Ecosystems (2019) 22: 1126–1144
https://doi.org/10.1007/s10021-018-0327-0

ECOSYSTEMS

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Isotopic Evidence that Nitrogen Enrichment Intensifies Nitrogen Losses to the Atmosphere from Subtropical Mangroves

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ABSTRACT

Nitrogen (N) enrichment can have large effects on mangroves' capacity to provide critical ecosystem services by affecting fundamental functions such as N cycling and primary productivity. However, our understanding of excess N input effects on N cycling in mangroves remains quite limited. To advance our understanding of how N enrichment via water or air pollution affects mangroves, we evaluated whether increasing N inputs would decrease biological N fixation (BNF), but intensify N dynamics and N losses to the atmosphere in these systems. We measured N concentrations in sediment and vegetation, rates of BNF in sediment and litter, and net sediment ammonification and nitrification rates. We also evaluated long-term integrated N dynamics and N losses to the atmosphere using the natural abundance of N stable isotopes (δ^{15}N) in the sediment–plant system and in estuarine water. We performed these analyses at non-N-enriched and N-enriched (that is, polluted) fringe and basin mangroves in southeastern Brazil. The δ^{15}N in the sediment–plant system was higher at N-enriched than non-N-enriched fringe sites, indicating increased N losses to the atmosphere from N-enriched sites. However, N concentrations in sediment and vegetation were similar or lower at N-enriched relative to non-N-enriched sites. BNF and net ammonification and nitrification rates were also similar between N-enriched and non-N-enriched sites. Excess N inputs intensified N losses to the atmosphere from mangroves, but N pools, BNF, and net ammonification and nitrification rates were not affected by N enrichment, likely because excess N was quickly lost from the system by direct denitrification and volatilization.

Key words: biological nitrogen fixation; denitrification; nitrification; nitrous oxide; phosphorous; stable isotopes.

HIGHLIGHTS

- Excess nitrogen (N) inputs intensified N losses to the atmosphere from mangroves.

Received 7 May 2018; accepted 15 November 2018;
published online 8 January 2019

Electronic supplementary material: The online version of this article (https://doi.org/10.1007/s10021-018-0327-0) contains supplementary material, which is available to authorized users.

Author Contributions CRGR and GBN conceived the ideas; CRGR, SCR, and GBN designed the methodology; CRGR collected and analyzed the data. All authors contributed to discussing the results and writing the manuscript.

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- Excess N was not stored in mangrove ecosystems, but rather lost to the atmosphere.
- Polluted mangroves may become a larger source of nitrous oxides to the atmosphere.

INTRODUCTION

Nitrogen (N) availability is one of the main factors regulating the structure and functioning of natural ecosystems (Vitousek and Howarth 1991; Elser and others 2007). Excess N input via water pollution and N deposition from the atmosphere can have several consequences for N cycling in terrestrial ecosystems (Galloway and others 2008). Potential consequences include reduced N input via biological N fixation (BNF) (Niu and others 2016; Reed and others 2011), altered N dynamics (that is, N transformations) and N availability, and increased N losses to the atmosphere and to aquatic systems (Erisman and others 2013; Niu and others 2016). The provision of many key ecosystem services is regulated by supporting services such as N cycling and primary productivity (de Vries and others 2014). As a consequence, services such as carbon (C) sequestration and the provision of clean air and water can be affected by excess N input and altered N cycling (for example, Compton and others 2011; Erisman and others 2013; de Vries and others 2014). However, quantifying the consequences of increased anthropogenic inputs of N remains an important research challenge.

Patterns in N dynamics and N losses to the atmosphere in terrestrial ecosystems can be assessed by the natural abundance of N stable isotopes ($\delta^{15}N$) in the soil–plant system. The $\delta^{15}N$ signal integrates long-term fractionation against the heavier ^{15}N isotope during N transformations such as nitrification, denitrification, and volatilization (Högberg 1997; Robinson 2001). Therefore, the $\delta^{15}N$ in the soil–plant system can be used as a measurement of the intensity of N cycling and N losses to the atmosphere from these systems (for example, Houlton and others 2006; Nardoto and others 2008). The $\delta^{15}N$ integrates N dynamics and N losses to the atmosphere over the leaf lifespan in leaf samples (up to 12 months for the mangrove species in the present study, for example, Ellison 2002; Menezes and others 2008), and over the decades that organic matter is decomposing in soil samples. Terrestrial ecosystems with intensified N dynamics and N losses to the atmosphere have higher $\delta^{15}N$ signatures in the soil–plant system (Craine and others 2009, 2015). Direct measurements of gaseous N losses to the atmosphere are highly variable in space and time, especially in mangrove ecosystems due to tidal regime and heterogeneous edaphic conditions (for example, Allen and others 2007; Alongi and others 2005; reviewed by Reis and others 2017a). Accordingly, point measurements of N loss to the atmosphere are difficult to scale. The ^{15}N approach offers a powerful way to integrate the large variability in gaseous N losses to the atmosphere. Thus, isotopic approaches offer opportunities for significantly improving our understanding of how increased anthropogenic inputs of N are affecting the structure and function of terrestrial systems.

Mangrove ecosystems are coastal woody wetlands that play fundamental roles in the biogeochemical cycling of tropical, subtropical, and warm temperate regions worldwide. Mangroves are among the most productive ecosystems on Earth, acting as substantial sinks for atmospheric CO_2 in coastal zones (for example, Donato and others 2011; Murdiyarso and others 2015). Mangroves also store a larger amount of C per unit area in soil and biomass than the often discussed C stocks found in upland tropical forests and in marine ecosystems (for example, Hutchison and others 2014; Alongi 2014). Furthermore, mangroves play critical roles in N cycling in coastal areas, as they are highly efficient at using excess N from tidal waters. Thus, mangroves help to improve coastal water quality. In turn, mangrove ecosystems represent a potentially larger per area source of nitrous oxide (N_2O) to the atmosphere than those found in terrestrial tropical forests (reviewed by Reis and others 2017a). Excess N input is expected to have large effects on mangrove N cycling and the provision of critical ecosystem services by these systems. Nonetheless, data for mangrove N cycling in the face of excess N inputs are quite limited. Collectively, the studies that do exist suggest that BNF can be dramatically reduced by increased N input (Romero and others 2012; Whigham and others 2009), whereas N losses to the atmosphere, including of N_2O, seem to be intensified by excess inputs of N (for example, Corredor and others 1999; Fernandes and others 2010). However, the range of rates reported by these studies for both non-N-enriched and N-enriched mangroves is quite large, suggesting that not all mangroves will fit these patterns (reviewed by Reis and others 2017a) and that measurement variability may constrain our ability to understand overall trends. More studies on the effects of excess N input to mangroves N dynamics and N losses to the atmosphere are needed, especially comprising a broader set of techniques to assess N pools and cycling pathways in different types of mangroves.

To advance our understanding of how N enrichment via water pollution and N deposition from the atmosphere affects mangrove ecosystems, we sampled various N cycling pools and fluxes and used a ^{15}N approach to assess mangrove N cycling in the face of elevated N inputs. Specifically, we evaluated the hypothesis that N enrichment decreases biological N fixation, but intensifies N dynamics and N losses to the atmosphere in mangrove ecosystems. To test this hypothesis, we quantified N concentrations in sediment and vegetation, rates of BNF in sediment and leaf litter, sediment net ammonification and nitrification rates, and the δ^{15}N in the sediment–plant system and estuarine water. We explored these multiple aspects of the N cycle at non-N-enriched and N-enriched fringe and basin mangroves in southeastern Brazil.

MATERIALS AND METHODS

Study Area

The study was conducted at 6 mangrove sites along a gradient of anthropogenic N inputs in the Estuarine Lagunar Complex (ELC) of Cananeia–Iguape, southeastern Brazil, in 2016 and 2017 (Figure 1). The Cananeia region comprises the most protected and conserved mangrove areas in the São Paulo State (Cunha-Lignon and others 2011). The Ribeira de Iguape River forms one of the largest watersheds of the Brazilian coast, supporting more than a half million people (IBGE 2010). The Ribeira de Iguape River also drains large areas of cropland that together account for 80% of banana production in the state of São Paulo (IBGE 2006), and carries excess N from both sewage and agriculture through the Valo Grande canal to the estuary and mangroves in the Iguape region.

