UNIVERSIDADE DE BRASÍLIA
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Carla Roberta Gonçalves Reis

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Tese apresentada ao Programa de Pós-Graduação em Ecologia da Universidade de Brasília, para a obtenção do Título de Doutora em Ecologia.

Orientadora: Profa. Dra. Gabriela Bielefeld Nardoto

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Dedico a meus pais,
Tereza e Rubens

"*One, remember to look up at the stars and not down at your feet. Two, never give up work. Work gives you meaning and purpose and life is empty without it. Three, if you are lucky enough to find love, remember it is there and don't throw it away.*" [1]

Stephen Hawking

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[1] Conselho de Stephen Hawking para seus filhos, em entrevista à Diane Sawyer para a ABC *News* em Junho de 2010

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O meu muito obrigada

Resumo

Escopo e objetivos

Manguezais provem importantes bens e serviços ecossistêmicos à sociedade e embora o enriquecimento de nitrogênio (N) possa ter grandes consequências para a provisão de bens e serviços por esses sistemas, nosso conhecimento sobre os efeitos do enriquecimento de N para a ciclagem de N em ecossistemas de manguezal ainda é limitado. Para contribuir com o conhecimento sobre os efeitos do enriquecimento de N para a ciclagem N em manguezais, nós compilamos e analisamos um banco de dados secundários em escala global e conduzimos dois estudos de caso em áreas de manguezal no Complexo Estuarino-Lagunar (CEL) de Cananeia-Iguape, São Paulo. O banco de dados incluiu taxas de transformação e de fluxos de N (fixação biológica de N, amonificação, nitrificação, denitrificação e volatilização), fluxos de N dissolvido na interface sedimento-água e a abundância natural dos isótopos estáveis do N (δ^{15}N) no sistema sedimento-planta em manguezais não enriquecidos e enriquecidos por N. Os resultados obtidos foram comparados com dados de florestas tropicais terrestres relatados na literatura por serem os ecossistemas mais produtivos e com maiores taxas de ciclagem de N em todo o mundo. Em um dos estudos de caso no CEL de Cananeia-Iguape, nós avaliamos a hipótese de que o enriquecimento de N diminui as taxas de fixação biológica de N (FBN), mas intensifica a dinâmica e as perdas de N para a atmosfera em ecossistemas de manguezal. Para testar essa hipótese, nós avaliamos a ciclagem de N em manguezais de franja e de bacia não enriquecidos e enriquecidos por N através das concentrações de N no sedimento e na vegetação, taxas de FBN no sedimento e na serapilheira, taxas líquidas de amonificação e nitrificação no sedimento e o δ^{15}N no sistema sedimento-planta-serapilheira e na água do estuário, que integram a dinâmica e as perdas de N para a atmosfera no sistema. No segundo estudo de caso no CEL de Cananeia-Iguape, nós avaliamos o uso do δ^{15}N e de concentrações de N nas folhas como indicadores para o monitoramento da ciclagem de N em manguezais. Para tal, nós avaliamos as concentrações de N no sedimento e na vegetação e o δ^{15}N foliar em parcelas permanentes de bosque de franja que diferem em dominância de espécies, estágio de sucessão ecológica e desenvolvimento estrutural, tanto em manguezais conservados não enriquecidos por N como em manguezais sujeitos ao enriquecimento de N e manguezais invadidos por macrófitas aquáticas.

Conclusões

Manguezais e florestas tropicais terrestres exibem intervalos semelhantes de taxas de FBN, denitrificação e de emissões de óxido nitroso (N_2O) para a atmosfera. Em função das elevadas taxas de transformação e de perdas de N para a atmosfera que frequentemente exibem, manguezais desempenham um importante papel na ciclagem de N em sistemas costeiros de regiões tropicais e subtropicais em todo o mundo. Os principais fatores limitantes das taxas de transformação e de fluxos de N no sedimento de manguezais são a disponibilidade de N inorgânico dissolvido e a sua imobilização na biomassa microbiana. Manguezais são altamente eficientes em utilizar formas de N inorgânico dissolvido da água das marés, relacionado a uma rápida absorção pela vegetação de mangue e a uma eficiente imobilização de N no sedimento pela atividade microbiana. No entanto, em manguezais sujeitos ao enriquecimento de N, o excesso de N pode ser principalmente perdido para a atmosfera ao invés de assimilado pela vegetação de mangue ou conservado no sedimento. Como consequência, o excesso de N pode não afetar a FBN, mas intensifica as perdas de N para a atmosfera em manguezais, principalmente as perdas de óxidos de N via denitrificação direta incompleta quando comparado às perdas de N atmosférico (N_2) via denitrificação completa acoplada. Considerando os cenários atuais e futuros de enriquecimento de N via poluição da água e deposição atmosférica, manguezais podem se tornar uma maior fonte de N_2O para a atmosfera. Em adição aos efeitos do enriquecimento de N, as macrófitas aquáticas invasoras intensificaram a dinâmica e as perdas de N para a atmosfera nos manguezais invadidos com a introdução de matéria orgânica com elevadas concentrações de N no sedimento. Apesar da grande variabilidade estrutural de manguezais de franja, o $\delta^{15}N$ foliar é um indicador consistente de alterações na ciclagem de N decorrentes do enriquecimento de N e invasão por macrófitas aquáticas em ecossistemas de manguezal.

Palavras-chave: amonificação, denitrificação, fixação biológica de nitrogênio, fluxos de nitrogênio na interface sedimento-água, invasão biológica, isótopos estáveis do nitrogênio, nitrificação, óxido nitroso

Abstract

Background and goals

Mangrove ecosystems provide critical societal goods and services, and although nitrogen (N) enrichment can have large effects on their provision, our understanding of excess N input effects on N cycling in mangrove ecosystems remains quite limited. To advance our understanding of how N enrichment affect N cycling in mangrove ecosystems, we compiled and evaluated literature data on N transformation and flux rates (biological N fixation, ammonification, nitrification, denitrification, and volatilization), dissolved N (DN) fluxes across the sediment-water interface, and the natural abundance of N stable isotopes ($\delta^{15}N$) in the sediment-plant system in non-N-enriched and N-enriched mangroves at the global scale. Data of tropical terrestrial forests were presented for comparative purposes since they are well recognized as being the most productive forested ecosystems on Earth, with the highest rates of N cycling. We also evaluated the hypothesis that excess N input decreases biological N fixation (BNF), but intensifies N dynamics and losses to the atmosphere from mangrove ecosystems in a study case in the Estuarine Lagunar-Complex (ELC) of Cananeia-Iguape, southeastern Brazil. To test this hypothesis, we evaluated N concentrations in sediment and vegetation, rates of BNF in sediment and leaf litter, net ammonification and nitrification rates in sediment, and the overall N dynamics and losses to the atmosphere using the $\delta^{15}N$ in the sediment-plant-leaf litter system and estuarine water at non-N-enriched and N-enriched fringe and basin mangroves. In another study in the ELC of Cananeia-Iguape, we evaluated the applicability of leaf $\delta^{15}N$ and N concentrations as indicators of N cycling in mangrove ecosystems for monitoring purposes. We evaluated N concentrations in sediment and vegetation and leaf $\delta^{15}N$ at permanent study plots of fringe forests differing in species dominance, stage of ecological succession and structural development in non-N-enriched and N-enriched mangroves, and mangroves invaded by aquatic macrophytes.

Conclusions

Mangroves and terrestrial tropical forests exhibit a great overlap in rates of BNF and denitrification and nitrous oxide (N_2O) flux rates. Because of the high rates of N transformation and fluxes to the atmosphere that mangroves often exhibit, mangrove ecosystems play a substantial role in N cycling in coastal areas of tropical and subtropical regions worldwide. The main factors limiting N transformation rates in mangrove sediment are inorganic DN availability and microbial immobilization. Mangroves are highly efficient users of DN forms from tidal waters, related to a rapid plant uptake and an efficient immobilization of DN in sediment by microbial activity. However, under N enrichment, excess N input can be mainly lost to the atmosphere rather than assimilated by mangrove vegetation or conserved in sediment. As a consequence, excess N input can not affect BNF, but intensifies N losses to the atmosphere from mangrove ecosystems, likely including N oxides losses via incomplete direct denitrification, rather than atmospheric N (N_2) losses via complete coupled denitrification. Considering current and future scenarios of N enrichment via water pollution and N deposition from the atmosphere, mangroves can become a larger source of N_2O to the atmosphere. In addition to the excess N input, invasive aquatic macrophytes further intensified N dynamics and losses to the atmosphere by introducing organic matter of high N concentrations into mangrove sediment. Despite of the large variability on vegetation structure of fringe forests, leaf $\delta^{15}N$ is a consistent indicator of alterations in N cycling following N pollution and biological invasions in mangrove ecosystems.

Keywords: ammonification, biological invasion, biological nitrogen fixation, denitrification, nitrification, nitrogen stable isotopes, nitrous oxide, nitrogen sediment-water interface fluxes

Sumário

1. Introdução Geral

A disponibilidade de nitrogênio (N) é um dos principais fatores reguladores da estrutura e do funcionamento de ecossistemas terrestres e aquáticos (Vitousek and Howarth 1991; Elser et al. 2007). A fixação biológica de N (FBN), que é o principal processo em que o N entra nos ecossistemas naturais, possui um elevado custo energético de maneira que a maioria dos ecossistemas são limitados ou colimitados pela disponibilidade desse nutriente (Chapin III et al. 2011). A conversão de N atmosférico em amônia para a produção de fertilizantes tem aumentado involuntariamente a disponibilidade de N nos ecossistemas naturais. Uma grande proporção do N aplicado na agricultura é perdida para a atmosfera e sistemas aquáticos (Erisman et al. 2008, 2013), enquanto que o esgoto quando coletado, não é eficientemente tratado para a redução do excesso de N (e.g., Mulder 2003). A maior entrada de N nos ecossistemas naturais via poluição de sistemas aquáticos e deposição atmosférica pode ter várias consequências para a ciclagem de N nesses sistemas como a diminuição nas entradas de N via FBN (Reed et al. 2011; Niu et al. 2016), alterações na dinâmica e em perdas de N para a atmosfera (Erisman et al. 2013; Niu et al. 2016) e alterações em *feedbacks* climáticos com o aumento das emissões de óxido nitroso (N_2O) (Erisman et al. 2011, 2013). As alterações na ciclagem N podem ainda resultar em alterações de produtividade (Tian et al. 2016), favorecer invasões biológicas (e.g., Dukes and Mooney 1999; Bradley et al. 2010) e a perda de biodiversidade (e.g., Eisenhauer et al. 2012; Payne et al. 2017), resultando também em alterações na provisão de bens e serviços ecossistêmicos para a sociedade (Compton et al. 2011; de Vries et al. 2014).

A ciclagem de N em ecossistemas terrestres pode ser avaliada indiretamente através da abundância natural dos isótopos estáveis do N ($\delta^{15}N$) no sistema solo-planta. Como o $\delta^{15}N$ integra o fracionamento contra o isótopo pesado ^{15}N durante as reações de transformação de N no solo como a nitrificação, denitrificação e volatilização (Högberg 1997; Robinson 2001), o $\delta^{15}N$ no sistema solo-planta pode ser utilizado como uma medida da intensidade da dinâmica e perdas de N para a atmosfera (e.g., Houlton et al. 2006; Nardoto et al. 2008). Ecossistemas terrestres que exibem maiores valores de $\delta^{15}N$ foliar possuem maior disponibilidade de N no sistema solo-planta e tendem a ter maiores perdas de N para a atmosfera (Craine et al. 2009). Já ecossistemas terrestres que exibem maiores valores de $\delta^{15}N$ no solo possuem maiores taxas de decomposição da matéria orgânica e dinâmica de N mais intensa (Craine et al. 2015). Desta forma, o $\delta^{15}N$ foliar tem sido utilizado como um integrador da disponibilidade de N no sistema solo-planta e o $\delta^{15}N$ do solo como um integrador da dinâmica da matéria orgânica nesse compartimento. O $\delta^{15}N$ possui ainda a vantagem de integrar a dinâmica e perdas de N para a atmosfera em escalas temporais de meses (nas folhas) a décadas (no solo), sendo uma medida mais robusta da dinâmica N no sistema do que medidas que integram menores escalas de tempo, como as concentrações de N no solo (Wolters et al. 2016). O $\delta^{15}N$ também integra a assinatura isotópica das diferentes fontes de N para o sistema e pode também ser utilizado como um indicador de fontes de N oriundas de atividades humanas como fertilizantes e esgoto (e.g., Costanzo et al. 2001, 2003, 2005).

