of the treatments on the pH of interstitial water (Table 2). The pH differed little between years (2001 = 7.61 ± 0.03 , 2002 = 7.60 ± 0.02 , 2003 = 7.55 ± 0.02) or treatments (control = 7.56 ± 0.03 , fertilized = 7.61 ± 0.02).

Nitrate concentrations in interstitial water were higher in the fertilized plots (fertilized = 1.89 ± 0.35 mg/l; control = 0.87 ± 0.22 mg/l) and the means differed across the 3 years (data not shown) but there was not a significant overall model effect (Table 2). There was a distinct model effect for ammonium with a significant year and year \times treatment interaction (Table 2). Ammonium concentrations were noticeably lower in 2003 (0.81 ± 0.07 mg/l) compared to similar values in 2001 (1.10 ± 0.09) and 2002 (1.02 ± 0.07).

The significant interaction was due to higher ammonium concentrations in the control plots in 2001 and 2002 (1.05 ± 0.11 and 1.15 ± 0.11 , respectively) and higher concentrations in fertilized plots in 2002 (1.18 ± 0.14).

Soil samples collected in February 2002 were analyzed for Total N, P, and K to determine if there had been any effects after 1 year of the experimental treatments on total standing stocks of nutrients in the top 10 cm of the plots. There were no significant differences (Table 2) between treatments for N (control= 0.60 ± 0.09 mg/g dry soil; fertilized= 0.58 ± 0.06), P (control= 0.71 ± 0.01 mg/g dry soil; fertilized= 0.71 ± 0.01) or K (control= 0.55 ± 0.03 mg/g dry soil; fertilized= 0.54 ± 0.04) and the ratio of N:P (control= 0.85 ± 0.12 ; fertilized= 0.82 ± 0.08).

Denitrification and Nitrous Oxide Emission

There were significant year and treatment effects on denitrification as measured through lab incubations of cores and nitrous oxide emissions measured in field chambers but the interaction between year and treatment was not significant for either method (Table 3). For 10-cm-deep soil cores (Fig. 1a), denitrification was much higher in fertilized (2.46 ± 0.27 mg N/m²/h) compared to control

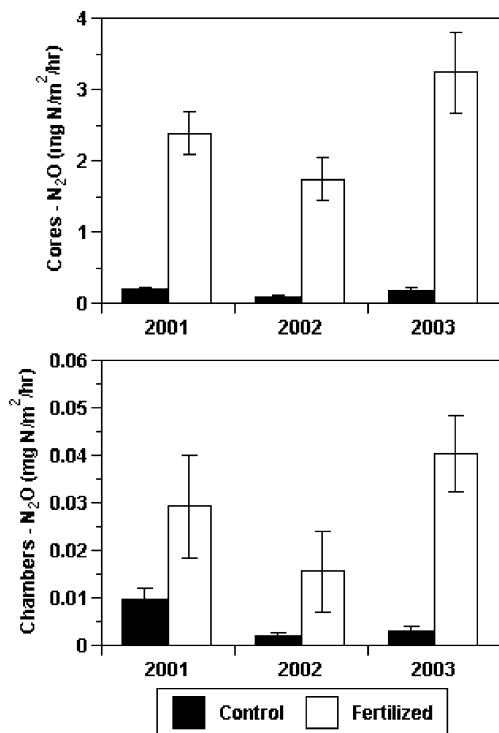


Fig. 1 Denitrification rates from cores incubated in the lab and N₂O fluxes measured in field chambers. Values are means ± 1 standard error. The means for fertilized plots differed significantly from Control plots in all 3 years for both measurement techniques and there were no significant interactions between treatment and time (Table 3)