We selected a fringe and a basin mangrove in a conserved mangrove area under great marine influence in the Cardoso Island State Park as the non-N-enriched control sites (Control) (Figure 1). We also selected a fringe and a basin mangrove that receive medium N inputs from sewage discharge of a small urban area in the Cananeia Island supporting about 12,000 people (IBGE 2010) (+N). Finally, we selected a fringe and a basin mangrove in the Iguape region strongly affected by N enrichment from the Ribeira de Iguape River watershed (++N). Average N–NH$_4^+$ concentration in estuarine water is 0.10 (up to 0.13) mg/l at Control sites, 0.13 (up to 0.35) mg/l at +N sites, and 0.35 (up to 1.43) mg/l at ++N sites. Although N–NO$_3^-$ concentrations in estuarine water are usually below detection limit ($<$ 0.20 mg/l) at Control and +N sites, the average is 0.49 (up to 3.55) mg/l at the ++N sites. Estuarine water is mesotrophic at Control sites averaging 4.6 (up to 8.5) µg/l of chlorophyll a, eutrophic at +N sites, 9.3 (up to 29.4) µg/l of chlorophyll a, and from eutrophic to hypereu-

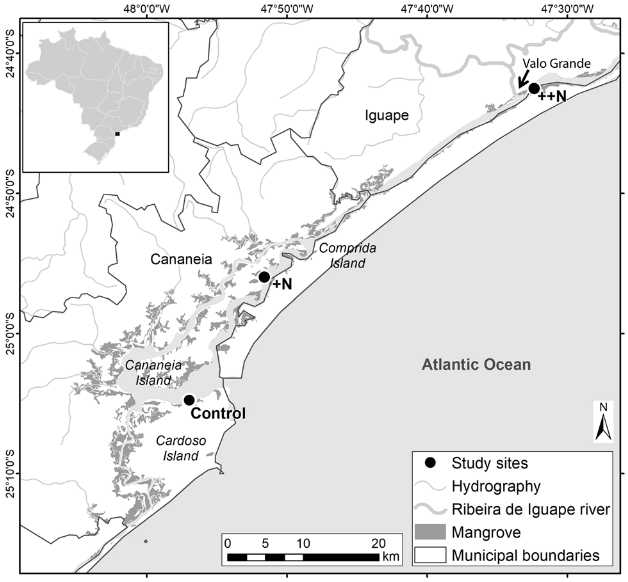

Figure 1. Location of the non-N-enriched (Control) mangrove study sites, and those sites subjected to medium (+N) and high (++N) N inputs in the Estuarine Lagunar Complex of Cananeia–Iguape, southeastern Brazil. The shapefiles of mangrove forest areas were provided by Marília Cunha-Lignon. *Source*: Cunha-Lignon and others (2011).

trophic at ++N sites, 10.4 (up to 47.6 µg/l) of chlorophyll a (data available at https://servicos.cetesb.sp.gov.br/infoaguas/).

Climate in the ELC of Cananeia–Iguape is classified as ''Cfa'' in the Köppen–Geiger climate classification, either named as a subtropical (Alvares and others 2013) or as a temperate climate (for example, Kottek and others 2006; Peel and others 2007). Mean monthly air temperature ranges from 24 °C in January to 8 °C in July. The ELC of Cananeia–Iguape was previously reported to experience sporadic frosts (Schaeffer-Novelli and others 1990), but frost events were not recorded at our study sites from 2008 to 2012 (see Lima and Galvani 2013; Lima and others 2013), or in the Iguape region from 2012 to the present (data available at http://www.inmet.gov.br). Mean annual rainfall is about 2300 mm, without a marked dry season. Tides are predominantly semidiurnal with mean amplitude of 0.8 m and of 1.2 m during spring tides (Schaeffer-Novelli and others 1990).

Mangrove vegetation in the ELC of Cananeia–Iguape is composed by *Rhizophora mangle* L. (Rhizophoraceae), *Laguncularia racemosa* (L.) C. F. Gaernt. (Combretaceae), and *Avicennia schaueriana* Stapf and Leechm. ex Moldenke (Acanthaceae) (families according to APG III 2009). *Laguncularia racemosa* dominates mangroves at the ++N sites, whereas *R. mangle* dominates mangrove areas at both Control and +N sites. To avoid confounding effects, we evaluated if differences between study sites on leaf parameters reflected actual N cycling differences between study sites, or merely differences between species and on species composition and abundance between study sites. To do that, we compared leaf results between species (study sites pooled together), between study sites (species pooled together), and also between study sites for each species.

Because of the natural variability of sedimentary dynamics within the estuarine system, and also the influence of the Ribeira de Iguape River discharge at ++N sites, the study sites also differ in sediment properties (Table 1). Sediment is approximately 70% silt and clay at the +N and ++N sites, whereas about 80% sand in the Control sites, which have a greater marine influence. As a consequence, organic matter concentrations are about 2–4 times higher in the +N and ++N than the Control sites. As we were not able to standardize these sediment properties among our study sites, we carefully considered these differences when interpreting the results. More information for the Control sites can be found in Reis and others (2017b).

Table 1. Physical and Chemical Properties of Sediment from Mangrove Study Sites

	Control		+N		++ N	
	F	B	F	B	F	B
Sand (%)	77	82	16	41	34	24
Silt (%)	7	6	49	41	40	56
Clay (%)	16	12	35	18	26	20
Texture class	SaLo	SaLo	SiClLo	Lo	Lo	SiLo
OM (g/kg)	52	58	116	115	97	218
Na^+ (mmol$_c$/kg)	344	246	873	245	344	27
K^+ (mmol$_c$/kg)	14	12	51	16	21	6
Ca^{2+} (mmol$_c$/kg)	50	43	135	109	71	119
Mg^{2+} (mmol$_c$/kg)	120	97	232	161	153	115
Al^{3+} (mmol$_c$/kg)	27	19	8	2	1	21
SEB (mmol$_c$/kg)	528	399	1291	531	589	266
CEC (mmol$_c$/kg)	609	483	1401	593	648	428

Data of a composite sample at 0–20 cm depth from non-N-enriched (Control) fringe (F) and basin (B) mangroves, and those subjected to medium (+N) and high (++N) N inputs. Data for the Control sites are from Reis and others (2017b). Data for the +N and ++N sites are from 5 samples collected in November 2016 and combined into a composite sample for each site and analyzed using the methods from Embrapa (1997, 1999). Sediment texture was analyzed using the hydrometer method (Bouyoucos 1932) and classified using the US Department of Agriculture textural triangle. Organic matter was extracted with potassium dichromate in sulfuric medium and quantified by titration using ammonium ferrous sulfate. Available exchangeable bases were extracted with ammonium acetate 1 M. Concentrations of sodium (Na^+) were analyzed by flame photometry, of potassium (K^+) by atomic emission, and of calcium (Ca^{2+}) and magnesium (Mg^{2+}) by atomic absorption spectrophotometry. Aluminum (Al^{3+}) was extracted with KCl 1 M and quantified by titration with NaOH (unpublished data).

Sa Sand, Si silt, Cl clay, Lo loam, OM organic matter, SEB sum of exchangeable bases, CEC cation exchange capacity.

Sampling Design

Three transects of 60 m length each and with a distance of 30 m in between were established perpendicularly to the shoreline at each study site. For each transect, 3 sampling points were established: one at the shoreward transect end, one 30 m away from the shore, and one 60 m away, at the landward transect end. At each sampling point, mature leaf samples (that is, green and fully expanded) were obtained from the upper canopy of 3 arboreal individuals with diameter at breast height (DBH) ≥ 4 cm (about 24 leaves per tree), as well as a composite leaf litter sample (that is, yellow and dark leaves on varying stages of decomposition, 10–20 leaves per sample), hand-collected from the forest floor below the sampled trees. Leaves were collected to assess N and P concentrations (N = 27 for N, and 9–19 for P concentrations for each site), and δ^{15}N was assessed in leaves (N = 27 for each site) and leaf litter (N = 9 for each site) (methods below).