Manguezais são ecossistemas lenhosos costeiros sujeitos ao regime de marés que provem importantes bens e serviços ecossistêmicos à sociedade, incluindo o considerável sequestro de carbono (C) em áreas costeiras (Alongi 2014a); o maior estoque de C por unidade de área do que florestas terrestres e outros ecossistemas marinhos (Donato et al. 2011; Alongi 2014b); a interceptação do excesso de nutrientes na água de rios e estuários, contribuindo com a melhor qualidade da água em sistemas costeiros (e.g., Alongi and McKinnon 2005; Jordan et al. 2011); e o suporte da produção pesqueira costeira (e.g., Aburto-Oropeza et al. 2008; Carrasquilla-Henao and Juanes 2017). Apesar da importância da disponibilidade de N para a provisão de bens e serviços ecossistêmicos por esses sistemas, informações sobre os efeitos do enriquecimento de N para a dinâmica e perdas de N em ecossistemas de manguezal ainda são limitadas. Os poucos estudos disponíveis sugerem que a FBN pode ser grandemente reduzida (Whigham et al. 2009; Romero et al. 2012), enquanto que as perdas de N para a atmosfera, incluindo as emissões de N_2O, podem ser intensificadas (e.g., Corredor et al. 1999; Fernandes et al. 2010). No entanto, a amplitude das taxas relatadas por esses estudos para manguezais enriquecidos e não enriquecidos por N é grande, de maneira que nem todos os manguezais devem apresentar as mesmas respostas ao enriquecimento de N. Mais estudos sobre os efeitos do enriquecimento de N para a dinâmica e perdas de N em manguezais são necessários, especialmente considerando um maior conjunto de transformações e fluxos de N no sedimento e em diferentes tipos de manguezal.

Manguezais são classificados em bosques de franja e de bacia (Figura 1). Bosques de franja ocorrem em declives margeando estuários, canais de deltas, lagoas costeiras e rios, sob grande influência das marés. Os bosques de bacia ocorrem em depressões rasas mais distantes da margem, inundados somente durante marés de sizígia (i.e., marés de maior amplitude durante a Lua cheia e nova), elevações sazonais do nível do mar ou pulsos de água doce. Em bosques de bacia, a água se desloca lentamente mantendo condições de estagnação de água por longos períodos. A maior frequência de inundação e amplitude das marés em bosques de franja resulta em uma maior entrada de nutrientes e no maior desenvolvimento estrutural da vegetação comparado a bosques de bacia (Schaeffer-Novelli et al. 2000). Em um estudo sobre a dinâmica de N em manguezais de franja e de bacia conservados no Parque Estadual da Ilha do Cardoso (Cananeia-SP), o bosque de franja exibiu maior dinâmica e perdas de N para a atmosfera indicados indiretamente por maiores valores de $\delta^{15}N$ no sistema sedimento-planta-serapilheira comparado ao bosque de bacia (Reis et al. 2017b). Diferentes tipos de manguezal podem, portanto, exibir respostas distintas frente ao enriquecimento de N.

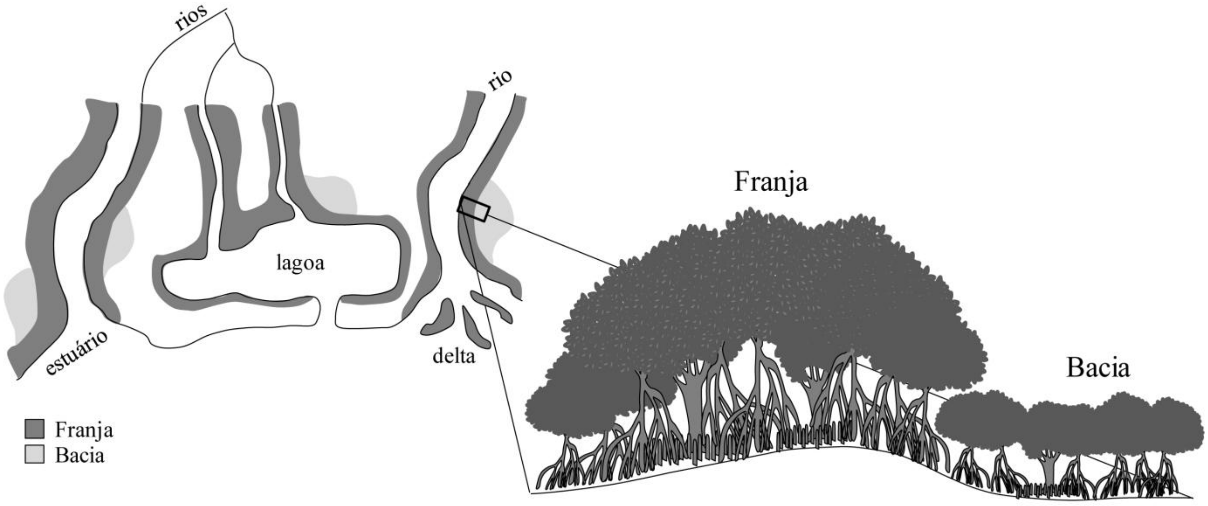

Figura 1. Áreas de ocorrência de bosques de franja e de bacia em ambientes costeiros. No detalhe, visão em perfil de diferenças estruturais e de relevo entre bosques de franja e de bacia

Para contribuir com o conhecimento sobre os efeitos do enriquecimento de N em ecossistemas de manguezal e aprimorar nossa capacidade de tomar decisões que mantenham resilientes os bens e serviços ecossistêmicos prestados por esses sistemas, no presente estudo nós avaliamos os efeitos do enriquecimento de N para a ciclagem de N em manguezais através da compilação e análise de um banco de dados secundários em escala global e estudos de caso em áreas de manguezal no Complexo Estuarino-Lagunar (CEL) de Cananeia-Iguape (Patrimônio Mundial da UNESCO e site RAMSAR), em São Paulo (Figura 2). A porção sul do complexo na região de Cananeia abriga as áreas de manguezal mais bem conservadas do Estado de São Paulo (Cunha-Lignon et al. 2011), enquanto que a porção norte na região de Iguape sofre a influência de esgoto e fertilizantes provenientes do Rio Ribeira de Iguape que forma uma das maiores bacias de drenagem na costa brasileira, com mais de meio milhão de habitantes (IBGE 2010). Apenas 64% do esgoto produzido na porção paulista da bacia do Rio Ribeira de Iguape é coletado, do qual 87% são tratados (CETESB 2014), mas não para a redução do excesso de N. A bacia do Rio Ribeira de Iguape também possui áreas agrícolas responsáveis por 80% da produção de banana no Estado de São Paulo (IBGE 2006), com aplicação de 130 a 270 kgN.ha^{-1}.ano^{-1} (Godoy et al. 2006).

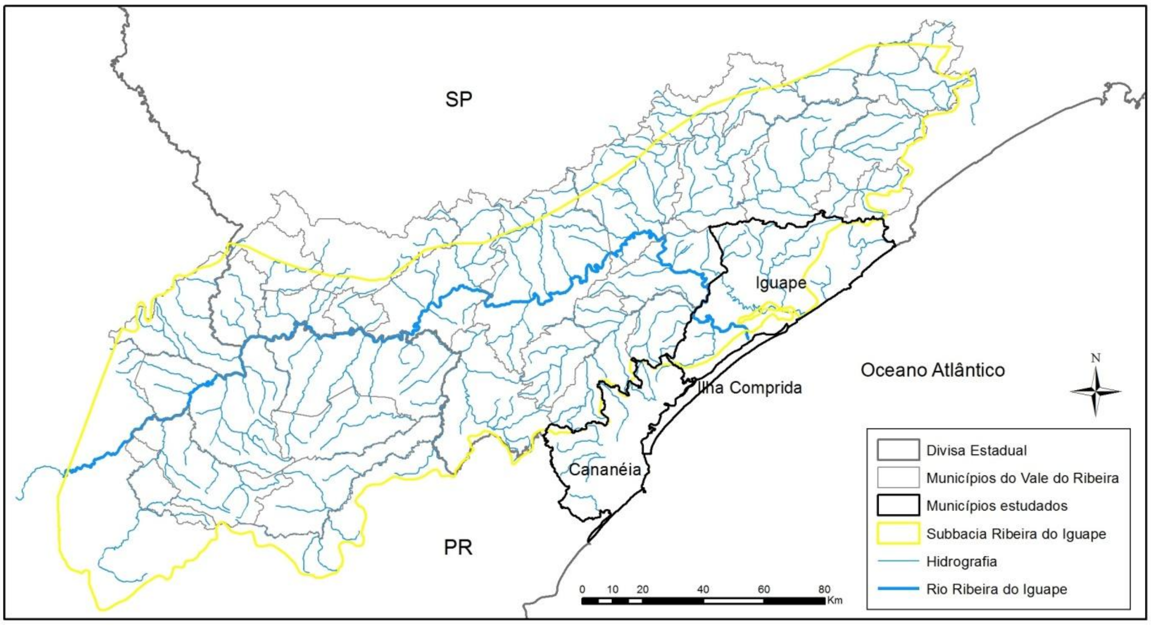

Figura 2. Localização do Complexo Esturino-Lagunar de Cananeia-Iguape, São Paulo, com destaque para a hidrografia desses municípios e a influência do Rio Ribeira de Iguape e seus afluentes na região de Iguape

2. Objetivos Gerais e Estrutura da Tese

O presente estudo teve por objetivo verificar os efeitos do enriquecimento de N para a ciclagem desse nutriente em ecossistemas de manguezal. Para atender esse objetivo, os seguintes estudos foram conduzidos:

No capítulo 1 investigamos padrões gerais, diferenças entre manguezais de franja e de bacia e alterações com o enriquecimento de N em entradas, dinâmica (i.e., transformações de N no sedimento) e perdas de N em manguezais. Esse estudo foi realizado através de uma ampla revisão da literatura associada à compilação e avaliação de um banco de dados secundários em escala global sobre a ciclagem de N em manguezais enriquecidos e não enriquecidos por N. O banco de dados incluiu taxas de FBN, amonificação, nitrificação, denitrificação e volatilização, fluxos de N dissolvido na interface sedimento-água e o δ^{15}N no sistema sedimento-planta. Os resultados obtidos foram comparados com dados de florestas tropicais terrestres relatados na literatura por serem os ecossistemas mais produtivos e com maiores taxas de ciclagem de N em todo o mundo. Além de contribuir para que os objetivos do presente estudo fossem atendidos, o capítulo 1 também teve por finalidade evidenciar lacunas no conhecimento e prioridades de investigação que foram abordadas nos capítulos 2 e 3 e prover informações que serviram de base para a interpretação e comparação dos resultados obtidos nos mesmos. O capítulo 1 foi publicado na revista *Plant and Soil* (doi: 10.1007/s11104-016-3123-7).

No capítulo 2 testamos a hipótese de que o enriquecimento de N diminui as taxas de FBN, mas intensifica a dinâmica e as perdas de N para a atmosfera em manguezais de franja e de bacia. Para testar essa hipótese, nós comparamos a ciclagem de N entre manguezais de franja não enriquecidos e enriquecidos por N, e entre manguezais de bacia não enriquecidos e enriquecidos por N no CEL de Cananeia-Iguape. Para tal, avaliamos as concentrações de N no sedimento e na vegetação, taxas de FBN no sedimento e na serapilheira, taxas líquidas de amonificação e nitrificação no sedimento e o δ^{15}N na água do estuário e no sistema sedimento-planta-serapilheira, que integram a dinâmica e as perdas de N para a atmosfera no sistema. O capítulo 2 encontra-se em processo de revisão para publicação na revista *Journal of Ecology*.

No capítulo 3 avaliamos o uso do δ^{15}N e de concentrações de N nas folhas como indicadores para o monitoramento da ciclagem de N em manguezais. Para tal, nós avaliamos as concentrações de N no sedimento e na vegetação e o δ^{15}N foliar em parcelas permanentes de bosque de franja que diferem em dominância de espécies, estágio de sucessão ecológica e desenvolvimento estrutural, tanto em manguezais conservados não enriquecidos por N como em manguezais sujeitos ao enriquecimento de N e em manguezais invadidos por macrófitas aquáticas no CEL de Cananeia-Iguape. O capítulo 3 será submetido para apreciação da revista *Conservation Physiology*.