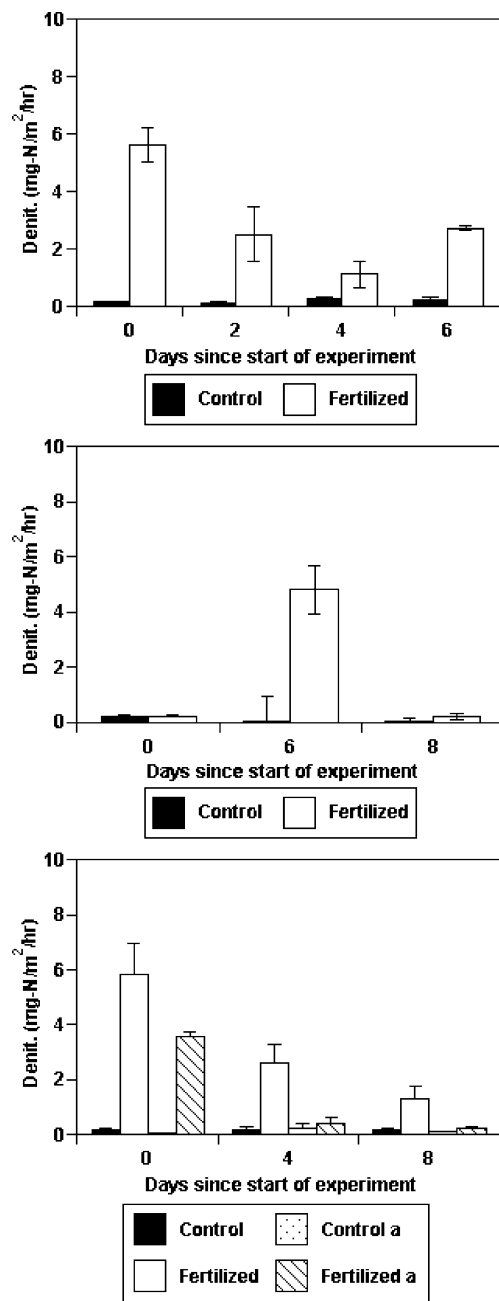


Fig. 2 Rates of denitrification in cores in 2001–2003. Values are means ± 1 standard error. In 2003, we also measured denitrification (see “Methods” section) in a second set of sites (Fertilized a and Control a) that were used to determine if the application of N to sites had resulted in any long-term changes in the rate of denitrification

(0.16 ± 0.03 mg/m²/h) plots and the measured rates differed between years (data not shown)

Nitrous oxide emission rates from chambers (Fig. 1b) were almost six times higher in fertilized plots (0.028 ± 0.006 compared to 0.005 ± 0.001 in control plots). The N₂O fluxes from chambers also varied between years of measurements; being substantially lower in 2002 ($0.009 \pm$

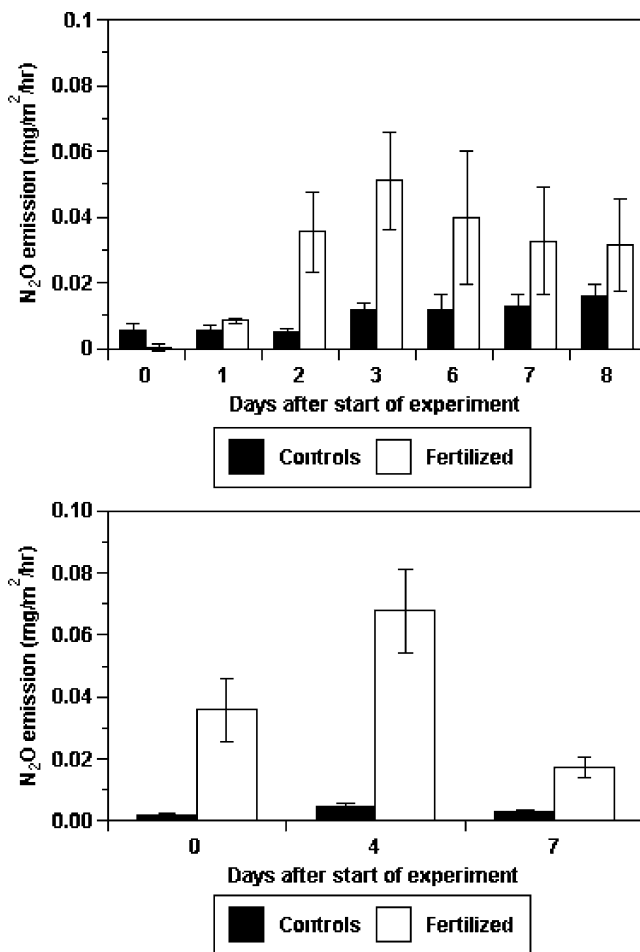


Fig. 3 Rates of nitrous oxide emissions in 2001 and 2003. Values are means ± 1 standard error

0.005) compared to 2001 (0.019 ± 0.006) and 2003 (0.021 ± 0.007).