Sediment samples at 0–10 cm depth (about 300 g each) were also obtained from each sampling point to characterize the δ^{15}N, total N concentrations, net ammonification and nitrification rates, and the concentrations of available NH_4^+, NO_3^-, and P (N = 9 for each site). Redox potentials and pH of sediment interstitial water at 0–5 cm depth were measured in situ with a portable meter (HI 991003) (Hanna Instruments, Woonsocket, Rhode Island). Composite leaf litter and 0–10 cm depth sediment samples were also obtained from 7 sampling points at each study site to estimate BNF rates (N = 7 for each site).

Estuarine water samples (1.5 l each) were collected during flood tide at 3 sampling points in the shoreline of each fringe site (N = 3 for each fringe site), and at 1 sampling point in the Valo Grande canal (VG) to characterize the δ^{15}N of NH_4^+ and NO_3^-. Estuarine water and sediment samples were kept refrigerated until analysis. Leaf and leaf litter samples were rinsed with tap water and dried at 40 °C over 48 h immediately after fieldwork.

Vegetation Structure

Vegetation structure was measured in a plot comprising 20 arboreal individuals established at the center sampling point of each of the six study sites (Schaeffer-Novelli and Cintrón 1986). At each plot, individuals taller than 1 m were identified and had their DBH and height (H) recorded.

From the census data, we estimated basal area (BA) and live aboveground biomass (AGB) according to the following equations available in Medeiros and Sampaio (2008):

$AGB = 0.2752*(DBH^2*H)^{0.8529}$ for *R. mangle* and
$AGB = 0.1214*(DBH^2*H)^{0.8615}$ for *L. racemosa*.

δ^{15}N of NH_4^+ and NO_3^- in Estuarine Water

For the analysis of δ^{15}N of NH_4^+, subsamples (350 ml) were filtered through 0.45-μm filter membrane and preserved by decreasing the pH with concentrated H_2SO_4 (Hannon and Böhlke 2008). The δ^{15}N of NH_4^+ was analyzed by the conversion of NH_4^+ into NH_3 gas by raising the pH of samples with magnesium oxide (Holmes and others 1998) and quantified using an elemental analyzer (EA) (Elementar vario EL cube), coupled with an isotope-ratio mass spectrometer (IRMS) (isoprime VisION) (Elementar Analysensysteme GmbH, Langenselbold, Germany) in the Stable Isotope Facility of the University of California, Davis. Bovine liver, glutamic acid, enriched alanine, and nylon 6 were used as reference materials. Uncertainty was up to 0.1‰.

For the analysis of δ^{15}N of NO_3^-, subsamples (100 ml) were filtered through 0.2-μm filter membrane and preserved by raising the pH with NaOH (Coplen and others 2012). The δ^{15}N of NO_3^- was analyzed by the enzymatic conversion of NO_3^- to N_2O by the denitrifying bacteria *Pseudomonas aureofaciens* (Sigman and others 2001) and quantified using an IRMS (Thermo Fischer MAT 253) coupled with a Thermo Fisher modified denitrification Gasbench via ConFlo IV (Thermo Fisher Scientific GmbH, Bremen, Germany) in the Stable Isotope Ratio Facility for Environmental Research of the University of Utah. The US Geological Survey 34, USGS35, and IAEA-NO-3 were used as reference materials. Uncertainty was up to 0.5‰. Atmospheric air was used as a standard for δ^{15}N analysis.

Biological N Fixation Rates in Sediment and Leaf Litter

Sediment samples at 0–10 cm depth were collected by inserting acrylic tubes (20 cm length, 5 cm inner diameter) into the sediment. Leaf litter samples were collected in a 1-m^2 area and placed inside acrylic tubes. The tubes were sealed with rubber stoppers, and 10% of the atmosphere inside the headspace of the tubes was replaced with acetylene (C_2H_2), immediately after field collections (Hardy and others 1968). After 2 h of incubation at ambient temperature, the atmosphere inside the tubes was collected and analyzed for ethylene (C_2H_4) concentrations using a gas chromatograph (Shimadzu Scientific Instruments, Kyoto, Japan) with a flame ionization detector. Control incubations were performed with samples without C_2H_2 addition to account for endogenous C_2H_4 production, and with C_2H_2 addition without samples to account for C_2H_4 concentrations in the acetylene gas.

Acetylene reduction activity was converted to estimated N fixation rates using the conversion factor of 4 (C_2H_4:N_2 ratio of 4:1) (Postgate 1982), because studies using $^{15}N_2$ calibration in mangroves have shown ratios ranging from 1.9 to 6.3 (Potts 1984; Hicks and Silvester 1985). After incubations, samples were dried and weighed. BNF rates were calculated on both a dry-weight and an areal basis.

Net ammonification and Nitrification Rates

Net ammonification and nitrification rates in sediment were quantified according to the incubation and extraction procedures described by Reis and others (2017b), modified from Piccolo and others (1994). Extracts were analyzed for concentrations of N–NH_4^+ with a Nessler's reagent (Greweling and Peech 1960) and N–NO_3^- (Meier 1991, cited in Sutton and others 2014) using an UV spectrophotometer in the Laboratório de Ecossistemas of the Universidade de Brasília.

$\delta^{15}N$ in the Sediment–Plant–Leaf Litter System and Leaf N Concentrations

Sediment, leaf, and leaf litter samples were prepared according to the procedures described by Reis and others (2017b) and analyzed for total C and N, C:N ratio, and $\delta^{15}N$ using an EA (Carlo Erba) coupled with an ThermoQuest-Finnigan Delta Plus IRMS (Thermo Fisher Scientific GmbH, Bremen, Germany) in the Laboratório de Ecologia Isotópica of the Centro de Energia Nuclear na Agricultura at Universidade de São Paulo. Atmospheric air was used as a standard, and sugarcane and tropical soil were used as reference materials. Analytical error was 0.15% for C, 0.01% for N, and 0.30‰ for ^{15}N.

Sediment Available P and Leaf P Concentrations

Available phosphorus (P) was extracted with Mehlich 1 solution and quantified with ammonium molybdate using a spectrophotometer (Embrapa 1999) in the Departamento de Ciências do Solo of the Escola Superior de Agricultura Luiz de Queiroz at Universidade de São Paulo.

Leaf P concentrations were quantified by spectrophotometry using the reagent ammonium metavanadate + ammonium molybdenum, after digestion with nitric–perchloric solution (Embrapa 2000) in the Laboratório de Fertilidade do Solo of the Centro de Energia Nuclear na Agricultura at Universidade de São Paulo.

Statistical Analysis

Spatial autocorrelation and comparisons between data groups with spatial autocorrelation were tested according to the R code proposed by Eisenlohr (2014) with improvements, using one-way ANOVA *F* test followed by post hoc Tukey HSD test, discounting the effects of spatial autocorrelation.

Normality was tested using the Shapiro–Wilk *W* test. Without spatial autocorrelation, comparisons of data with normal distribution were tested by one-way ANOVA *F* test followed by *post hoc* Tukey HSD. Comparisons of data without normal distribution were tested by nonparametric Kruskal–Wallis *H* test, followed by *post hoc* pairwise comparisons described by Siegel and Castellan (1988).

Post hoc Tukey HSD tests discounting spatial autocorrelation effects were performed using Statistica software (StatSoft 2011). All other analyses were performed using R software (R Core Team 2014).

RESULTS

Vegetation Structure

Aboveground biomass was similar between Control and +N fringe sites, but about 2 times higher at these sites than at ++N fringe (Table 2 and Supplemental Table 1). Aboveground biomass was about 2 times higher at the Control basin site than at the ++N basin site, and the Control basin site was about 5 to 6 times higher than the +N basin site. Aboveground biomass was also up to 10 times higher at fringe relative to basin sites. Considering both fringe and basin sites, *R. mangle* dominated 99% of BA in Control and 94% in +N sites, whereas *L. racemosa* dominated 89% of BA in ++N sites. Due to its low abundance at the sites, *A. schaueriana* was completely absent from our vegetation structure sampling plots.