3. Resultados e Discussão Geral

No capítulo 1, verificamos que as taxas de FBN livre em manguezais (0 a 199 $mgN.m^{-2}.d^{-1}$) e associada à raízes de plantas de mangue (2 a 10 $mgN.m^{-2}.d^{-1}$) por unidade de área ocorrem em taxas semelhantes às taxas de FBN livre (0 a 16 $mgN.m^{-2}.d^{-1}$, Reed et al. 2011) e simbiótica (2 a 17 $mgN.m^{-2}.d^{-1}$, Sylvester-Bradley et al. 1980; Cleveland et al. 1999; Reed et al. 2011; Sullivan et al. 2014) em florestas tropicais terrestres. No entanto, taxas com grande variabilidade e abaixo do limite de detecção foram frequentemente relatadas na literatura, de maneira que nem todos os manguezais devem sustentar elevadas taxas de FBN. Os dados de fluxos de N inorgânico e orgânico dissolvidos na interface sedimento-água indicaram que manguezais são altamente eficientes em importar formas de N dissolvido da água das marés, mesmo contra gradientes de concentração, o que tem sido atribuído a uma rápida absorção pelas plantas de mangue e à imobilização na biomassa microbiana (e.g., Alongi et al. 1993; Kristensen et al. 1998). No entanto, assim como para as taxas de FBN, a variabilidade em taxas de fluxo de N dissolvido também foi bastante grande, de maneira que enquanto alguns manguezais tendem a importar, outros devem principalmente exportar formas dissolvidas de N para a maré.

As taxas líquidas de amonificação (0 a 0,005 $mgN.g^{-1}.d^{-1}$) e nitrificação (0 a 0,003 $mgN.g^{-1}.d^{-1}$) por unidade de peso em manguezais também são semelhantes às taxas de líquidas de amonificação (-0,02 a 0,008 $mgN.g^{-1}.d^{-1}$) e nitrificação (-0,002 a 0,005 $mgN.g^{-1}.d^{-1}$) de florestas tropicais terrestres (Vitousek and Matson 1988; Luizao et al. 2004; Silver et al. 2005). Grandes diferenças nas magnitudes de taxas de amonificação brutas (92 a 575 $mgN.m^{-2}.d^{-1}$) e líquidas (0 a 19 $mgN.m^{-2}.d^{-1}$) no sedimento de manguezais indicaram que a imobilização de amônio (NH_4^+) na biomassa microbiana é um importante fator limitante da disponibilidade de N nesses sistemas. Grandes diferenças nas magnitudes de taxas líquidas de amonificação e nitrificação e de taxas de denitrificação (0 a 0,5 $mgN.g^{-1}.d^{-1}$) também indicaram que a denitrificação direta (i.e., que ocorre a partir do nitrato importado da maré) é uma importante via de perda de N para a atmosfera em manguezais. As taxas de denitrificação (0,2 a 308 $mgN.m^{-2}.d^{-1}$) e de fluxos de N_2O (0,01 a 20 $mgN.m^{-2}.d^{-1}$) em termos de área em manguezais também ocorrem no mesmo intervalo das taxas de denitrificação (0,5 to 8,2 $mgN.m^{-2}.d^{-1}$) e de emissões de N_2O (0,02 to 13,7 $mgN.m^{-2}.d^{-1}$) em florestas tropicais terrestres (Silver et al. 2000; Kiese et al. 2003; Fang et al. 2015). As maiores taxas de denitrificação em manguezais comparado a florestas tropicais terrestres devem em parte refletir a importância da denitrificação direta em manguezais. Assim como para as demais taxas, as amplitudes das taxas de denitrificação e de emissões de N_2O também foram grandes. Logo, nem todos os manguezais devem exibir elevadas taxas de perdas de N para a atmosfera.

Poucos estudos avaliaram possíveis diferenças na dinâmica e perdas de N para a atmosfera entre diferentes tipos de manguezal. Esses estudos sugeriram que manguezais de franja exibem maiores taxas de amonificação líquidas e de denitrificação direta, mas taxas de nitrificação líquidas similares a manguezais de bacia (Rivera-Monroy et al. 1995b; Reis et al. 2017b). Enquanto manguezais de franja tendem a importar N inorgânico dissolvido, manguezais de bacia tendem a importar N orgânico dissolvido (Rivera-Monroy et al. 1995a; Davis et al. 2001a, b). Manguezais anões (i.e., subtipo de manguezal de bacia com desenvolvimento estrutural mais restrito) exibiram maiores taxas de FBN (Sheridan 1991; Lee and Joye 2006) e de volatilização da amônia comparado a manguezais de franja (Fogel et al. 2008). O $\delta^{15}N$ no sistema sedimento-planta-serapilheira de manguezais de franja é consistentemente maior do que em manguezais de bacia e anões (e.g., Mckee et al. 2002; Reis et al. 2017b), o que indica que manguezais de franja exibem maior dinâmica e perdas de N para a atmosfera comparado aos demais tipos de manguezal.

Informações sobre os efeitos do enriquecimento de N para a dinâmica e perdas de N para a atmosfera em manguezais foram limitadas. As taxas líquidas de amonificação (4 a 86 $mgN.m^{-2}.d^{-1}$) e taxas de emissões de N_2O (0 a 37 $mgN.m^{-2}.d^{-1}$) foram maiores em manguezais sob enriquecimento de N. Não foram observadas diferenças nas taxas de FBN no sedimento (0 a 8 $mgN.m^{-2}.d^{-1}$) e de denitrificação (0 a 106 $mgN.m^{-2}.d^{-1}$) em manguezais enriquecidos e não enriquecidos por N. No entanto, estudos anteriores verificaram reduções de até 75% na FBN (Whigham et al. 2009; Romero et al. 2012) e repetidamente maiores taxas de denitrificação em manguezais enriquecidos por N (e.g., Corredor et al. 1999; Fernandes et al. 2010). A maior dinâmica de N com o enriquecimento de N não resulta necessariamente no aumento da assimilação do excesso de N pela vegetação de mangue, uma vez que a imobilização na biomassa microbiana e as perdas para a atmosfera podem ser significativas. Mesmo que a assimilação de N pela vegetação seja maior, a produtividade primária líquida (PPL) não será obrigatoriamente maior, o que também irá depender da disponibilidade de outros fatores limitantes à PPL. Por exemplo, em manguezais limitados por N, o excesso de N pode aumentar a PPL, ao menos até um limite de saturação por esse nutriente (Tian et al. 2016). Em manguezais limitados ou colimitados por fósforo (P), no entanto, o excesso de N pode limitar a PPL nesses sistemas.

No capítulo 2, o $\delta^{15}N$ na água do estuário caracterizou o excesso de N oriundo de atividades humanas chegando às áreas de manguezal sujeitos ao enriquecimento de N. Os elevados valores de $\delta^{15}N$ do nitrato (NO_3^-) no canal artificial Valo Grande (9,8‰), que conecta o Rio Ribeira de Iguape ao estuário na porção norte do CEL de Cananeia-Iguape sob grande enriquecimento de N, e nos sítios de estudo nessa região, com mediana (e amplitude) igual a 3,9 (3,5 a 4,8) ‰, comparado aos valores de $\delta^{15}N$ do NO_3^- na porção sul do CEL, 0,4 (-0,4 a 1,3) ‰, indicaram o excesso de NO_3^- oriundo de esgoto e agricultura na bacia do Rio Ribeira de Iguape chegando ao estuário na porção norte do CEL. Não foi possível quantificar o $\delta^{15}N$ do NO_3^- na água do estuário na Ilha do Cardoso (sítio controle não enriquecido por N na porção sul do CEL), pois as concentrações de NO_3^- estavam abaixo do limite de detecção. Os maiores valores de $\delta^{15}N$ do NH_4^+ na porção sul do CEL na Ilha de Cananeia, 3,6 (1,2 a 9,1) ‰, comparado ao sítio controle na Ilha do Cardoso também na porção sul do CEL, 0,6 (-0,1 a 1,7) ‰, indicaram o excesso de NH_4^+ proveniente de fontes locais de esgoto de pequenas áreas urbanas chegando aos sítios de estudo na Ilha de Cananeia. Os maiores valores de $\delta^{15}N$ no sedimento (franja: 4,6‰ e bacia: 1,8‰) e nas folhas (franja: 3,6‰ e bacia: 1,8‰) nos manguezais sob grande enriquecimento de N na porção norte do CEL, e nas folhas (franja: 4,0‰ e bacia: 1,1‰) nos manguezais sob menor enriquecimento de N na Ilha de Cananeia, comparado aos valores de $\delta^{15}N$ no sedimento (franja: 3,2‰ e bacia: 1,1‰) e nas folhas (franja: 2,2‰ e bacia: 0,5‰) no sítio controle na Ilha do Cardoso, caracterizaram as maiores perdas de N para a atmosfera nos manguezais enriquecidos do que não enriquecidos por N. O $\delta^{15}N$ nos bosques de franja foi consistentemente maior no sistema sedimento-planta-serapilheira do que nos bosques de bacia, indicando a maior dinâmica e perdas de N para a atmosfera em bosques de franja comparados a bosques de bacia, tanto em manguezais não enriquecidos como enriquecidos por N.

As concentrações de N foliar foram maiores em *A. shaueriana*, com mediana (1º e 3º quartis) igual a 23,1 (22,6 a 24,0) g/kg, do que em *R. mangle*, 14,9 (13,7 a 16,5) g/kg, e maiores nessas espécies do que em *L. racemosa*, 10,9 (10,0 a 12,7) g/kg. No entanto, as concentrações de N foliar e razões C:N nas folhas das diferentes espécies foram similares entre os sítios de estudo. Por outro lado, as concentrações de P foliar foram similares entre as espécies de mangue e maiores no bosque de franja sob maior enriquecimento de N, 1,4 (1,3 e 1,5) g/kg, comparado aos bosques de franja sob menor enriquecimento de N, 1,2 (1,0 e 1,3) g/kg, e no sítio controle, 1,1 (1,0 e 1,2) g/kg, $F(5,74) = 3.70$, $P = 0.008$. As concentrações de P foliar também foram maiores no bosque de bacia sob maior enriquecimento de N, 1,1 (1,1 e 1,3) g/kg, comparado à bacia no sítio controle, 0,8 (0,8 e 0,9) g/kg. Esses resultados caracterizaram o excesso de P proveniente de atividades humanas na bacia do Rio Ribeira de Iguape, incluindo esgoto e a produção de fertilizantes, chegando ao estuário na porção norte do CEL de Cananeia-Iguape. As concentrações de $N\text{-}NH_4^+$ no sedimento foram similares entre os sítios de estudo, 16,7 (6,3 e 32,8) mg/kg. As concentrações de $N\text{-}NO_3^-$ foram duas vezes maiores na franja do sítio controle, 0,08 (0,06 e

0,08) mg/kg, do que na franja sob grande enriquecimento de N, 0,04 (0 e 0,05) mg/kg, mas similar entre esses bosques e a franja sob menor enriquecimento de N, 0,07 (0,05 e 0,07) mg/kg. As concentrações de N-NO_3^- no sedimento foram similares entre os bosques de bacia, 0,05 (0,03 e 0,07) mg/kg, e entre os bosques de franja e de bacia. As taxas líquidas de amonificação, 5,6 (3,4 e 24,2) mgN.kg^{-1}.d^{-1}, e de nitrificação, 0,05 (0,03 a 0,09) mgN.kg^{-1}.d^{-1}, também foram similares entre os sítios de estudo. As taxas de FBN em termos de peso e área foram similares entre os sítios de estudo enriquecidos por N e sítios controle, tanto no sedimento, 0,003 (0 e 0,01) µgN.g^{-1}.d^{-1} e 0,2 (0 e 0,5) mgN.m^{-2}.d^{-1}, como na serapilheira, 4,7 (0,6 e 19,8) µgN.g^{-1}.d^{-1} e 0,01 (0 e 0,1) mgN.m^{-2}.d^{-1}. As taxas de FBN também foram similares entre bosques de franja e de bacia.

Considerando a dominância por *L. racemosa* (89% da área basal) nos manguezais sob grande enriquecimento de N, espécie que exibiu as menores concentrações de N foliar, e a menor biomassa acima do solo nos manguezais sob grande enriquecimento de N (franja: 144 Mg/ha; bacia: 102 Mg/ha) comparado aos manguezais no sítio controle (franja: 226 Mg/ha; bacia: 144 Mg/ha), um menor estoque de N acima do solo seria esperado nos manguezais sob grande enriquecimento de N. Isso indica que a vegetação de mangue não foi um importante dreno do excesso de N. Como as concentrações de N-NH_4^+ e N-NO_3^- no sedimento foram similares ou menores nos manguezais enriquecidos por N e a vegetação não foi um importante dreno do excesso de N, o excesso de NH_4^+ e NO_3^- deve ter sido principalmente imobilizado na biomassa microbiana e perdido para a atmosfera. Como as taxas líquidas de nitrificação também foram similares entre os sítios de estudo, o excesso de NH_4^+ foi provavelmente principalmente perdido para a atmosfera via volatilização, especialmente considerando os valores de pH registrados nos sítios de estudo (cerca de 6,8 com máxima de 8,6), favoráveis à ocorrência de volatilização. As taxas líquidas de nitrificação similares entre os sítios de estudo também indicaram que o excesso de NO_3^- foi provavelmente principalmente perdido via denitrificação direta, a partir do NO_3^- importado da maré, ao invés de denitrificação acoplada, a partir do NO_3^- proveniente da nitrificação no próprio sistema. Os valores de potencial redox foram semelhantes entre os sítios de estudo, -36 (-162 and 18) mV, em amplitude moderadamente redutora, favorável à ocorrência de denitrificação em manguezais (Patrick Jr. & Mahapatra, 1968; Reef, Feller, & Lovelock 2010). Como discutido anteriormente, maiores emissões de N_2O em manguezais sob enriquecimento de N têm sido consistentemente relatadas na literatura, de maneira que a denitrificação direta incompleta foi provavelmente uma importante via de perda do excesso de NO_3^- para a atmosfera nos manguezais enriquecidos por N. As taxas de FBN similares entre manguezais enriquecidos e não enriquecidos por N também indicaram que o excesso de N foi rapidamente perdido do sistema, de maneira que a comunidade diazotrófica permaneceu limitada por N.