In 2003, we measured denitrification from cores in a separate set of 12 plots (see the “Methods” section) to compare rates after 2 years of fertilization with rates of denitrification after a single application of fertilizer. There were significant treatment and year differences observed, but the interaction term was not statistically significant (Table 3). Denitrification was about eight times higher in fertilized plots (2.33 ± 0.48 mg/m²/h compared to 0.16 ± 0.04 mg/m²/h in control plots) and plots that had been fertilized for 2 years had higher rates of denitrification (1.72 ± 0.53 mg/m²/h) compared to plots that only received one application of fertilizer (0.77 ± 0.34 mg/m²/h).

The time-courses of denitrification (Fig. 2) and N₂O emissions (Fig. 3) were also similar across the course of the experiment. For the soil cores, the rate of denitrification in the fertilized plots increased sharply within 1 h of application of the N (day 0) and decreased gradually in a few days afterwards in 2001 and 2003. In 2002, the site

(see above) was flooded during most of the 2-week study period and the peak measurement was delayed until the second set of measurements on day 6 (Fig. 2b). N₂O emissions from the chambers peaked 3–4 days after the fertilizer was added in 2001 and 2003 (Fig. 3). Only one measurement of N₂O emission was made in 2002 because of surface flooding of the plots.

Nitrogen Fixation

Nitrogen fixation was measured in February 2002 and 2003 and July 2002 (Fig. 4). There was a significant treatment and time effects on the rate of nitrogen fixation, and no significant interactions between treatment and time (Table 3). For 5-cm-deep soil cores, and averaging across all months, N₂ fixation was substantially lower in fertilized than control plots (0.041 ± 0.022 versus 0.331 ± 0.080 mg N/m²/day). Averaging across treatments, rates measured in February 2003 (0.098 ± 0.049 mg N/m²/day) were noticeably lower than rates measured in February 2002 (0.203 ± 0.054 mg N/m²/day) and July 2002 (0.186 ± 0.059 mg N/m²/h).

Black Mangrove Measurements

There was no significant experimental effect on annual shoot growth, the number of new leaves produced on the tagged *Avicennia* shoots, or the N concentration of recently senesced leaves (Table 3 and Fig. 5). There was a significant year and treatment effect on the number of new branches on the tagged shoots with shoots on fertilized plants producing more branches per year ($0.92 \pm 0.20 =$ fertilized; $0.36 \pm 0.89 =$ control) and a significantly greater number of branches produced in year 1 (0.81 ± 0.16) compared to year 2 (0.43 ± 0.14).

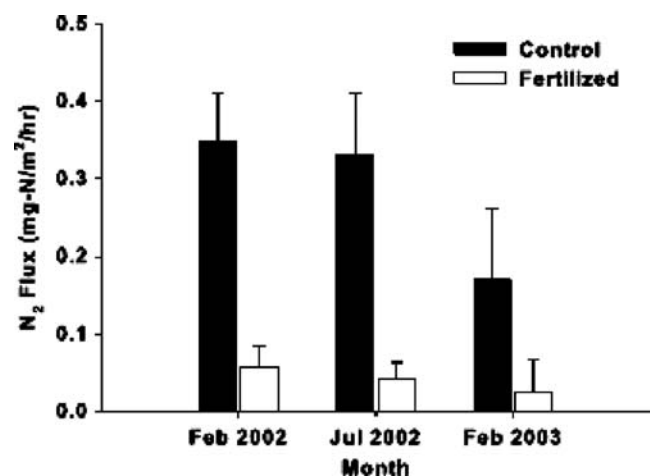
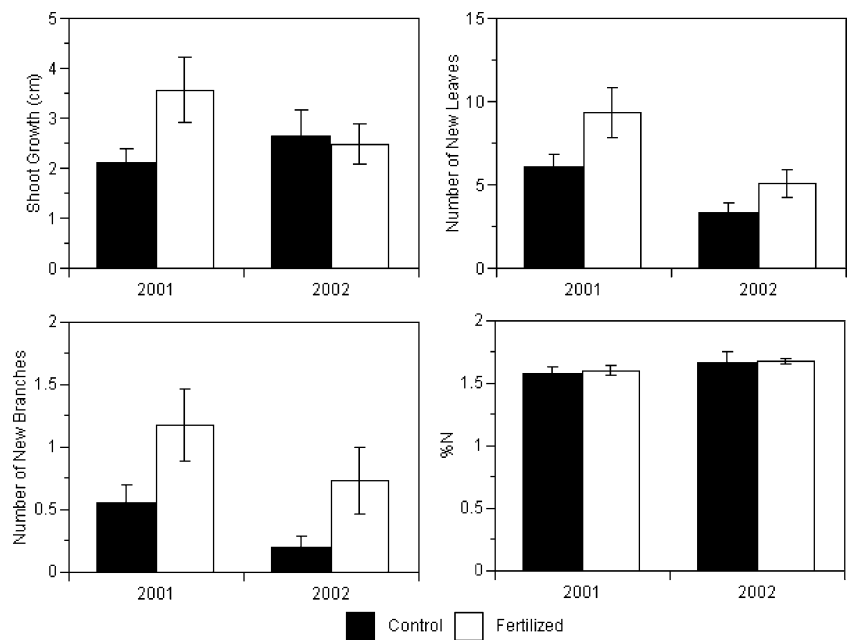