$\delta^{15}N$ of NH_4^+ and NO_3^- in Estuarine Water

The $\delta^{15}N$ of NH_4^+ in estuarine water was 3.0‰ at VG and ranged from − 2.8 to 0.1‰ at the ++N site (Table 3). The $\delta^{15}N$ of NH_4^+ was also 6 times higher at the +N (3.6‰) than the Control site (0.6‰). The $\delta^{15}N$ of NO_3^- was 9.8‰ at VG and ~ 10 times higher at ++N (3.9‰) than +N (0.4‰) site. Concentrations of NO_3^- were below detection limits for $\delta^{15}N$ analysis in Control samples.

BNF Rates in Sediment and Leaf Litter

Biological N fixation rates on a dry-weight and an areal basis in sediment were higher at ++N than +N fringe, but values did not differ between these sites and Control fringe (Figure 2). Dry-weight and areal BNF rates in sediment were also similar among basin sites, and between fringe and basin sites. Dry-weight and areal BNF rates in leaf litter were similar among study sites.

Table 2. Live Aboveground Biomass and Basal Area at the Mangrove Sites

	Control		+N		++ N	
	F	B	F	B	F	B
AGB (Mg/ha)	226	155	252	25	144	102
R. mangle BA (m^2/ha)	22.2	25.1	20.4	6.0	6.5	0
L. racemosa BA (m^2/ha)	0.5	0	1.6	0.2	17.9	37.3

Live aboveground biomass (AGB) and basal area (BA) of Rhizophora mangle and Laguncularia racemosa estimated from one plot comprising 20 arboreal individuals taller than 1 m per site. Data from fringe (F) and basin (B) mangroves at the non-N-enriched (Control) sites, and those subjected to medium (+N) and high (++N) N inputs in April 2017.

Table 3. Natural Abundance of Nitrogen Stable Isotopes (δ^{15}N) in Estuarine Water

	Control	+N	++ N	VG
NH_4^+	0.6 (− 0.1 and 1.7)	3.6 (1.2 and 9.1)	− 1.2 (− 2.8 and 0.1)	3.0
NO_3^-	B.D.	0.4 (− 0.4 and 1.3)	3.9 (3.5 and 4.8)	9.8

Median (and range) values of δ^{15}N (‰) of ammonium (NH_4^+) and nitrate (NO_3^-) from 3 samples collected during flood tide at the shoreline of each fringe site, and 1 sample in the Valo Grande (VG) canal in April 2017. Data from non-N-enriched (Control) mangroves, and those subjected to medium (+N) and high (++N) N inputs.
B.D. below detection limit.

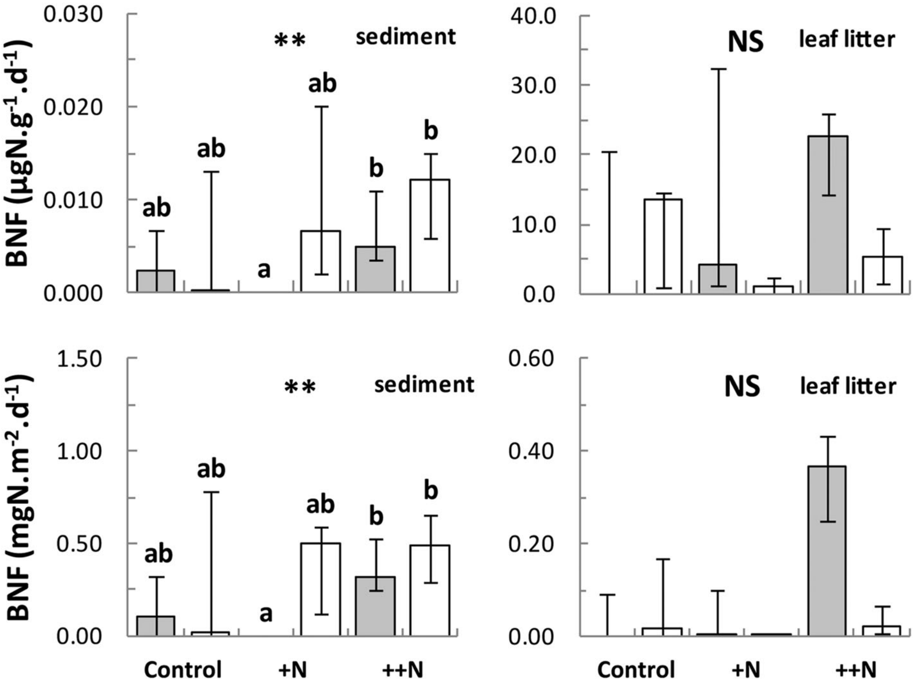

Figure 2. Biological nitrogen fixation (BNF) rates on dry-weight and areal basis in sediment at 0–10 cm depth and leaf litter from the mangrove study sites in April 2017. Data from non-N-enriched (Control) mangroves, and those subjected to medium (+N) and high (++N) N inputs. Gray bars indicate fringe mangroves, and white bars indicate basin mangroves. Median and first and third quartiles values are presented. *NS*, no significant statistical difference, $^{**}P \leq 0.01$ (Kruskal–Wallis *H* test, followed by post hoc pairwise comparisons described by Siegel and Castellan 1988). Different letters indicate significant statistical difference among study sites (N = 7 for each study site).

Net Ammonification and Nitrification Rates and Available N and P

Concentrations of N–NO_3^- in sediment were 2 times higher at Control than ++N fringe, but were not significantly different between these sites and +N fringe, $F(5,48) = 3.23$, $P = 0.014$. Median (first and third quartiles) values of N–NO_3^- concentrations at fringe sites were 0.08 (0.06 and 0.08) mg/kg in Control, 0.07 (0.05 and 0.07) mg/kg in +N, and 0.04 (0 and 0.05) in ++N sites. Concentrations of N–NO_3^- were also similar among basin sites, 0.05 (0.03 and 0.07) mg/kg, and between fringe and basin sites ($P > 0.05$).

Concentrations of N–NH_4^+ were similar among study sites, 16.7 (6.3 and 32.8) mg/kg, $F(5,41) = 1.84$, $P = 0.123$. Net ammonification, 5.6 (3.4 and 24.2) mgN kg^{-1} d^{-1}, and net nitrification rates, 0.05 (0.03–0.09) mgN kg^{-1} d^{-1}, were also similar among study sites (net ammonification: $F(5,44) = 0.55$, $P = 0.739$; net nitrification: $F(5,42) = 0.96$, $P = 0.447$).

Available P concentrations were similar among study sites, 26.7 (17.6 and 34.9) mg/kg, $H(5,28) = 7.83$, $P = 0.166$. Redox potentials, − 36 (− 162 and 18) mV, and pH values, 6.6 (6.8 and 7.2), were also similar among study sites (Eh: $F(5,43) = 1.02$, $P = 0.456$; pH: $F(5,45) = 1.14$, $P = 0.342$).

δ^{15}N in the Sediment–Plant–Leaf Litter System

Sediment δ^{15}N values were higher at ++N than Control and +N fringe sites (Figure 3). Sediment δ^{15}N values were also 2 times higher at ++N than +N basin, but there was no significant difference between these sites and the Control basin. Leaf δ^{15}N values were about 2 times higher at ++N and +N than Control fringe sites. Leaf δ^{15}N values were also 4 times higher at ++N than Control basin, but were not significantly different between these sites and +N basin. Leaf litter δ^{15}N values were similar between fringe sites, and between basin sites.