No capítulo 3, o $\delta^{15}N$ foliar foi similar entre *L. racemosa* e *A. shaueriana*, e duas vezes maior nessas espécies do que em *R. mangle*. O $\delta^{15}N$ foliar de *L. racemosa* e *A. shaueriana* foi maior na porção norte sob grande enriquecimento de N (5,5‰) do que na porção sul (4,7‰) do CEL de Cananeia-Iguape, $U(40,32) = 465$, $P = 0.023$. O $\delta^{15}N$ foliar dessas espécies também foi cerca de duas vezes maior nos manguezais próximos ao Valo Grande (5,9‰) do que nos manguezais mais distantes desse canal e sob maior influência marinha (3,7‰) na porção norte do CEL, indicando a menor poluição por N nos manguezais mais distantes do Valo Grande. Em função da dominância por *L. racemosa* nos manguezais próximos ao Valo Grande, somente um indivíduo de *R. mangle* foi amostrado nesses sítios. Considerando os demais sítios de estudo, o $\delta^{15}N$ foliar de *R. mangle* foi similar nos manguezais da porção sul e porção norte distante do Valo Grande no CEL (3,2‰), o que também indicou a menor poluição por N no estuário mais distante do Valo Grande e sob maior influência marinha. Esses resultados indicaram que apesar da grande variabilidade estrutural em manguezais de franja, o $\delta^{15}N$ foliar é um indicador consistente da ciclagem de N em manguezais.

O maior $\delta^{15}N$ e maiores concentrações de N nas folhas das macrófitas aquáticas invasoras (25,4 g/kg e 6,8‰) em relação à vegetação de mangue (13,9 g/kg e 5,8‰) nos sítios invadidos indicaram a maior demanda por N das espécies invasoras. As menores concentrações de $N\text{-}NO_3^-$ no sedimento de manguezais sob grande enriquecimento de N e invadidos por macrófitas aquáticas, 0,07 (0,05 e 0,11) mg/kg, comparado aos manguezais sob grande enriquecimento de N mas não invadidos e os manguezais na porção sul do CEL, 0,20 (0,12 e 0,24) mg/kg, provavelmente refletem a maior absorção de N pelas macrófitas aquáticas invasoras e maiores perdas de N para a atmosfera com a intensificação da dinâmica de N com o enriquecimento de N e invasão por macrófitas aquáticas. Como consequência da maior demanda por N, as macrófitas aquáticas invasoras introduzem no sedimento uma matéria orgânica com menor razão C:N (14,9) em relação à vegetação de mangue (23,5), provavelmente promovendo uma maior atividade microbiana e maiores taxas de mineralização de N no sedimento (e.g., Vilà et al. 2011; Lee et al. 2017). A maior disponibilidade de N e atividade microbiana também resultam em maiores perdas de N para a atmosfera, o que provavelmente inclui emissões de N_2O (e.g., Chen et al. 2010; Fernandes et al. 2010; revisado por Reis et al. 2017a), e contribuem com o maior $\delta^{15}N$ foliar da vegetação de mangue nas áreas invadidas.

As concentrações de N foliar foram maiores na porção norte do que na porção sul do CEL para *R. mangle*, mas similares entre os sítios de estudo para *A. shaueriana* e *L. racemosa*. As razões C:N nas folhas foram similares entre os sítios de estudo para *R. mangle* e *L. racemosa*, e cerca de duas vezes maiores nos sítios sob maior enriquecimento de N do que na porção sul do CEL para *A. shaueriana*. Considerando a dominância por *L. racemosa* nos sítios de estudo próximos ao Valo Grande, que exibiu as menores concentrações de N foliar dentre as espécies de mangue, e a elevada mortalidade de troncos de árvore (até 60% da área basal) nesses sítios em relação às áreas de estudo na porção sul do CEL (até 20%) (Cunha-Lignon and Menguini 2016), um menor estoque de N acima do solo é esperado nos manguezais próximos ao Valo Grande. Como a vegetação de mangue não foi um importante dreno do excesso de N, o qual deve ter sido principalmente assimilado pelas macrófitas aquáticas invasoras e perdido para a atmosfera, as concentrações e razões de N nas folhas da vegetação de mangue não foram bons indicadores da ciclagem de N no sistema.

4. Conclusões

Manguezais e florestas tropicais terrestres exibem intervalos semelhantes de taxas de FBN, denitrificação e de emissões de óxido nitroso (N_2O) para a atmosfera. Em função das elevadas taxas de transformação e de perdas de N para a atmosfera que frequentemente exibem, ecossistemas de manguezal desempenham um importante papel na ciclagem de N em sistemas costeiros de regiões tropicais e subtropicais em todo o mundo. Os principais fatores limitantes das taxas de transformação e de fluxos de N no sedimento de manguezais são a disponibilidade de N inorgânico dissolvido e a sua imobilização na biomassa microbiana. Manguezais são altamente eficientes em utilizar formas de N inorgânico dissolvido da água das marés, relacionado a uma rápida absorção pela vegetação de mangue e a uma eficiente imobilização de N no sedimento pela atividade microbiana. No entanto, em manguezais sujeitos ao enriquecimento de N, o excesso de N pode ser principalmente perdido para a atmosfera ao invés de assimilado pela vegetação de mangue ou conservado no sedimento. Como consequência, o excesso de N pode não afetar a FBN em manguezais, mas intensifica as perdas de N para a atmosfera, principalmente as perdas de óxidos de N via denitrificação direta incompleta quando comparado às perdas de N atmosférico (N_2) via denitrificação completa acoplada. Considerando os cenários atuais e futuros de enriquecimento de N via poluição da água e deposição atmosférica, manguezais podem se tornar uma maior fonte de N_2O para a atmosfera. Em adição aos efeitos do enriquecimento de N, as macrófitas aquáticas invasoras intensificaram a dinâmica e as perdas de N para a atmosfera nos manguezais invadidos com a introdução de matéria orgânica com elevadas concentrações de N no sedimento. Apesar da grande variabilidade estrutural em manguezais de franja, o $\delta^{15}N$ foliar é um indicador consistente de alterações na ciclagem de N decorrentes do enriquecimento de N e invasão por macrófitas aquáticas em ecossistemas de manguezal.

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Capítulo 1

Global overview on nitrogen dynamics in mangroves and consequences of increasing nitrogen availability for these systems

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CRGR compilou e analisou o banco de dados e liderou a escrita do manuscrito. Todos os autores contribuíram criticamente para a interpretação dos resultados e com a versão final do manuscrito.

Title: Global overview on nitrogen dynamics in mangroves and consequences of increasing nitrogen availability for these systems

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Abstract

Background and Scope: Mangroves provide several ecosystem goods and services to society. However, mangroves are frequently subjected to land conversion, overharvesting, and pollution including increased nitrogen (N) availability. Aiming to provide useful information to predict effects of N enrichment on mangroves, we evaluated literature data on N transformation rates in sediment, dissolved N (DN) fluxes across the sediment-water interface, and natural abundance of N stable isotopes ($\delta^{15}N$) in the sediment-plant system in conserved mangroves and those subjected to anthropogenic N enrichment.

Conclusions: Mangroves and terrestrial tropical forests exhibit a great overlap in rates of biological N fixation (BNF) and denitrification and nitrous oxide flux rates. Mangroves can be highly efficient users of DN forms from tidal waters, related to rapid plant uptake and an efficient conservation of DN in sediment by microbial activity. The main factors limiting N transformation rates in mangrove sediment are inorganic DN availability and microbial immobilization. The $\delta^{15}N$ data indicated that fringe forests exhibit higher N transformation rates in sediment and higher N losses to atmosphere, compared to other mangrove types. Except for BNF, all other N transformation and flux rates seem to be intensified by increasing N availability.

Keywords: biological nitrogen fixation, nitrogen mineralization, sediment-water interface fluxes, denitrification, nitrous oxide, nitrogen stable isotopes.

Abbreviations: ARA, acetylene reduction activity; BNF, biological nitrogen fixation; C, carbon; DIN; dissolved inorganic nitrogen; DN, dissolved nitrogen; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; HC, heterocystous cyanobacteria; H_2S, sulfide; N, nitrogen; NH_3, ammonia; NH_4^+, ammonium; NO_2^-, nitrite; NO_3^-, nitrate; NO_x^-, nitrite plus nitrate; N_2, atmospheric nitrogen; N_2O, nitrous oxide; $\delta^{15}N$, nitrogen stable isotope ratio; O_2, oxygen; P, phosphorus; PN, particulate nitrogen.

1. Introduction

Mangroves are intertidal coastal wetlands colonized by a group of 62 plant species in the Indo-West Pacific and only 12 species in the Atlantic and Eastern Pacific, including trees, shrubs, a palm, and a ground fern (also termed mangroves) sharing adaptations to periodic flooding, highly variable salinities, and oxygen depleted sediments (Spalding et al. 2010; Schaeffer-Novelli et al. 2000). There are about 152,000 km^2 of mangroves distributed in 123 countries and territories of tropical, subtropical and warm temperate regions. Despite their broad distribution, only 12 countries have about 68% of the world's mangroves, with the top three being Indonesia (31,894 km^2), Brazil (13,000 km^2), and Australia (9,910 km^2) (Spalding et al. 2010). Mangroves originally occupied more than 200,000 km^2 of coastlines (Spalding et al. 2010). However, about 25 to 35% of mangrove forest area has already been lost due to land conversion to aquaculture, agriculture, urbanization, tourism, overharvesting, and pollution (Valiela et al. 2001; Alongi 2002; van Lavieren et al. 2012). Beyond these regional pressures affecting mangrove forests around the world, these ecosystems are also threatened by human-induced global environmental changes such as climate change and the associated sea-level rise (*e.g.*, Alongi 2008; Gilman et al. 2008; Lovelock et al. 2015), and the increased reactive nitrogen creation related to the large-scale use of nitrogen (N) fertilizers, legume cultivation, and N emissions to the atmosphere during fossil-fuel combustion (Bleeker et al. 2011; see also Erisman et al. 2008),

Nitrogen availability strongly regulates the structure and functioning of both terrestrial and aquatic ecosystems (Elser et al. 2007; Lebauer and Tresender 2008; Chapin III et al. 2011). Nitrogen enrichment is therefore recognized as one of the major threats to conservation of natural ecosystems and maintenance of human activities (Rockström et al. 2009; Erisman et al. 2013). In terrestrial ecosystems, the excess of N favors biological invasions and modifies the competitive ability among species, resulting in changes of dominance patterns, and loss of plant and soil biota biodiversity (*e.g.*, Bobbink et al. 2010; Bradley et al. 2010; Eisenhauer et al. 2012). Nitrogen enrichment in terrestrial ecosystems also has the potential to increase soil N mineralization rates, nitrous oxide (N_2O) emissions to atmosphere, and nitrate (NO_3^-) losses to aquatic systems, modifying productivity patterns and increasing mortality (*e.g.*, Fenn et al. 1998; Matson et al. 1999). Biodiversity loss associated with alterations in ecosystems functioning may adversely affect the provision of important ecosystem services to society (Díaz et al. 2006; Chapin III et al. 2000; Comptom et al. 2011).