Fig. 4 N-fixation from Control and Fertilized plots. Values are means ± 1 standard error

Fig. 5 Shoot growth, the number of new leaves, the number of branches per shoot, and %N of senesced leaves on tagged *Avicennia germinans* shoots. Values are means ± 1 standard error



Discussion

Historically, dwarf growth forms of mangrove trees have been associated with a variety of factors including high salinity, poorly aerated sulfate-rich and compacted soils, and nutrient limitations (e.g., Lugo and Snedaker 1974; Tomlinson 1986; McKee 1993; Ball 1996; Nandoo 2006). Whether or not individual factors or suites of interrelated factors are responsible for dwarf growth forms remains an open question, but recent nutrient-addition experiments have shed light on some of the key factors. Feller and colleagues (Feller 1995; Feller et al. 2002; McKee et al. 2002; Feller et al. 2003; Lovelock et al. 2006a, b, 2007) have been conducting a series of nutrient-addition experiments in mangroves in Florida, Belize, Panama, New Zealand, and Australia. They found that the addition of a limiting nutrient (nitrogen, phosphorus or a combination of N and P) at all study sites resulted in a substantial increase in growth and an especially dramatic increase in the growth of shoots of dwarf mangroves. The underlying mechanisms thought to be responsible for the dramatic growth response of dwarf growth forms (e.g., as much as a tenfold increase in annual shoot elongation) were summarized by Lovelock et al. (2006a, b). In P-limited habitats, a significant increase in several growth-related measurements was associated with an increase in water conductance which, in turn, was associated with an increase in the average diameter of water-conducting vessels in red mangrove shoots. Stomatal conductance and photosynthetic rates of dwarf red mangrove shoots also increased to levels that were similar to much taller trees in fringe habitats (Lovelock et al. 2006a).

In the same area where the current study was conducted, Lovelock et al. (2006b) measured a sevenfold increase in shoot growth in dwarf *A. germinans* compared to dwarf *L. racemosa* following the addition of N. Even though there were dramatic differences in growth, there were no differences in stem hydraulic conductivity and the only difference between the two species was that leaf-specific conductance was higher in *A. germinans*. Lovelock et al. concluded that the response of *A. germinans* was primarily due to the allocation of biomass to leaf area, as a lower leaf area per unit of water consumption may result in less vulnerability to xylem embolism.

The most striking response of *Avicennia* to N addition in our study was the lack of a clear and dramatic growth response (Fig. 5 and Table 3) in all the parameters measured. Shoot growth, the number of new leaves, and the number of branches per shoot were all higher in N-fertilized plants (Fig. 5) but the differences were only significant for the number of branches (Table 3). Also, in contrast to the distinctly higher N content of senesced leaves of fertilized *Avicennia* in the adjacent impoundment (Feller et al. 2003), the means for fertilized and control plants were almost identical in this experiment (Fig. 5).

The reasons for the lack of clear growth responses of dwarf *Avicennia* in this study are unknown but at least two factors need to be considered. First, our study sites were distinctly hypersaline (57; see Table 1). Increases in salinity have been shown to have a negative effect on growth, mortality, and hydraulic conductivity of *A. germinans* (Suárez and Medina 2005; Lovelock et al. 2006b). However, there is also evidence that seedlings and dwarf growth forms of black mangrove are adapted to higher

salinities and higher ambient light conditions (Christian 2005), are capable of maintaining adequate water uptake under hypersaline conditions (Sobrado and Ewe 2006), and experience minimal effects on growth at salinities up to at least 35 (Ye et al. 2005). Feller et al. (2003) found that black mangrove responded positively to N addition at salinities that averaged 41–57 and that there was an almost tenfold increase in growth of fertilized dwarf black mangroves at the sites with the highest salinities. It does not, therefore, seem likely, that the lack of a significant growth response of trees in this study was due to hypersaline conditions.