Leaf δ^{15}N values were about 2–3 times higher in *L. racemosa* and *A. schaueriana* than in *R. mangle* (Supplemental Table 2). *Laguncularia racemosa* leaf δ^{15}N values were higher at ++N and +N than Control fringe sites, but similar between basin sites, $F(5,48) = 7.37$, $P = 0.001$ (data not shown). *Rhizophora mangle* leaf δ^{15}N values were higher at ++N and +N than Control fringe sites, and 2 orders of magnitude higher at +N than Control basin sites, $F(4,75) = 3.42$, $P = 0.014$ (data not shown). Because of the low abundance of *A. schaueriana* in the

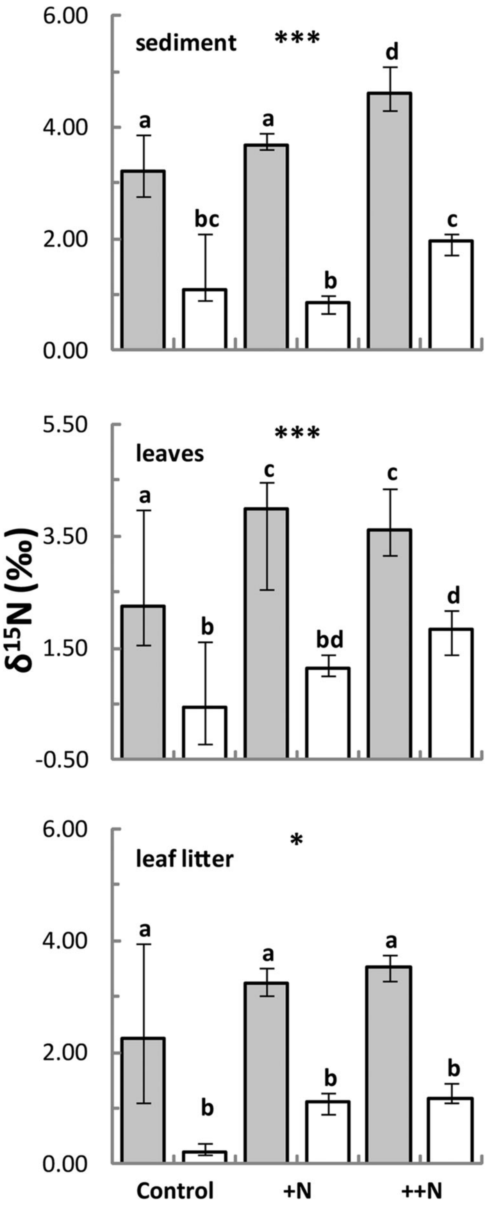

Figure 3. Natural abundance of N stable isotopes (δ^{15}N) in the sediment–plant–leaf litter system from the mangrove study sites in November 2016. Data from non-N-enriched (Control) mangroves, and those subjected to medium (+N) and high (++N) N inputs. Gray bars indicate fringe mangroves, and white bars indicate basin mangroves. Median and first and third quartiles values are presented. ***$P \leq 0.001$, *$P \leq 0.05$ (one-way ANOVA *F* test followed by post hoc Tukey HSD test, discounting the effects of spatial autocorrelation as described by Eisenlohr (2014) with improvements). Different letters indicate significant statistical difference between study sites (N = 9 for sediment and leaf litter samples and N = 27 for leaf samples for each study site).

study sites, only three individuals of this species were sampled for leaf analyses, one individual at each fringe site.

The δ^{15}N values from fringe sites were about 3 times higher in sediment, about 2–5 times higher in leaves, and 3–11 times higher in leaf litter than from basin sites. Leaf δ^{15}N values of both *R. mangle* and *L. racemosa* were higher at fringe than basin sites ($P < 0.05$).

Leaf N and P Concentrations

Leaf N concentrations were higher at +N than ++N fringe sites, but similar between Control fringe and +N or ++N fringe sites (Figure 4). Leaf N concentrations were lower at ++N basin than Control and +N basin sites. Leaf N concentrations were also higher at fringe than basin across all sites. Leaf N concentrations were about 2 times higher in *A. schaueriana* than in *R. mangle* and *L. racemosa*, and higher in *R. mangle* than in *L. racemosa* (Supplemental Table 2). Leaf N concentrations of *L. racemosa* were similar between fringe sites, and between basin sites ($P \geq 0.05$). Leaf N concentrations of *L. racemosa* were also similar between fringe and basin sites at Control and +N sites, but higher at ++N fringe than basin site, $F(5,53) = 3.46$, $P = 0.009$ (data not shown). Leaf N concentrations of *R. mangle* were similar among study sites, $F(4,75) = 1.61$, $P = 0.182$ (data not shown).

Leaf P concentrations were similar between mangrove species. Across species, leaf P concentrations were higher at ++N than Control and +N fringe sites, and higher at ++N and +N relative to the Control basin site. Leaf P concentrations were similar between fringe and basin at ++N and +N sites, but higher at Control fringe than the Control basin site.

Leaf N:P ratios were higher at the Control and +N sites relative to the ++N fringe sites. Leaf N:P ratios were also about 2 times higher at Control and +N than ++N basin sites. Leaf N:P ratios were similar

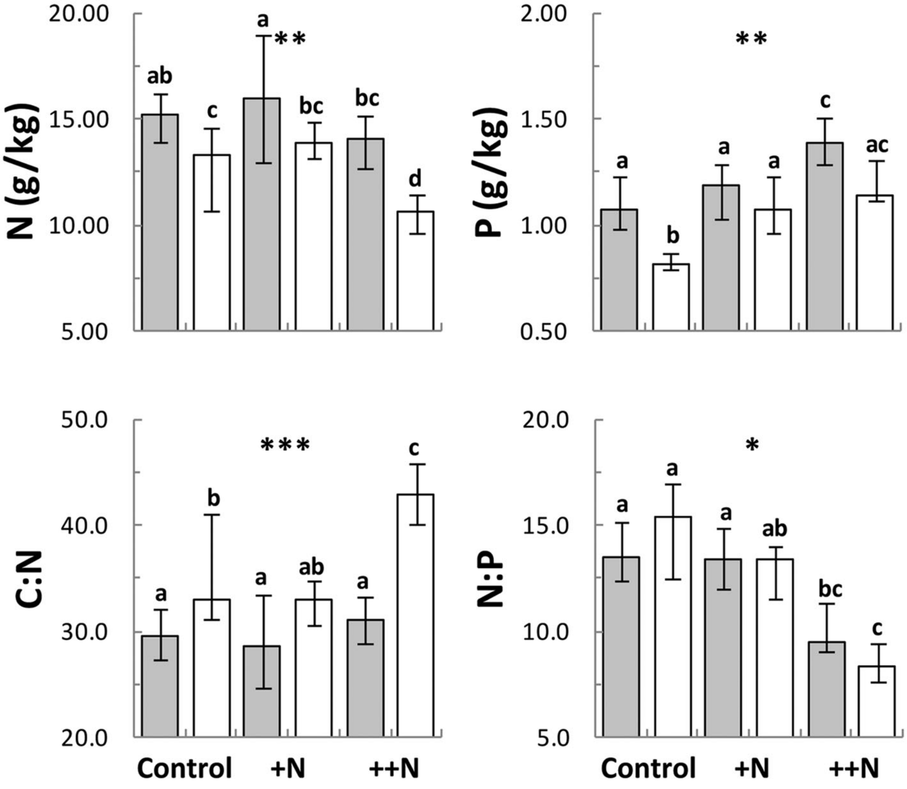

Figure 4. Concentrations of nitrogen (N) and phosphorous (P), and nitrogen/phosphorous (N:P) and carbon/nitrogen (C:N) ratios in leaves from the mangrove study sites in November 2016. Data from non-N-enriched (Control) mangroves, and those subjected to medium (+N) and high (++N) N inputs. Gray bars indicate fringe mangroves, and white bars indicate basin mangroves. Median and first and third quartiles values are presented. $^{***}P \leq 0.001$, $^{**}P \leq 0.01$, $^{*}P \leq 0.05$ (one-way ANOVA *F* test followed by post hoc Tukey HSD test, discounting the effects of spatial autocorrelation as described by Eisenlohr (2014) with improvements). Different letters indicate significant statistical difference between study sites (N = 27 for N concentrations and C:N ratios, and N = 9–19 for P concentrations and N:P ratios for each study site).

between fringe and basin sites. Leaf N:P ratios were similar between *R. mangle* and *A. schaueriana*, but higher in these species than the N:P ratios for *L. racemosa*. Leaf N:P ratios of *R. mangle* were about 2 times higher at Control and +N sites relative to the ++N fringe site, and higher at Control than +N basin, $F(4,31) = 4.74$, $P = 0.006$ (data not shown). Leaf N:P ratios of *R. mangle* were higher at basin than fringe in the Control site, while higher at fringe than basin in the +N site, $F(4,31) = 4.74$, $P = 0.006$ (data not shown). Leaf N:P ratios of *L. racemosa* were similar among study sites, $F(5,33) = 1.15$, $P = 0.356$ (data not shown).