Mangroves potentially play an important role in the global context of N enrichment. They provide many ecosystem goods and services to society, which can be impaired by increasing N availability, including the following: (1) support of coastal fisheries by providing food, shelter, and/or nursery grounds for commercially important species that spend at least part of their life cycle in mangroves (*e.g.*, Mumby et al. 2004; Crona and Rönnbäck 2005; Aburto-Oropeza et al. 2008); (2) access to food sources (*e.g.*, fish, molluscs, crustaceans, fruits, sugar, and honey), wood products (firewood, charcoal, and timber for construction), and non-wood products (*e.g.*, thatch, fodder, tannins, wax, dyes, and herbal remedies) associated with the livelihood of human communities living in or near mangroves (*e.g.*, Bandaranayake 1998; Glaser 2003; Walters et al. 2008; Hussain and Badola 2010; Warren-Rodes et al. 2011; Baba et al. 2013); (3) trapping and storage of sediment, organic matter, nutrients, and heavy metals from surrounding waters originating from anthropogenic sources (*e.g.*, Tam and Wong 1995; 1996; Alongi and Mckinnon 2005; Jordan et al. 2011); and (4) efficient carbon (C) sequestration because of high primary productivity and C allocation in belowground biomass, low sediment respiration rates, substantial long-term organic C burial in sediments, and considerable exportation of refractory dissolved organic C to the ocean, contributing to the C burial in marine sediments (*e.g.*, Komiyama et al. 2008; Kristensen et al. 2008; Donato et al. 2011; Duarte et al. 2013). In addition, mangrove forests are the only forest formations occurring in the confluence of terrestrial, marine, and freshwater systems (Alongi 2002). Therefore, alterations in mangroves functioning as a consequence of N enrichment may affect not only the important ecosystem goods and services they provide to society but also affect surrounding ecosystems.

There are still many knowledge gaps in the functioning of mangroves that need to be addressed to allow us to infer the consequences of global environmental changes to mangroves and the ecosystem goods and services they provide. Among these gaps are included N dynamics and alterations following N enrichment in the different types of mangroves. In the Americas, at the local scale, two main types of mangrove forests (fringe and basin) are currently recognized by physiographic aspects determined by water flow and the type of landform occupied, but some authors have also recognized other subtypes of mangroves (riverine, overwash, dwarf, and scrub) (Schaeffer-Novelli et al. 2000). Fringe forests develop on inclined slopes bordering estuaries, deltaic channels, coastal lagoons, and rivers (termed as riverine), under great influence of tidal flushing or river discharge. Overwash designates small self-enveloping fringe islands entirely covered by water during high tide. Basin forests develop in shallow depressions of more inland areas flooded by spring tides, seasonal sea level elevations, or freshwater pulses. At basin stands, water moves slowly as sheet flows maintaining waterlogged conditions for long periods of time. Dwarf is a subtype of basin forest that develops in carbonate or deep peat sediments and exhibit low stature (usually up to 1.5 m) stunted by nutrient limitations. The term "scrub" is employed as a descriptor of both fringe and basin forests of reduced structural development imposed by other stressors than nutrient limitations (Schaeffer-Novelli et al. 2000; 2005; Spalding et al. 2010). Currently, there are no data on the extension area occupied by each mangrove physiographic type in the Americas, but fringe forests are usually more conspicuous formations, while basin forests occupy smaller inland areas in coastal systems. However, there are places where dwarf mangroves are found occupying extensive areas such as in southern Florida (Davis III et al. 2003), Panama (Lovelock et al. 2005), and Belize (Feller 1995). These mangrove types differ in water movement, hydroperiod, edaphic conditions, and inputs of freshwater and nutrients, which result in differences of structural development (Schaeffer-Novelli et al. 1990; 2000; see Cunha-Lignon et al. 2011) and of nutrient dynamics and ecosystem functioning (*e.g.*, Twilley et al. 1986a; Twilley 1988; Rivera-Monroy et al. 1995a; Rivera-Monroy and Twilley 1996). These mangrove types may, therefore, exhibit different responses to N enrichment, which can also modulate different responses to climatic changes.

In order to provide useful information for the discussion about the major consequences of N enrichment on mangroves, here we provide an updated overview on the general patterns of N dynamics in mangrove forests from previous synthesis and advance: 1. At regional scale on the understanding of N dynamics in mangroves by evaluating differences in N dynamics between mangrove physiographic types in the Americas and 2. At global scale on major alterations of N dynamics following N enrichment, identified with an extensive review of the literature and evaluation from a global database.

2. Material and Methods

The search for publications was performed in the databases of Springer (http://www.springer.com), JSTOR (http://www.jstor.org), and Web of Science (http://thomsonreuters.com/web-of-science) using the word "mangrove" in keyword, title, and abstract searches. From the resulting lists, publications containing information on N dynamics in conserved and subjected to anthropogenic N enrichment mangroves were selected and data of N transformation rates in sediment (biological nitrogen fixation, mineralization, ammonification, nitrification, denitrification, and volatilization), N fluxes across the sediment-water interface, and natural abundance of N stable isotopes ($\delta^{15}N$) in the sediment-plant system were collected and tabulated. Data published as figures were extracted using the software Plot Digitizer 2.6.3 (Huwaldt and Steinhorst 2013). To expand the search, each publication had its reference list assessed as well as the new publications obtained until no additional item was found. Data of sediment within mudflats, tidal creeks or coastal lagoons were not included. The biological N fixation data published as acetylene reduction activity (ARA) rates were converted to N fixation rates using the conversion factor of 4 (C_2H_4:N_2 ratio of 4:1) (Postgate 1982), which is a reasonable assumption for mangroves, since studies using $^{15}N_2$ calibration have shown ratios ranging from 1.9 to 6.3 (Potts 1984; Hicks and Silvester 1985). In all, data were obtained from 62 studies, but also data from previous reviews by Howarth et al. (1988), Alongi et al. (1992), and Purvaja et al. (2008), totaling 123 mangrove sites comprising areas in the Atlantic Ocean, Caribbean Sea, Gulf of Mexico, and Indo-Pacific coasts (Fig. 1). The countries with a greater number of study sites include Australia (22), India (21), the United States of America (18), Belize (12), Puerto Rico (10), China (7), and Papua New Guinea (7). Other countries had four or fewer study sites each. Data from N-enriched mangrove stands included sites affected by sewage pollution (Corredor and Morell 1994; Corredor et al. 1999; Kreuzwieser et al. 2003; Allen et al. 2007; Purvaja et al. 2008; Chen et al 2010; 2012), fertilizers from agriculture (Kreuzwieser et al. 2003, Chauhan et al. 2008, Chen et al. 2012), aquaculture pond effluent (Alongi et al. 2005, Chauhan et al. 2008, Chen et al. 2012), and ferromanganese mine discharge effluent (Fernandes et al. 2010; Fernandes and LokaBharathi 2011; Fernandes et al. 2012a,b), and also mangrove stands that were sites of a failed shrimp farm (Alongi et al. 2002). Data of tropical terrestrial forests are presented for comparative purposes since they are well recognized as being the most productive forested ecosystems on Earth (see Chapin III et al. 2011) with the highest rates of N cycling (Vitousek and Sanford Jr 1986; Martinelli et al. 1999; Vitousek et al. 2002). For data analysis, normality was evaluated using the Shapiro-Wilk *W*-test. Comparisons between two data groups were performed using the nonparametric Mann-Whitney *U*-test. Comparisons between more than two groups were assessed by the nonparametric test of Kruskal-Wallis *H*-test followed by *post hoc* pairwise comparisons as described by Siegel and Castellan (1988). Statistical analysis was performed using the software R (R Core Team 2014). Differences at the 0.05-level were considered significant.

3. Results and Discussion

3.1. Biological N fixation

One of the main pathways by which N enters mangrove ecosystems is biological N fixation (Alongi 2002; 2009), the process whereby atmospheric N (N_2) is reduced to ammonia (NH_3) carried out by microorganisms that possess the nitrogenase enzyme complex (termed as diazotrophs). Biological N fixation (BNF) has been detected in mangrove stands associated with plant roots (associative BNF) and free in sediments, microbial mats (*i.e.*, centimeter-thick multilayered structures of microorganisms as defined by Reitner 2011), leaf litter (and senescent leaves incubated on sediment), pneumatophores, and cyanobacterial crusts growing on trunks (free-living BNF) (Fig. 2), at rates that overlap those reported for tropical terrestrial forests. Mean estimates of free-living BNF in tropical terrestrial forests (including rainforests, deciduous forests, and forested floodplains) fall within 0.9 and 2.1 $mgN.m^{-2}.d^{-1}$ with rates ranging from 0.03 to 16 $mgN.m^{-2}.d^{-1}$ (Reed et al. 2011). There is a wide range of rates of BNF in symbiotic associations with plant roots reported for tropical terrestrial forests, from 0.07 to 66.5 $mgN.m^{-2}.d^{-1}$, but global estimates fall within 1.5 to 16.6 $mgN.m^{-2}.d^{-1}$ (Sylvester-Bradley et al. 1980; Cleveland et al. 1999; Reed et al. 2011; Sullivan et al. 2014). Comparisons to associative BNF rates in mangroves, from which plants can also directly benefit, are difficult due to the reduced number of estimates reported on an areal basis for mangroves. Nevertheless, these estimates range from 2 to 10 $mgN.m^{-2}.d^{-1}$, falling within the global estimates range of symbiotic BNF in tropical terrestrial forests. There was no evidence of regional differences in BNF rates from our database. However, negligible and highly variable rates were often reported (*e.g.*, Nedwell et al. 1994; Alongi et al. 2004a; Romero et al. 2012), so that not all mangroves may sustain high BNF rates, such as the Australian mangroves studied by Alongi (2013).

Several factors that can regulate aboveground BNF rates in mangroves have been reported. Potts (1979) pointed out reduced light intensity, water content, and phosphorus (P) availability, as well as photorespiration and to a lesser extent endogenous oxygen (O_2) production and reduced dissolved organic C (DOC) availability constrained N fixation rates in heterocystous cyanobacterial (HC) communities on pneumatophores of *Avicennia marina* (Forssk.) Vierh. in mangrove stands in Sinai, Egypt. Sheridan (1991; 1992) demonstrated that high salinity and low percent moisture restricted N fixation rates in HC communities growing on trunks of *Avicennia germinans* (L.) L. in mangrove stands in Guadeloupe, Lesser Antilles. Joye and Lee (2004) and Lee and Joye (2006) found that light intensity, endogenous O_2 production, water content, sulfide (H_2S) concentration, DOC availability, and the dominance by HC or non HC species regulated N fixation rates in mangrove microbial mats in Twin Cays, Belize. BNF rates were enhanced after DOC amendments, which was attributed to stimulation of O_2 respiration and, consequently, of H_2S oxidation and alleviation of H_2S-inhibition, as well as stimulation of heterotrophic N fixation. Gotto and Taylor (1976) and Pelegrí and Twilley (1998) reported an O_2 suppression and a C stimulation, respectively, of BNF rates in leaf litter from mangrove sites in Florida, US, while Zuberer and Silver (1978) and Hicks and Silvester (1985) found no effect of these factors on BNF rates in mangrove leaf litter from Florida and Auckland, New Zealand, respectively. These conflicting results on factors regulating N fixation in mangrove leaf litter may rely on the community type of N fixers, which seems to be more variable for leaf litter than other mangrove compartments (see Gotto and Taylor 1976; Hicks and Silvester 1985; Pelegrí et al. 1997; Pelegrí and Twilley 1998).

On the other hand, only few studies investigated factors regulating N fixation rates in mangrove belowground compartments. Zuberer and Silver (1978) and Pelegrí and Twilley (1998) reported marked increases in BNF rates in sediment after C sources amendments in mangrove stands in Florida. Because of the relatively long lag periods before BNF rate enhancement following C additions (12 to 24 h), Zuberer and Silver (1978) suggested that the increase in BNF rates would primarily result from diazotrophic population growth and/or nitrogenase synthesis, instead of an immediate increase in energy sources. Zuberer and Silver (1978) also added C sources to washed excised roots of *A. germinans*, *Rhizophora mangle* L., and *Laguncularia racemosa* (L.) C.F. Gaertn. seedlings but found no response, suggesting that the diazotrophic bacteria attached to mangrove roots may have had ample access to C and energy sources. Sengputa and Chaudhuri (1991) isolated and identified diazotrophic bacteria associated with root samples of several mangrove species including *Acanthus ilicifolius* L., *Avicennia spp.*, *Bruguiera spp.*, *Ceriops decandra* (Griff.) W. Theob., *Rhizophora mucronata* Lam., *Sonneratia apetala* Buch-Ham, *Aegialitis rotundifolia* Roxb., and *Excoecaria agallocha* L. from mangrove stands in Sundarban, India. There was no specificity of any of the bacterial isolates to any of the plant species. Regardless of plant identity, root samples from tidally inundated mangrove sites sustained higher BNF rates compared to root samples from occasionally inundated or drier highland sites. This was attributed to the presence of a larger number of more efficient N_2-fixing bacterial strains belonging to a greater number of O_2 response groups in the tidally inundated mangrove sites.