The mode of fertilizer application is another factor that may be related to the lack of a strong growth response of dwarf black mangroves in this study. The inter-continental fertilization experiments being conducted by Feller and colleagues (see citations above) employ the addition of a slow-release N fertilizer into 30-cm-deep holes that are then plugged with the substrate that was removed to make the hole. The goal of this application procedure was to make the fertilizer available to the tree roots. The N fertilizer used in their experiments is urea (N:P:K, 45:0:0) and it is placed into dialysis tubing before it is inserted into the holes. This mode of application results in the slow release of N at depths that is probably below the surface microbial community.

In this study, we applied N at the same annual rate that is used in the study by Feller and colleagues, but the form of the fertilizer was different. We applied the N as NaNO_3 dissolved in 5 l of estuarine water that was sprinkled onto the soil surface when the substrate was not flooded; thus ensuring that the NO_3^- infiltrated the substrate. Our goal was to mimic an increase in nitrate input in tidal flooding that would be associated with increased eutrophication of the Indian River. We anticipated that the added nitrogen would be available to the microbial community in the surface soils where most of the N retention and losses from mangrove soils apparently occur (Chiu et al. 2004).

A portion of the microbial community involved in N-cycling responded rapidly to the addition of NO_3^- to the surface of the soil by increasing the rates of denitrification and N_2O emission. Denitrification increased within an hour of addition of the NO_3^- in 2001 and 2003 (Fig. 2). The denitrification response was delayed in 2002, probably because the groundwater was at or very close to the soil surface at the time the N was added.

Rapid uptake of N by the microbial community in mangroves has previously been measured (Corredor et al. 1999). Others found that mangrove soils were a sink for N due to microbial assimilation, especially in vegetated sites (e.g., Rivera-Monroy et al. 1995; Kristensen et al. 1998). We also found that the rates of denitrification were higher in plots that had been fertilized for 1 year compared to plots that had not been previously fertilized (see the “Results” section), suggesting that the biomass or denitrifying enzyme content

of the denitrifying microbial community increased over time, even though nitrate additions to the soil were episodic.

While we did not attempt to separate N uptake by plants versus microorganisms, a comparison of our results to those of Feller and colleagues, clearly suggests that the N that was added was mostly assimilated and processed by the microbial community. Our study suggests that mangrove systems in the N-limited (Feller et al. 2002) Indian River Lagoon are likely to be long-term sinks for any increases in N loading that result from local cultural eutrophication. The microbial community is clearly an important component of N-processing and the dwarf mangroves in the interior of the wetlands are likely to remain N-limited unless or until N addition to the lagoon reaches much higher level. N_2O fluxes in non-fertilized plots of studied hypersaline mangrove wetland are currently within the range reported for other pristine mangroves (Barnes et al. 2006). Enhancing N loading of the lagoon will stimulate nitrification and denitrification in mangrove soils and increase N_2O flux to the atmosphere.

The long-term availability of N in mangrove systems is also determined by the extent to which outputs are balanced by inputs from tides, atmospheric deposition, and microbial N_2 fixation. N_2 fixation rates are governed by the availability of carbon (i.e., energy), soil NH_4^+ availability, and other factors (Holguin et al. 2001). In control plots, rates of N_2 fixation exceeded denitrification by twofold even though the N_2 fixation cores were only half as deep (5 cm versus 10 cm). This suggests these sites are either accumulating N or acting a net source of N to the surrounding system. Rates of both N_2 fixation and denitrification were at the low end of the range of previously reported values for mangrove soils (Lee and Joye 2006). The low rates at our site may be caused by hypersaline conditions (Table 1) or rapid desiccation of the sandy soils between flood events.

We found that N fertilization suppressed inputs from N_2 fixation, a potentially important N source, by about 75%. This 0.24 mg $\text{N}/\text{m}^2/\text{h}$ decline in N_2 fixation inputs was small compared to the 2.3 mg $\text{N}/\text{m}^2/\text{h}$ increase in denitrification, indicating that denitrification is the more important process to consider for mitigation of cultural N eutrophication.

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