C:N Ratios in the Sediment–Plant–Leaf Litter System

Leaf C:N ratios were higher at ++N than Control and +N basin sites, but similar between fringe sites (Figure 4). Leaf C:N ratios were also higher at basin than fringe in Control and ++N sites, but similar at the +N site. Leaf C:N ratios were up to 2 times higher in *L. racemosa* than in *R. mangle* and *A. schaueriana*, and 2 times higher in *R. mangle* than in *A. schaueriana* (Supplemental Table 2). Leaf C:N ratios of *L. racemosa* were similar between fringe sites, and between basin sites ($P > 0.05$) (data not shown). Leaf C:N ratios of *L. racemosa* were also similar between fringe and basin at Control and +N sites, but higher at ++N basin than fringe, $F(5,53) = 3.08$, $P = 0.019$ (data not shown). Leaf C:N ratios of *Rhizophora mangle* were similar among study sites, $F(4,76) = 1.03$, $P = 0.413$ (data not shown).

Leaf litter C:N ratios were similar among study sites, with median (first and third quartiles) values of 61 (54 and 67), $F(5,47) = 0.41$, $P = 0.831$.

Total N concentrations in sediment, 2.0 (0.8 and 3.1) mg/kg, were similar among study sites, $F(5,44) = 0.77$, $P = 0.575$. Sediment C:N ratios were higher at Control than +N and ++N fringe sites, but similar between basin sites, 27 (22 and 30), $F(5,43) = 3.96$, $P = 0.013$. Sediment C:N ratios at fringe sites were 26 (23 and 28) in Control, 22 (21 and 22) in +N, 19 (16 and 21) in ++N. Sediment C:N ratios were also about 2 times higher at basin than fringe sites in +N and ++N, but similar in Control ($P > 0.05$).

DISCUSSION

Collectively, our results indicate that excess N stemming from anthropogenic activities intensified N losses to the atmosphere from mangrove sediment. However, biological N fixation, net ammonification and nitrification rates, and N pools in sediment and vegetation were not affected by N enrichment, likely because excess N was quickly lost from the system (Figure 5). The results further suggest that a large proportion of excess N was mainly lost as nitrous oxides and N_2 via direct rather than coupled denitrification, and by NH_3 volatilization. Considering the current and future scenarios of N enrichment via water pollution and N deposition from the atmosphere, increased N losses would suggest that mangroves will become a larger source of N_2O to the atmosphere.

The $\delta^{15}N$ in estuarine water suggested that the excess N originating from human activities did affect mangrove areas at the ++N and +N sites. The high $\delta^{15}N$ of NO_3^- in the Valo Grande canal and ++N showed the isotopic signal of excess NO_3^- from sewage and agricultural sources in the upstream watershed reaching mangrove areas in the Iguape region. Despite the fact that inorganic N fertilizers have lower $\delta^{15}N$ signatures than organic matter, excess N inputs from both sources, ^{15}N-enriched NH_4^+ and NO_3^- pools, led to supporting the idea of intensified N dynamics and N losses to the atmosphere in the upstream watershed (Fry and others 2003). The negative values of $\delta^{15}N$ of NH_4^+ at the ++N site, however, suggested that a predominance of NH_4^+ in the estuarine water may have originated from BNF and ammonification in sediments in the Iguape region. These results also suggested that NH_3 volatilization and nitrification of these locally originated NH_4^+ pools were relatively less important N cycling pathways in the estuarine water. In contrast, the data suggested that denitrification of NO_3^- from N pollution sources was an important pathway of N loss to the atmosphere from estuarine water in the Iguape region. Because the estuary in the Cananeia region is influenced only by water springs located within the Cananeia municipality, the high $\delta^{15}N$ of NH_4^+ at the +N site indicated inputs of NH_4^+ from local sewage sources. Thus, the results suggest that both sewage and inorganic N fertilizers from the upstream watershed and local sources could be contributing to the excess N affecting mangrove areas in the ELC of Cananeia–Iguape.

The higher $\delta^{15}N$ in the sediment–plant system at the ++N and +N sites relative to Control fringe sites also underscored the likelihood of excess N inputs from human activities reaching the ++N and +N sites and affecting the N cycle there. Nitrogen sources from both sewage and agriculture in the upstream watershed can result in high leaf $\delta^{15}N$ values in mangroves (Costanzo and others 2003; Gritcan and others 2016). Previous studies consis-

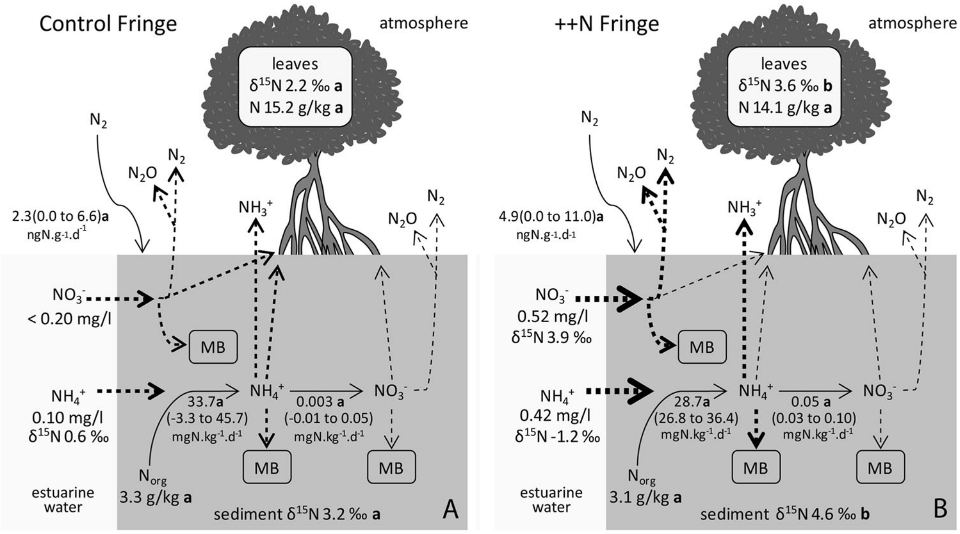

Figure 5. Schematic view of nitrogen cycling at the non-N-enriched Control and ++N fringe sites. *MB*, microbial biomass. Median and first and third quartiles are presented. Different letters indicate significant statistical difference between study sites. Solid arrows indicate rates directly measured in the present study, and dashed arrows indicate rates and fluxes indirectly inferred by δ^{15}N results. The arrows' width indicates the intensity of N transformation and flux rates in the studied sites.

tently reported higher leaf δ^{15}N values from N-enriched relative to non-N-enriched mangroves (Table 4), except for studies that performed N fertilization experiments with direct addition of urea, which is less enriched in the ^{15}N isotope (that is, Mckee and others 2002; Fogel and others 2008). The high δ^{15}N values in the sediment–plant system from ++N and +N fringe sites not only showed excess N inputs from human activities to mangroves, but also suggested intensified N losses to the atmosphere from these systems as a consequence of the excess N input (for example, Costanzo and others 2004; Fry and Cormier 2011; Reef and others 2014). Because the δ^{15}N does not change when a N pool is divided but not transformed (Robinson 2001), such as in N exchange with tidal waters or leaching, the higher δ^{15}N at N-enriched sites indicated higher N losses to the atmosphere rather than losses to the aquatic system. In addition, despite the loamy sediment texture and higher organic matter concentrations in sediment at N-enriched sites, total N concentrations and net ammonification and nitrification rates in sediment were similar between N-enriched and non-N-enriched sites. This suggests that the differences in N cycling between the N-enriched and Control fringe sites reflected differences in inorganic N inputs and N losses to the atmosphere rather than potential differences in organic N inputs and organic matter decomposition rates between these sites (reviewed by Craine and others 2015a).