Comparing BNF rates on a dry-weight basis between mangrove compartments (Fig. 2), leaf litter sustained the highest "intrinsic capacity" of fixing N followed by roots and cyanobacterial crusts on trunks, while incubated senescent leaves, sediment, and pneumatophores exhibited the lowest dry-weight based rates (H = 23.64, df = 5,54, p = 0.0002). Pelegrí et al. (1997) and Pelegrí and Twilley (1998) also found higher BNF rates on a dry-weight basis for leaf litter (up to 0.09 $mgN.g^{-1}.d^{-1}$) compared to incubated senescent leaves (up to 0.01 $mgN.g^{-1}.d^{-1}$), sediments (up to 0.005 $mgN.g^{-1}.d^{-1}$), and pneumatophores (up to 0.0008 $mgN.g^{-1}.d^{-1}$) in mangrove stands at the Everglades National Park, with the latter two not significantly differing from each other. These differences in "intrinsic capacity" of fixing N can be related to differences in the community type of N fixers and in C availability between compartments. N fixation in mangrove sediment and roots are dominated by heterotrophic bacteria, and pneumatophores is dominated by cyanobacteria, while mangrove leaf litter seems to have a variable contribution of both groups. Mangrove leaf litter and roots were reported to provide ample C sources for N fixation (in intermediate stages of leaf litter decay) while N fixation in sediment and pneumatophores were demonstrated to be C-limited (Zuberer and Silver 1978; Potts 1979; Pelegrí and Twilley 1998). Despite the differences in N fixing "intrinsic capacity", mangrove roots, pneumatophores, sediment, and microbial mats exhibited similar BNF rates on an areal basis, suggesting that these compartments make similar contributions to the total N input in mangroves (H = 2.84, df = 3,51, p = 0.4167). No BNF rates on an areal basis for mangrove leaf litter were found in the literature. Despite their high "intrinsic capacity" of fixing N, many mangroves exhibit low-standing leaf litter, ranging from about 0.02 to 5 $Mg.ha^{-1}$ (Twilley et al. 1986b; Twilley et al. 1997), suggesting that the total contribution of leaf litter to N input in many mangroves can be low compared to other compartments.

3.2. N fluxes across the sediment-water interface

Another important pathway by which N enters (and leaves) mangrove ecosystems is tidal exchange (Alongi 2002; 2009). Several studies have attempted to determine whether mangroves act as an N sink or source for coastal waters, but results are often highly variable and controversial, especially regarding dissolved N forms (Adame and Lovelock 2011). Studies investigating dissolved N (DN) fluxes between mangrove sediment and overlying water based on direct measurements have shown that mangroves can be highly efficient users of DN forms from tidal waters (Fig. 2). Even when concentration gradients between porewater and overlying water suggested dissolved N release into overlying water, DN net fluxes were often negligible or into the sediment (*e.g.*, Kristensen et al. 1988; Alongi et al. 1993; Kristensen et al. 1998). This ability has been attributed to a rapid uptake of DN by plants and benthic microalgae and an efficient conservation of DN in sediment by microbial activity (*e.g.*, Kristensen et al. 1988; Alongi et al. 1993; Kristensen et al. 1998; Alongi 2013). Findings concluding that DN fluxes at the sediment-overlaying water interface are largely driven by sediment microbial activity include Stanley et al. (1987), who found measurable amino acid efflux from sediment only after poisoning microbial populations, and Alongi et al. (1993) and Mohammed and Johnstone (2002), who reported high DN fluxes into sediment associated with high microbial productivity and biomass, respectively. However, the range of DN flux rates were high, indicating that while some mangroves tend to act as net "sinks" for DN from tides, others may primarily export DN for adjacent water bodies. Studies that quantified DN fluxes between tidal creek or estuarine waters in mangrove areas and coastal or ocean waters indicated that mangroves can also act as a DN source for adjacent water bodies (Adame and Lovelock 2011).

3.3. N mineralization

Together with BNF and tidal exchange, N also becomes available to plants through N mineralization. It is the microbial mediated process by which organic N is converted to inorganic forms, including the steps of ammonium (NH_4^+) production termed ammonification, and NH_4^+ oxidation to nitrite (NO_2^-) and NO_3^- termed nitrification. Mangroves and tropical terrestrial forests differ in the most common DIN form in sediment and soil, respectively, NH_4^+ for mangroves (Alongi et al. 1992) and NO_3^- for tropical terrestrial forests (*e.g.*, Reiners et al. 1994, Silver et al. 2000). However, net ammonification and nitrification rates on a weight basis reported for mangrove sediments (Fig. 2) overlap net N mineralization (-0.02 to 0.008 $mgN.g^{-1}.d^{-1}$) and nitrification rates (-0.002 to 0.005 $mgN.g^{-1}.d^{-1}$) reported for terrestrial tropical forests (Vitousek and Matson 1987; Luizão et al. 2004; Silver et al. 2005). The differences in magnitude between gross and net ammonification rates (*i.e.*, gross production minus microbial immobilization) in mangrove sediments (Fig. 2) indicate that an efficient microbial immobilization of NH_4^+ may constrain net ammonification rates in mangrove sediment. Only Chen and Twilley (1999) investigated factors regulating net ammonification rates under natural conditions in mangroves. They found a very strong positive correlation of net ammonification rates with P availability in mangrove stands in Florida, suggesting a P-limitation of microbial activities. Factors limiting nitrification in mangroves have received little attention. Rivera-Monroy and Twilley (1996) conducted sediment incubation experiments with $^{15}NH_4^+$ and found that nitrification (coupled with denitrification) was constrained by NH_4^+ availability and microbial immobilization in sediment. Kristensen et al. (1998) reported higher potential nitrification rates with depth in vegetated-mangrove compared to non-vegetated tidal flat sediments from Phuket Island, Thailand. This was attributed to more oxic conditions provided by root activity in vegetated compared to non-vegetated sediments. Krishnan and LokaBharathi (2009), however, found that nitrification rates were regulated by Mn availability, suggesting the occurrence of anoxic nitrification at the expense of this element in mangrove sediments from Goa, India. Other factors reported to regulate nitrification rates by Krishnan and LokaBharathi (2009) were NH_4^+ and organic C availability, the latter as a C source for heterotrophic nitrifiers.

3.4. N losses to atmosphere

Besides N losses through tidal exchange, some microbial mediated N transformations in sediment also result in gaseous N losses from mangroves. Denitrification is the reduction process of NO_3^- and NO_2^- to N_2O and N_2 mainly. There are two types of denitrification: direct denitrification, which is supported by NO_3^- that diffuses from overlying water into sediment, and coupled denitrification, which is supplied with NO_3^- from nitrification. The differences in magnitude between denitrification and net ammonification and nitrification rates reported for mangrove sediments (Fig. 2) suggest that direct denitrification is an important pathway contributing to gaseous N losses in mangroves. Few studies investigated direct and coupled denitrification rates in mangrove sediment. Using ^{15}N enrichment techniques, Rivera-Monroy and Twilley (1996) found higher potential direct denitrification rates, up to 74 $mgN.m^{-2}.d^{-1}$, compared to potential coupled denitrification rates, up to about 10 $mgN.m^{-2}.d^{-1}$, in mangrove sediments from Isla del Carmen, Mexico. These authors also reported that in sediments subjected to $^{15}NH_4^+$ enrichment, none of the added ^{15}N was denitrified but was recovered in the non-extractable sediment N pool, suggesting that coupled denitrification rates were constrained by microbial immobilization of NH_4^+ and NO_3^- in sediment. Earlier, Rivera-Monroy et al. (1995b) reported that less than 10% of the $^{15}NO_3^-$ added to mangrove sediments was denitrified, while the remaining was recovered as particulate N (PN) in sediment, suggesting that direct denitrification rates were also constrained by NO_3^- availability and microbial immobilization in sediment. The denitrification and N_2O fluxes rates on areal basis reported for mangroves also greatly overlap denitrification (0.5 to 8.2 $mgN.m^{-2}.d^{-1}$) and N_2O fluxes rates (0.02 to 13.7 $mgN.m^{-2}.d^{-1}$) reported for tropical terrestrial forests (Silver et al. 2000, Kiese et al. 2003, Fang et al. 2015). The higher maximum denitrification rates reported for mangroves may in part reflect the importance of direct denitrification in mangroves, supplied with NO_3^- from flood tides (*e.g.*, Rivera-Monroy and Twilley 1996). However, the range of denitrification and N_2O fluxes rates in mangrove sediments were large, indicating that not all mangroves may exhibit high rates of N losses to atmosphere, such as some Australian mangroves (Alongi 2013).

3.5. N stocks

Few studies provided direct measurements on N stocks in mangroves. Alongi et al. (2003) and Bulmer et al. (2016) reported total N stocks of up to 12.2 and 15.4 ± 1.0 $MgN.ha^{-1}$ for mangrove forests in Australia and New Zealand, respectively. Khan et al. (2007) estimated a total N stock of 3.5 $MgN.ha^{-1}$ for a pioneer mangrove forest in Japan, while Fujimoto et al. (1999) estimated N stocks in sediment of up to 56 $MgN.ha^{-1}$ for mangrove forests in Micronesia (Fujimoto et al. 1999). We estimated a total N stock for mangroves of about 20 $MgN.ha^{-1}$, from indirect calculation considering the global averages of C stocks in sediment (about 718 $MgC.ha^{-1}$) and in above and below ground compartments in mangroves (99 and 138 $MgC.ha^{-1}$, respectively) (Alongi 2014), and the C:N ratios reported for sediment (40) (Rivera-Monroy et al. 1995b; Rivera-Monroy and Twilley 1996), for litter (100) (Kristensen et al. 1995), and for roots (79) in these ecosystems (Alongi et al. 2003, 2004b). This estimate is similar to the N stocks for non-flooded lowland evergreen tropical forest in Brazilian Amazon, which is up to 22 MgN.ha^{-1}, including soil and above and below ground biomass compartments (PBMC 2014).

3.6. Differences in N dynamics between mangrove physiographic types

Few studies investigated possible differences in BNF between mangrove physiographic types. Sheridan (1991) reported mean ARA rates of 1,943 and 3,046 nmolC_2H_4.mgChlorophylla^{-1}.h^{-1} for cyanobacterial crusts on trunks in dwarf mangrove stands, while no activity was found in a seaward fringe forest in Guadeloupe, Lesser Antilles. Laboratory and field experiments revealed that ARA rates were constrained by increasing salinity and desiccation. As ARA rates in the fringe remained below the detection limit after 2 days of heavy rainfall, it can be supposed that a higher wind-borne salt delivery in fringe compared to inland dwarf stands may have constrained BNF. Lee and Joye (2006) reported a greater importance of BNF in cyanobacterial dominated microbial mats in dwarf stands compared to fringe forests in Twin Cays, Belize (Table 1). According to their findings, this could be attributed to the presence of O_2 and desiccation-tolerant HC species in dwarf microbial mats, while fringe microbial mats were dominated by non-HC species, and also to a higher benthic surface light availability in dwarf stands compared to fringe forests. Studies that evaluated BNF rates in mangrove sediment also suggested that dwarf mangroves can sustain higher BNF rates compared to other mangrove types (Table 1). Despite some evidence that mangrove physiographic types may differ in BNF rates, none of the other studies surveyed for this review evaluated possible differences in BNF between mangrove physiographic types. More studies are needed to evaluate the importance of BNF in different compartments and of total N input via BNF in the different physiographic types of mangroves.

Concerning N fluxes between mangrove sediment and overlying water, Rivera-Monroy et al. (1995a) investigated net PN and DN fluxes between a fringe forest and an adjacent tidal creek, and an inland basin forest in Isla del Carmen, Mexico. The fringe forest imported NH_4^+ and NO_x^- from both tidal creek and basin forest, while exported DON and PN to both basin forest and tidal creek. These results suggest that fringe forests might primarily act as sink for DIN and a source of organic N forms, while basin forests may exhibit the opposite pattern (Table 1). Davis III et al. (2001a,b) evaluated net DIN fluxes between sediment and overlying water from fringe and dwarf stands in southern

Everglades, Florida. Higher fluxes were reported for the dwarf mangrove. While the fringe forest tended to import DIN, the dwarf mangrove imported NH_4^+, but exported greater amounts of NO_x^-, resulting in a net DIN export (Table 1).

Regarding N transformation rates in sediment, Reis et al. (2017) evaluated net N mineralization and nitrification rates in fringe and basin forests in Cardoso Island, Brazil. The fringe forest exhibited higher mean net N mineralization rate but similar mean net nitrification rate compared to the basin forest (Table 1). However, there were evidences that gross nitrification might have been higher in fringe compared to basin forest. Rivera-Monroy et al. (1995b) investigated direct denitrification rates in sediment of fringe and basin forests in Isla del Carmen. The fringe forest exhibited double the highest direct denitrification rate reported for the basin forest (Table 1). Rivera-Monroy and Twilley (1996) reported negligible coupled denitrification rates in sediment from fringe and basin forests in Isla del Carmen. These authors also evaluated potential direct, coupled and total denitrification rates for a riverine forest. Potential direct denitrification fluxes attained greater values than potential coupled denitrification (Table 1). Nitrous oxide fluxes were only reported for fringe mangroves (Table 1). Lee and Joye (2006) also evaluated denitrification rates in microbial mats from fringe and dwarf mangrove stands in Twin Cays. Fringe microbial mats attained higher denitrification rates at 1 cm depth compared to dwarf microbial mats, and higher potential denitrification rates following NO_3^- addition during short- and long-term experiments. However, dwarf microbial mats at 5 cm depth attained a similar maximum denitrification rate compared to fringe microbial mats at 1 cm depth (Table 1). Fogel et al. (2008) quantified NH_3 volatilization rates in fringe and dwarf mangroves in Twin Cays. Higher NH_3 emissions were found in the dwarf mangrove stand compared to the fringe forest (Table 1). These differences were attributed to a greater development of microbial mats on the ground in the dwarf mangrove compared to the fringe forest.

3.7. N dynamics of mangrove physiographic types from $\delta^{15}N$ studies

Differences in N dynamics between terrestrial ecosystems can also be evaluated through the analysis of natural abundance of N stable isotopes ($\delta^{15}N$) in the soil-plant system (Högberg 1997; Robinson 2001; Craine et al. 2015). The $\delta^{15}N$ expresses the $^{15}N/^{14}N$ ratio in a sample (*e.g.*, leaves, leaf litter, and soil) in relation to the $^{15}N/^{14}N$ ratio of the international standard, the atmospheric N, equals to 0.0036765. The $\delta^{15}N$ integrates the

N stable isotope signature of N sources and isotope fractionations against the heavier isotope ^{15}N during N transformation reactions, mainly those mediated by microorganisms in soil such as nitrification, denitrification, and NH_3 volatilization. The lighter isotope ^{14}N reacts faster than ^{15}N, so that products are maintained enriched in ^{14}N, while substrates are maintained enriched in ^{15}N (Högberg 1997; Robinson 2001). Terrestrial ecosystems under lower N limitations usually exhibit higher soil N transformation rates and N losses to atmosphere, which keeps the soil-plant system enriched in ^{15}N (*i.e.*, higher $\delta^{15}N$) (*e.g.*, Martinelli et al. 1999; Ometto et al. 2006; Nardoto et al. 2008; Craine et al. 2009), compared to terrestrial ecosystems under higher N limitations, which usually exhibit lower N transformation rates in soil and lower N losses to atmosphere, maintaining the soil-plant system enriched in ^{14}N (*i.e.*, lower $\delta^{15}N$) (*e.g.*, Martinelli et al. 1999; Bustamante et al. 2004; Nardoto et al. 2008; Craine et al. 2009; Mardegan et al. 2009).

Studies evaluating differences in N dynamics between mangrove physiographic types using $\delta^{15}N$ are presented in Table 2. The higher $\delta^{15}N$ in the sediment-plant-leaf litter system of fringe compared to dwarf mangroves suggests that fringe mangroves may exhibit higher N transformation rates in sediment and higher N losses to atmosphere, compared to dwarf and basin mangroves. These results are consistent with the higher net N mineralization and denitrification rates in sediment and microbial mats reported for fringe compared to basin and dwarf mangroves. The lower $\delta^{15}N$ in dwarf mangroves can also reflect a greater importance of BNF, as suggested by the higher BNF rates associated with cyanobacterial crusts and microbial mats in dwarf mangroves compared to fringe forests, discussed above.

Some of the studies that evaluated leaf $\delta^{15}N$ patterns in mangroves proposed other explanations for the higher leaf $\delta^{15}N$ in fringe compared to dwarf mangroves. Fry et al. (2000) proposed that the lower leaf $\delta^{15}N$ in dwarf stands would result of the isotopic fractionation during NH_4^+ uptake by plants associated with a slower plant growth and lower N demand by plants in dwarf compared to fringe mangroves. A lower N demand would increase fractionation during N uptake because not all available N would be used, resulting in lower leaf $\delta^{15}N$ in dwarf compared to fringe mangroves. Mckee et al. (2002) reached the same conclusions as Fry et al. (2000), and observing an increase in leaf $\delta^{15}N$ in dwarf stands following P amendments, these authors pointed that the enhanced P availability increased N demand by dwarf plants, reducing fractionation during N uptake and increasing leaf $\delta^{15}N$. Fogel et al. (2008) also observed an increase in leaf $\delta^{15}N$ after P amendments. They suggested that higher P availability could enhance root biomass and activate NH_4^+ transport in roots, reducing fractionation during N uptake. Fogel et al. (2008) also reported extremely low leaf $\delta^{15}N$ values in dwarf mangrove stands, up to -21.6‰, which was attributed to a foliar uptake of ^{15}N depleted atmospheric NH_3, given the higher volatilization rates observed in the dwarf compared to the fringe stand, especially in sites with greater microbial mat development. As isotope fractionations during N transformations in sediment such as nitrification, denitrification, and volatilization are much higher than fractionations in plants (Robinson 2001), it is most probable that leaf $\delta^{15}N$ reflects both $\delta^{15}N$ of N sources and isotope fractionations during N transformations in sediment, than fractionation during N uptake by plants. The influence of P availability on leaf $\delta^{15}N$ probably reflects the influence of a limiting nutrient on overall sediment microbial activity, and consequently, on N transformation rates in sediment, instead of an increased N uptake by plants.

3.8. Effects of N enrichment on N dynamics in mangroves

The patterns of N dynamics in mangroves discussed so far might be modified by anthropogenic N enrichment. Fernandes et al. (2012a) reported that BNF in sediment was only detected at a conserved mangrove stand, with rates reaching about 0.008 $mgN.g^{-1}.d^{-1}$, while no BNF activity was found in a mangrove stand receiving ammonium nitrate from ferromanganese mines in Goa, India. Studies that investigated long-term effects of N amendment on BNF rates in mangrove sediment also found marked reductions up to about 75% (Whigham et al. 2009; Romero et al. 2012). Alongi et al. (2002; 2005) quantified BNF rates in a mangrove plantation that was originally the site of a failed shrimp farm in Thailand and in mangrove stands subjected to intermittent discharge of aquaculture pond effluents in China. There were no difference, however, between BNF rates in sediment reported for these mangrove sites, ranging from about 0 to 8 $mgN.m^{-2}.d^{-1}$, and rates reported for non-N-enriched mangrove stands in the literature ($U = 173.00$, $df = 31,11$, $p = 0.9542$) (Fig. 3). Alongi et al. (2002) also quantified DN net fluxes across the sediment-overlying water interface. There were no differences between the mean NH_4^+ flux rates reported by these authors, from -10 to 10 $mgN.m^{-2}.d^{-1}$, and rates reported for non-N-enriched mangrove stands included in the literature ($U = 287.00$, $df = 10,64$, $p = 0.6066$); but all other mean flux rates reported by these authors, including of NO_2^- plus NO_3^- (NO_X^-) (-407 to -3 $mgN.m^{-2}.d^{-1}$), DIN (-413 to -3 $mgN.m^{-2}.d^{-1}$), dissolved organic N (DON) (-37 to 37 $mgN.m^{-2}.d^{-1}$), and DN (-440 to 27 $mgN.m^{-2}.d^{-1}$) were significantly lower (*i.e.*, higher fluxes into the sediment) (NO_x^-: *U* = 45.00, *df* = 10,40, *p* = 0.0001; DIN: *U* = 74.00, *df* = 10,38, *p* = 0.0017; DON: *U* = 48.00, *df* = 10,21, *p* = 0.0078; DN: *U* = 48.00, *df* = 10,21, *p* = 0.0085).

Concerning N mineralization, Alongi et al. (2002; 2005) also reported mean net ammonification rates from 4 to 86 $mgN.m^{-2}.d^{-1}$, significantly higher than net ammonification rates reported for non-N-enriched mangrove stands (*U* = 51.00, *df* = 8,7, *p* = 0.0030) (Fig. 3). More attention, however, has been given to possible alterations of denitrification and N_2O flux rates in response to N enrichment in mangroves. Denitrification rates reported for mangrove sediments affected by N enrichment ranged from 0 to 106 $mgN.m^{-2}.d^{-1}$ (Corredor and Morell 1994; Corredor et al. 1999; Alongi et al. 2002; 2005; Purvaja et al. 2008; Fernandes et al. 2010; Fernandes and LokaBharathi 2011), while N_2O flux mean rates ranged from -61 to 34 $mgN.m^{-2}.d^{-1}$ (Corredor et al. 1999; Alongi et al. 2005; Kreuzwieser et al. 2003; Allen et al. 2007; Chauhan et al. 2008; Purvaja et al. 2008; Chen et al. 2010; 2012; Fernandes et al. 2010). There were no differences between the denitrification rates reported for these N-enriched mangroves and non-N-enriched mangrove stands reported in the literature (*U* = 245.50, *df* = 11,35, *p* = 0.1763) (Fig.3), but N_2O flux rates were significantly higher in mangroves under N enrichment than non-N-enriched mangrove stands (*U* = 947.00, *df* = 87,30, *p* = 0.0128). Studies comparing denitrification (Corredor and Morell 1994; Fernandes et al. 2012a), N_2O flux rates (Kreuzwieser et al. 2003; Chen et al. 2010; Fernandes et al. 2012b), and both rates (Corredor et al. 1999; Purvaja et al. 2008; Fernandes et al. 2010) between N-enriched and non-N-enriched mangroves, however, repeatedly found that both denitrification and N_2O fluxes were higher in N-enriched mangrove stands. Studies investigating short- and long-term effects of N amendment on denitrification and N_2O flux rates in mangrove sediment also found higher rates at N-enriched sites (Muñoz-Hincapié et al. 2002; Whigham et al. 2009; Chen et al. 2011; Fernandes and LokaBharathi 2011). N availability and microbial activity are some of the main factors regulating N transformation rates in mangrove sediment. Except for BNF, which seems to be constrained by an increased availability of a lower cost N source, all other N transformation rates in mangrove sediment seem to be intensified by increasing N availability. However, the range of these N transformation rates in both N-enriched and non-N-enriched mangroves were large, suggesting that not all N-enriched mangrove may exhibit all the reported alterations in the N cycling.

Increasing N availability and N cycling rates in mangrove sediment do not imply strictly an increase in net primary productivity (NPP) and assimilation of extra N by mangrove vegetation. Mangroves have been shown to be N- (Feller et al. 2003; Lovelock and Feller 2003; Feller et al. 2007; Lovelock et al. 2007a,b; Naidoo 2009) or P-limited (Kock 1997; Kock and Snedaker 1997; Feller 1995; Feller et al. 1999; 2007), or even co-limited by N and P (Cheeseman and Lovelock 2004; Lovelock et al. 2004; 2007a), which can either switch along tidal height gradients (Feller et al. 2002; Cheeseman and Lovelock 2004; Lovelock et al. 2004; 2007a). At N-limited mangrove forests, the extra N may increase NPP, but at P-limited mangrove forests, the increased N availability might increase P-limitation, thus constraining NPP.

4. Conclusion

Despite large variability in N transformation and flux rates between mangrove forests, the results of the present study indicated that mangroves play a substantial role in N cycling in tropical and subtropical regions because of the high N cycling rates that mangrove forests often exhibit. Furthermore, anthropogenic N enrichment may result in extensive impact not only on the N cycling with direct effects on ecosystem functioning but also the potential indirect effects on ecosystem structure in mangrove forests. As a consequence of anthropogenic N enrichment, mangroves may increase N_2O fluxes to the atmosphere, also contributing to global warming. The results also indicated that different types of mangroves differ in N dynamics. Fringe forests have higher N transformation rates in sediment and higher N losses to atmosphere compared to other mangrove types. These differences may reveal different responses to N enrichment. While fringe forests may experience higher increases in N transformation rates in sediment on shorter timescales, basin forests may experience larger ecosystem functioning alterations given their higher nutrient limitation.

Finally, we also highlight in this review the paucity of studies that have evaluated N dynamics and alterations following anthropogenic N enrichment in mangroves, making it difficult to perform a comprehensive meta-analysis and evaluation of regional trends. More empirical and experimental data on consequences of N enrichment to N pools, fluxes, cycling rates, and stocks, as well as its relation with other nutrients availability to the resulting NPP in the different types of mangroves are needed, especially in underrepresented areas such as South America. The establishment of accessible monitoring tools to evaluate alterations following N enrichment in mangroves are also needed. There is also a critical need for the development of a single classification system of mangroves to be used worldwide, despite the richness differences between American and Indo-West Pacific mangroves. There is also a great need for developing coordinated research programs worldwide to conduct studies with standardized methodologies on higher spatial and temporal scales.