Interestingly, the concentrations of NH_4^+ in sediment were similar between N-enriched and non-N-enriched sites. This indicated that the excess NH_4^+ reaching N-enriched sites was lost, or possibly absorbed by mangrove plants, immobilized in the microbial biomass, and/or converted to other forms of N (Figure 5). Although we did not measure total N content in vegetation, considering the lower aboveground biomass and the dominance by *L. racemosa* at ++N sites, which had the lowest foliar N concentrations, a lower total N content would be expected at N-enriched relative to non-N-enriched sites. This suggests that aboveground vegetation was not a large sink for the excess N. We also were not able to measure microbial biomass N; however, it is recognized as an important fate of dissolved N in mangroves (for example, Alongi and others 1993; Rivera-Monroy and Twilley 1996; Reis and others 2017a), and therefore likely an important fate of excess N in N-enriched sites. Nevertheless, high δ^{15}N values in the sediment–plant system at N-enriched fringe sites indicate higher N losses to the atmosphere. Because sediment net nitrification rates were similar between N-enriched and non-N-enriched sites, higher losses of NH_4^+ via coupled nitrification–denitrification at N-enriched sites were less likely. Therefore, ammonia (NH_3) volatilization may be a particularly important pathway of N loss to the atmosphere, especially considering the pH range (around 6.8, up to 8.6) recorded in the studied sites. This pathway would

Table 4. Natural Abundance of $\delta^{15}N$ (‰) in the Sediment–Plant System from Mangroves

Sample	Species	Non-N-enriched				N-enriched			*P*-value	N source	Location	References
		Fringe	Basin/dwarf	NR	*P*-value	Fringe	Basin/dwarf	NR				
Sediment	*Rhizophora mangle*	0.1	− 0.5[d]		*						Twin Cays, Belize	Mckee and others (2002)[a]
	–	3.7				5.2			**	Shrimp farms	Thailand	Thimdee and others (2002)[b]
	Multispecies			3.5–4.4				4.9–5.6		Sewage	Santa Catarina, Brazil	Tognella and others (2016)[e]
	Multispecies	3.6	0.7		****						Cardoso Island, Brazil	Reis and others (2017b)[f]
Leaves	*R. mangle*	2	− 5 to 2[d]			7–10	− 3 to − 1[d]			Agriculture	Florida, USA	Fry and others (2000)
	Avicennia marina			1.6 to 2.2				3.9–12.2		Sewage	Moreton Bay, Australia	Costanzo and others (2001)
	Avicennia schaueriana	4.68 to 6.10	4.95[d]								Pará, Brazil	Medina and others (2001)
	R. mangle	0.1	− 5.4[d]			− 3.4	− 8.1[d]			Urea	Twin Cays, Belize	Mckee and others (2002)[a]
	Multispecies	3.7				5.2			**	Shrimp farms	Thailand	Thimdee and others (2002)[b]
	Laguncularia racemosa	1.5	1.2			11.4–12.3				Bird guano	Belize	Wooller and others (2003a)
	R. mangle	0	− 10[d]		***						Twin Cays, Belize	Wooller and others (2003b)
	Rhizophora stylosa	2.4–4.4				5.1–5.6			*	Shrimp farms	Hinchinbrook channel, Australia	Costanzo and others (2004)
	R. mangle	− 0.6	− 6.8[d]		***						Twin Cays, Belize	Fogel and others (2008)

Table 4. continued

Sample	Species	Non-N-enriched				N-enriched			*P*-value	N source	Location	References
		Fringe	Basin/dwarf	NR	*P*-value	Fringe	Basin/dwarf	NR				
	R. mangle	0.2–0.8	− 7.3 to − 3.2[d]			− 4.1 to − 1	− 9.7 to − 4.8[d]		**	Urea	Twin Cays, Belize	Fogel and others (2008)
	R. mangle	0.2	− 11.1 to − 5.5[d]								Ceiba, Puerto Rico	Medina and others (2010)
	R. mangle			2.6				4.7–11.6	*	Watershed	Hawaii, USA	Fry and Cormier (2011)
Multispecies	− 1 to 2				0–4			*	Bat guano	Lizard Island, Australia	Reef and others (2014)[c]	
	A. marina	1.6–4				5			*	Kangaroo feces	Mangrove Bay, Australia	Reef and others (2014)[c]
	R. mangle	9				15			**	Bird guano	Celestun, Mexico	Adame and others (2015)
	Multispecies	3.6	0.9		****						Cardoso Island, Brazil	Reis and others (2017b)[f]
Leaf litter	*R. mangle*	0.1	− 3.3[d]		**						Twin Cays, Belize	Wooller and others (2003b)
	Multispecies	3	0.2		****						Cardoso Island, Brazil	Reis and others (2017b)[f]

Mean or median values or ranges (depending on what was provided in the published document) are presented. NR = mangrove type not reported.

*P-values reported by authors, ****$P \leq 0.0001$; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$. Data published as figures were extracted using the software Plot Digitizer 2.6.3 (Huwaldt and Steinhorst 2013).*

[a] *Data for sediment at 0–20 cm depth.*

[b] *Data for sediment at 0–5 cm depth, and for Rhizophora apiculata, Rhizophora mucronata, Ceriops decandra, Bruguiera gymnorrhiza, Xylocarpus moluccensis, and Avicennia alba pooled together.*

[c] *Data for R. stylosa, Ceriops tagal, and Lumnitzera rosea pooled together.*

[d] *Data for dwarf mangrove subtype.*

[e] *Data for sediment at 0–50 cm depth.*

[f] *Data for sediment at 0–10 cm depth, and for R. mangle, L. racemosa, and Avicennia schaueriana pooled together. Modified by permission from Springer Nature: Plant and Soil (Reis and others 2017a) © 2016.*

help explain the ^{15}N-enriched N pools in the sediment–plant system of N-enriched fringe sites. Ammonia volatilization rates were previously reported for mangrove sediment as a function of NH_4^+ availability, with rates up to about 2 mgN g^{-1} d^{-1} with N fertilization (Fogel and others 2008). Different from what we found for fringe mangroves, the differences in δ^{15}N between Control and N-enriched basin sites were inconclusive. Further investigation is required to clarify the potential effects of excess N input on sediment N dynamics in basin mangroves.

Nitrate concentrations in sediment were similar or lower at N-enriched than non-N-enriched sites. Because N uptake by plants did not seem to be higher at N-enriched sites, at least aboveground as discussed above, both NO_3^- immobilization in microbial biomass and denitrification were likely central sinks of excess NO_3^- in nitrate-enriched sites (Figure 5). Redox potentials were typically moderately reducing, which is favorable for denitrification in waterlogged conditions (Patrick and Mahapatra 1968; Reef and others 2010). Because net nitrification rates were similar between study sites, excess NO_3^- may have mainly been lost to the atmosphere via direct denitrification supported by NO_3^- that diffuses from tides into the sediment, rather than coupled denitrification supplied with NO_3^- from nitrification. Direct denitrification was previously reported as the most important denitrification pathway in mangrove sediments (Reis and others 2017a; Rivera-Monroy and Twilley 1996). In addition, previous studies consistently found increased N_2O fluxes from N-enriched relative to non-N-enriched mangroves (for example, Chen and others 2010; Fernandes and others 2010; reviewed by Reis and others 2017a). Therefore, direct incomplete denitrification was likely an important pathway of N loss to the atmosphere at nitrate-enriched fringe sites, quickly removing excess NO_3^- from the system as N_2O, and leading to ^{15}N-enriched NO_3^- pools in the sediment. The N_2O flux rates per unit area reported for non-N-enriched mangroves (0.01–20 mgN m^{-2} d^{-1}) greatly overlap those reported for terrestrial tropical forests (0.02–13.7 mgN m^{-2} d^{-1}; Silver and others 2000; Kiese and others 2003; Fang and others 2015). Furthermore, the rates reported for N-enriched mangroves can be even higher (up to 37 mgN m^{-2} d^{-1}) (reviewed by Reis and others 2017a) than those reported for N-enriched terrestrial tropical forest soils (up to 12 mgN m^{-2} d^{-1}) (for example, Keller and others 1993). Thus, mangroves could represent a significant and increasing source of N_2O to the atmosphere.

Biological N fixation rates in mangrove sediment were previously reported to be reduced up to about 50–75% with long-term N amendment (Romero and others 2012; Whigham and others 2009). In contrast to these results, we found BNF rates in sediment and leaf litter to be similar between N-enriched and non-N-enriched sites. The lack of BNF reduction in the face of excess N reaching mangrove sites in the ++N and +N sites supports a framework of N being relatively quickly lost from the system, such that acquiring N from BNF remains advantageous for diazotrophs (Figure 5). Recent findings also indicate that BNF rates in sediment at highly N-enriched mangroves can be similar to or even higher than at non-N-enriched mangroves (Ray and others 2014; Shiau and others 2017). The BNF rates reported for mangrove sediment in the present study are within the lower range of rates based on the acetylene reduction technique reported for both non-N-enriched (0–69 mgN m^2 d^{-1}) and N-enriched mangroves (0–8 mgN m^2 d^{-1}). The BNF rates reported for leaf litter are also within the lower range of rates reported for non-N-enriched mangroves (0–6 mgN g^{-1} d^{-1}), based on the same technique (reviewed by Reis and others 2017a). Our sampling effort was higher than previous studies quantifying BNF rates in mangroves (usually 3 samples per treatment, for example, Alongi and others 2002; Lee and Joye 2006). However, it was probably not large enough to capture clear patterns on N fixation given the large intrinsic spatial and temporal variability of N fixation rates. More studies considering larger sets of samples are needed to confirm these patterns.

In addition to differences in N cycling along the gradient of anthropogenic N inputs, the δ^{15}N in the sediment–plant–leaf litter system was consistently higher at fringe than basin sites, in both non-N-enriched and N-enriched areas. Previous studies have also reported higher δ^{15}N values at fringe sites relative to values in basin or dwarf sites in the sediment–plant–leaf litter system in non-N-enriched areas, and for leaves in N-enriched areas (Table 4). The results presented here add to this by indicating higher N availability at fringe than basin sites from either higher N inputs or higher N mineralization rates in sediment. Because biological N fixation and net ammonification rates were similar between fringe and basin sites in this study, higher inorganic N inputs from tidal waters was likely the main factor contributing to the higher N availability at fringe compared to basin sites. Indeed, fringe forests have been previously reported as sinks for dissolved inorganic N from both tidal creeks and basin mangroves via tidal water exchange (Rivera-Monroy and others 1995a, b). The sum of exchangeable bases was also higher at fringe than basin sites likely as a consequence of higher inundation frequency and nutrient inputs from tidal waters.

The higher δ^{15}N in the sediment–plant–leaf litter system at fringe sites also indicated intensified N losses to the atmosphere in these systems compared to basin mangroves (Reis and others 2017a, b). Because of higher aboveground biomass and N concentrations in leaves at fringe than basin sites, vegetation may be a more important fate of the higher N input from tidal waters at fringe sites. In turn, the different N dynamics in the fringe sites may help sustain a larger mangrove aboveground biomass. Because of similar NH_4^+ and NO_3^- concentrations and net nitrification rates in the sediment of fringe and basin sites, the higher N input at fringe sites may also be immobilized in microbial biomass in sediment and/or lost to the atmosphere. The results suggested that NH_3 volatilization and direct denitrification were important pathways of N loss to the atmosphere at fringe sites, resulting in ^{15}N-enriched NH_4^+ and NO_3^- pools in the sediment of these systems compared to basin mangroves. Previous studies also reported similar net nitrification rates (Reis and others 2017b) and higher direct denitrification rates at fringe than basin sites (Rivera-Monroy and others 1995a, b). As a result of intensified N losses to the atmosphere, fringe mangroves may represent a larger source of N_2O to the atmosphere than basin mangroves.

Despite similar available P concentrations in sediment among study sites, leaf P concentrations were higher at ++N than Control sites, which characterized the excess P input at ++N sites originating from human activities. Because of the higher P availability at ++N sites, the lower aboveground biomass at these sites was not likely a result of excess N input, which could increase P limitation. Concentrations of available P and of NH_4^+ and NO_3^- in mangrove sediment were not good indicators of the excess P and N reaching mangroves in the ++N and +N sites. Nutrient concentrations in sediments represent the net balance of several gross processes, as discussed above, and therefore may be less sensitive indicators of long-term nutrient status. Integrated indicators such as mangrove δ^{15}N signature and leaf nutrient concentrations may lend more insight into mangrove nutrient status than indicators that integrate over shorter timescales, particularly for highly dynamic processes such as many components of the N cycle (for example, Wolters and others 2016; see Reis and others 2017b).

Mangrove ecosystems in the Control and +N sites were dominated by *R. mangle*, while mangrove systems in ++N sites were dominated by *L. racemosa*. To our knowledge, there is no record of mangrove species dominance in the Iguape region before the Valo Grande opening. However, *L. racemosa* was reported to outcompete *R. mangle* during the seedling stage at high nutrient availability (McKee 1995) and to dominate mangrove stands under low salinity (Estrada and others 2013). The diversion of freshwater into the estuary dramatically reduced sediment porewater salinity in the Iguape region, with values ranging from 0 to 4 psu, while porewater salinity ranges from 20 to 28 psu in the Cananeia region (Cunha-Lignon and others 2015). Whether the Valo Grande opening promoted *L. racemosa* dominance in mangrove stands in the Iguape region is unknown, but it likely contributes to the maintenance of this species dominance in this region.

The diversion of upstream watershed waters into the estuary in the Iguape region not only affects N dynamics and losses to the atmosphere and P availability in mangrove sediment, but also promotes the invasion by aquatic macrophytes in mangrove areas (Cunha-Lignon and others 2011). In the present study, we selected mangrove sites without a marked presence of aquatic macrophytes and avoided collecting data and samples in spots colonized by them. However, there are mangrove areas densely invaded by aquatic macrophytes in the Iguape region. The interactive effects of N enrichment and invasion by aquatic macrophytes on mangrove N dynamics were evaluated in a separate study (Reis and others, unpublished data), and the study of their consequences for C dynamics and storage in mangrove sediment is underway.

CONCLUSIONS

The results of this study indicate that the excess N related to anthropogenic inputs intensified N losses to the atmosphere from mangrove sediment, as indicated by the higher δ^{15}N values in the sediment–plant system of N-enriched relative to non-N-enriched fringe mangroves. Moreover, N pools in sediment and vegetation, biological N fixation, and net ammonification and nitrification rates were not affected by N enrichment, which suggests excess N may be quickly lost from the system. The results also suggest that excess N may be mainly lost as nitrous oxides and N_2 via direct rather than coupled denitrification, and by NH_3 volatilization. Fringe mangroves had higher N inputs from tidal waters and intensified N losses to the atmosphere, compared to basin mangroves in both N-enriched and non-N-enriched areas. Considering the current and future N pollution scenarios via water pollution and N deposition from the atmosphere, mangroves could become a larger source of N_2O to the atmosphere as fringe mangroves continue to cycle higher inputs of N. Further investigation is required to clarify sediment N dynamics responses to excess N inputs in basin mangroves and to quantify the multiple pools and fluxes that make up the mangrove N cycle. More studies on N pollution effects on climate feedbacks of mangrove ecosystems are also needed, especially considering a range of temperature regimes as higher temperatures could interact with excess N to affect N_2O efflux rates. The use of ^{15}N labeling techniques to trace N dynamics in sediment, and also the abundance of functional genes related to N dynamics and N losses to the atmosphere, which has been shown to be a powerful way to assess dynamic N cycling processes and responses to change (Reed and others 2010), are promising approaches to provide a detailed description of N pollution effects on N dynamics and N_2O emissions from mangrove ecosystems.

ACKNOWLEDGMENTS

CRGR was supported by the Rufford Foundation (Ref. 20243-1), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Processes 1422671 and 88881.132767/2016-01), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process 141069/2016-3), and Idea Wild. SCR was supported by the US Geological Survey. We would like to thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio—Brazil) and the Instituto Florestal (Secretaria do Meio Ambiente, São Paulo, Brazil) for the permission to conduct this research in the Cananeia-Iguape-Peruibe Protection Area (Process 47365) and Cardoso Island State Park (Process 260108-012.547/2014), respectively. The authors are also thankful to Pedro Eisenlohr for providing statistical assistance, to Marília Cunha-Lignon for providing the shapefiles of mangrove forests area, and to João Souza for providing the map of the study area. We also thank Ariel Lugo, Maga Gei, and anonymous reviewers whose suggestions significantly improved the manuscript. The authors declare that they have no conflict of interest. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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