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Table 1. Nitrogen cycling rates in sediment and microbial mats from mangrove physiographic types. Median rates (and ranges) are presented, including biological nitrogen fixation (BNF), net nitrous oxide (N_2O) flux, ammonia (NH_3) volatilization, and fluxes of ammonium (NH_4^+), nitrite plus nitrate (NO_x^-), dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), total dissolved nitrogen (DN), and particulate nitrogen (PN) between mangrove sediment and overlying water. Negative values indicate net flux into the sediment or microbial mat. When reported by authors, P-values of statistical comparison tests are presented. Source: (1) Kimball and Teas (1975) *apud* Alongi et al. (1992); (2) Rivera-Monroy et al. (1995a); (3) Rivera-Monroy et al. (1995b); (4) Rivera-Monroy and Twilley (1996); (5) Davis III et al. (2001a); (6) Davis III et al. (2001b); (7) Bauza et al. (2002); (8) Muñoz-Hincapié et al. (2002); (9) Joye and Lee (2004); (10) Lee and Joye (2006); (11) Fogel et al. (2008); (12) Romero et al. (2012); and (13) Reis et al. (2017).

	Fringe	Basin	Dwarf	Unit	P-value	Location
Sediment						
BNF	0.255	0.118 and 0.189	(0.027 to 0.310)	mgN.m^{-2}.d^{-1}	-	Florida, EUA (1)
BNF	-	-	18.2 (0 to 68.5)	mgN.m^{-2}.d^{-1}	-	Twin Cays, Belize (12)
NH_4^+ flux	-1.1	0.2	-	mgN.m^{-2}.d^{-1}	-	Terminos Lagoon, Mexico (2)
NO_x^- flux	-0.05	0.003	-	mgN.m^{-2}.d^{-1}	-	Terminos Lagoon, Mexico (2)
DIN flux	-1	0.2	-	mgN.m^{-2}.d^{-1}	-	Terminos Lagoon, Mexico (2)
DON flux	0.08	-0.2	-	mgN.m^{-2}.d^{-1}	-	Terminos Lagoon, Mexico (2)
DN flux	-1	-0.05	-	mgN.m^{-2}.d^{-1}	-	Terminos Lagoon, Mexico (2)
PN flux	1.4	-0.2	-	mgN.m^{-2}.d^{-1}	-	Terminos Lagoon, Mexico (2)
NH_4^+ flux	-0.71 to 0.26	-	-7.0 (-2.2 to -10.6)	mgN.m^{-2}.d^{-1}	-	Florida, EUA (5; 6)
NO_x^- flux	-1.11 to 0.12	-	12.0 (2.4 to 47.7)	mgN.m^{-2}.d^{-1}	-	Florida, EUA (5; 6)

DIN flux	-1.34 to 0.38	-	5.04	mgN.m^{-2}.d^{-1}	-	Florida, EUA (5; 6)
Net N mineralization	0.440	0.171	-	mgN.g^{-1}.d^{-1}	0.0385	Cardoso Island, Brazil (13)
Net nitrification	0.017	0.031	-	mgN.g^{-1}.d^{-1}	0.1620	Cardoso Island, Brazil (13)
Direct denitrification	(0 to 3.2)	(0 to 1.5)	-	mgN.m^{-2}.d^{-1}	-	Isla del Carmen, Mexico (3)
Coupled denitrification	0	0	-	mgN.m^{-2}.d^{-1}	-	Isla del Carmen, Mexico (4)
Potential direct denitrification	(1.2 to74.3) [a]	-	-	mgN.m^{-2}.d^{-1}	-	Isla del Carmen, Mexico (4)
Potential coupled denitrification	(0.8 to 9.7) [a]	-	-	mgN.m^{-2}.d^{-1}	-	Isla del Carmen, Mexico (4)
Potential denitrification	(2.0 to 84.0) [a]	-	-	mgN.m^{-2}.d^{-1}	-	Isla del Carmen, Mexico (4)
Net N_2O flux	0.03 to 0.4 (0.01 to 0.9)	-	-	mgN.m^{-2}.d^{-1}	-	Magueyes Island, Puerto Rico (7; 8)
NH_3 volatilization	0.2	-	0.38 (0 to 1.05)	mgN.m^{-2}.d^{-1}	-	Twin Cays, Belize (11)
Microbial mats						
BNF	0.03 to 3.2	-	1.6 to 7.6	mgN.m^{-2}.d^{-1}	-	Twin Cays, Belize (10)
BNF	-	-	3.4 (0 to 6.7)	mgN.m^{-2}.d^{-1}	-	Twin Cays, Belize (9)
Denitrification	0.002 to 0.7	-	0 to 0.1	mgN.m^{-2}.d^{-1}	-	Twin Cays, Belize (10)
Denitrification	-	-	0.7	mgN.m^{-2}.d^{-1}	-	Twin Cays, Belize (9)
Potential denitrification	53.6 and 91.9	-	7.5 to 31.0 (6.7 to 67)	mgN.m^{-2}.d^{-1}	-	Twin Cays, Belize (9; 10)

(1) based on the acetylene reduction technique at 10.4 cm depth

(2) fluxes between tidal creek and fringe forest and between fringe and basin forests, based on flume technique

(3) based on ^{15}N enrichment technique at 25 cm depth

(4) based on ^{15}N enrichment technique at 25 cm depth, [a] indicate data for riverine subtype

(5) data for dwarf mangrove based on island enclosure technique

(6) data for fringe forest based on flume technique

(7; 8) based on enclosed chamber technique at 25 cm depth

(9) BNF data based on the acetylene reduction technique at 6 cm depth, and denitrification data based on the acetylene inhibition method at 5 cm depth

(10) BNF data based on the acetylene reduction technique, and denitrification data based on the acetylene inhibition method at 1 cm depth

(11) based on ammonia-sensing badges

(12) based on the acetylene reduction technique at 30 cm depth

(13) based on sediment incubation essays at 10 cm depth

Table 2. Mean or range of natural abundance of nitrogen stable isotopes ($\delta^{15}N$) (‰) of leaves, leaf litter and sediment from fringe, basin and dwarf mangrove forests dominated by *Rhizophora mangle*. When reported by authors, P-values of statistical comparison tests are presented. Source: (1) Fry et al. (2000); (2) Mckee et al. (2002); (3) Wooller et al. (2003); (4) Fogel et al. (2008); (5) Medina et al. (2010); (6) Reis et al. (2017)

Sample	Fringe	Basin/Dwarf	P-value	Location
leaves	2 to 7	-5 to 2	-	Florida, USA (1)
leaves	0.1	-5.4	-	Twin Cays, Belize (2)
leaves	0	-10	< 0.001	Twin Cays, Belize (3)
leaves	-0.6	-6.8	< 0.001	Twin Cays, Belize (4)
leaves	0.2	-11.1 to -5.5	-	Ceiba, Puerto Rico (5)
leaves	3.6	0.9 [a]	< 0.0001	Cardoso Island, Brazil (6)
leaf litter	0.1	-3.3	< 0.005	Twin Cays, Belize (3)
leaf litter	3.3	0.2 [a]	< 0.0001	Cardoso Island, Brazil (6)
sediment	0.1	-0.5	0.0159	Twin Cays, Belize (2)
sediment	3.6	0.7 [a]	< 0.0001	Cardoso Island, Brazil (6)

[a] Indicate data for basin forests. All other data are for dwarf mangroves.

Legend to figures

Fig.1 Location of the 123 study sites considered in this review from studies that quantified nitrogen cycling and flux rates and the natural abundance of nitrogen stable isotopes in mangroves. Because of the map scale, nearby study sites were overlaid.

Fig.2 Nitrogen cycling rates in mangroves. Median rates (and ranges) in $mgN.g^{-1}.d^{-1}$ (unbold values) and $mgN.m^{-2}.d^{-1}$ (bold values) are presented, including biological nitrogen fixation (BNF), nitrous oxide (N_2O) flux, and fluxes of ammonium (NH_4^+), nitrite plus nitrate (NO_x^-), dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), and total dissolved nitrogen (DN) between mangrove sediment and overlying water. Negative values indicate net flux into the sediment. For BNF, mean rates are presented. * incubated on the forest floor. Source: Hesse (1961); Kimball and Teas (1975) *apud* Alongi et al. (1992); Gotto and Taylor (1976); Viner (1979) *apud* Howarth et al. (1988); van der Valk and Attiwill (1984); Hicks and Silvester (1985); Iizumi (1986) *apud* Alongi et al. (1992); Myint (1986) *apud* Alongi et al. (1992); Shaiful et al. (1986) apud Alongi et al. (1992); Kristensen et al. (1988); Mann and Steike (1989) *apud* Alongi et al. (1992); Boto and Robertson (1990); Sengputa and Chaudhuri (1991); Sheridan (1991; 1992); Kristensen et al. (1992); Alongi et al. (1993); Nedwell et al. (1994); Rivera-Monroy et al. (1995a); Alongi (1996); Pelegrí et al. (1997); Woitchik et al. (1997); Kristensen et al. (1998); Pelegrí and Twilley (1998); Chen and Twilley (1999); Corredor et al. (1999); Alongi et al. (1999; 2000); Kristensen et al. (2000); Davis III et al. (2001a,b); Bauza et al. (2002); Lugomela and Bergman (2002); Mohammed and Johnstone (2002); Muñoz-Hincapié et al. (2002); Kyaruzi et al. (2003); Kreuzwieser et al. (2003); Alongi et al. (2004a); Joye and Lee (2004); Lee and Joye (2006); Chauhan et al. (2008); Purvaja et al. (2008); Krishnan and LokaBharathi (2009); Chen et al. (2010); Fernandes et al. (2010; 2012a,b); Romero et al. (2012); and Reis et al. (2017)

Fig.3 Nitrogen cycling in conserved (a) and N-enriched mangroves (b). Median rates (and ranges) in $mgN.m^{-2}.d^{-1}$ are presented, including biological nitrogen fixation (BNF), nitrous oxide (N_2O) flux, and fluxes of dissolved inorganic nitrogen (DIN), dissolved organic nitrogen (DON), and total dissolved nitrogen (DN) between mangrove sediment and overlying water are presented. Negative values indicate net flux into the sediment. For BNF, mean rates are presented. Data of conserved mangroves are based on studies cited in

Figure 2. Data of N-enriched mangroves are based on Corredor and Morell 1994; Corredor et al. 1999; Alongi et al. 2002; Kreuzwieser et al. 2003, Alongi et al. 2005; Purvaja et al. 2008; Chen et al. 2010; and Fernandes et al. 2010; 2012a,b

Figure 1

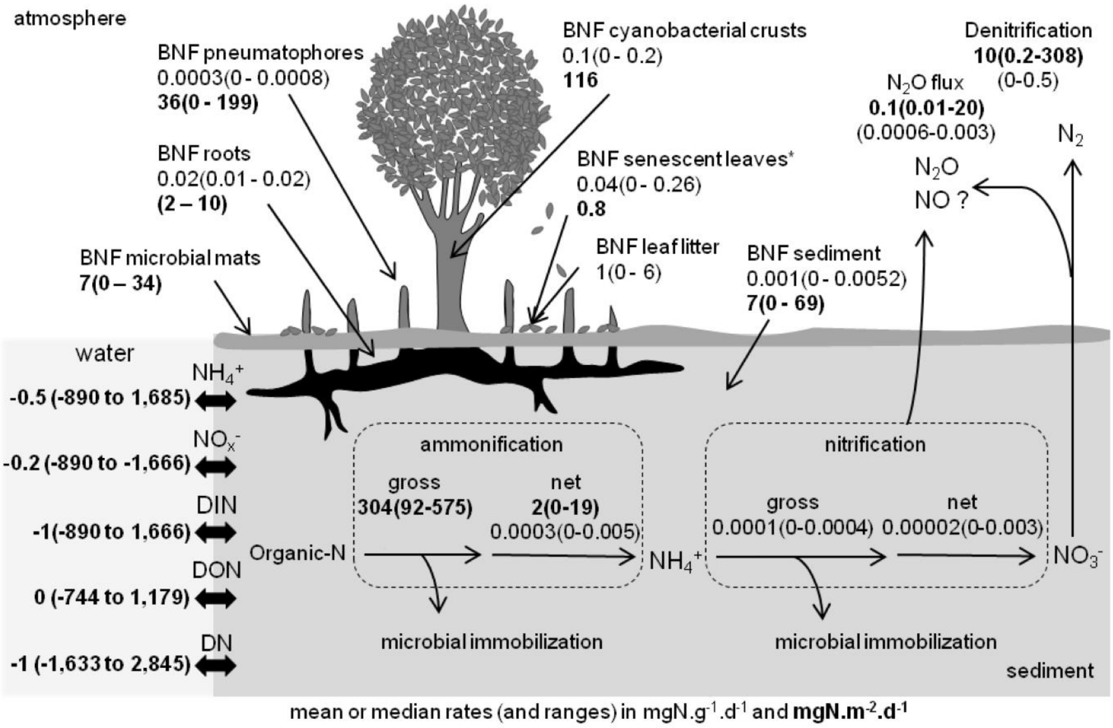
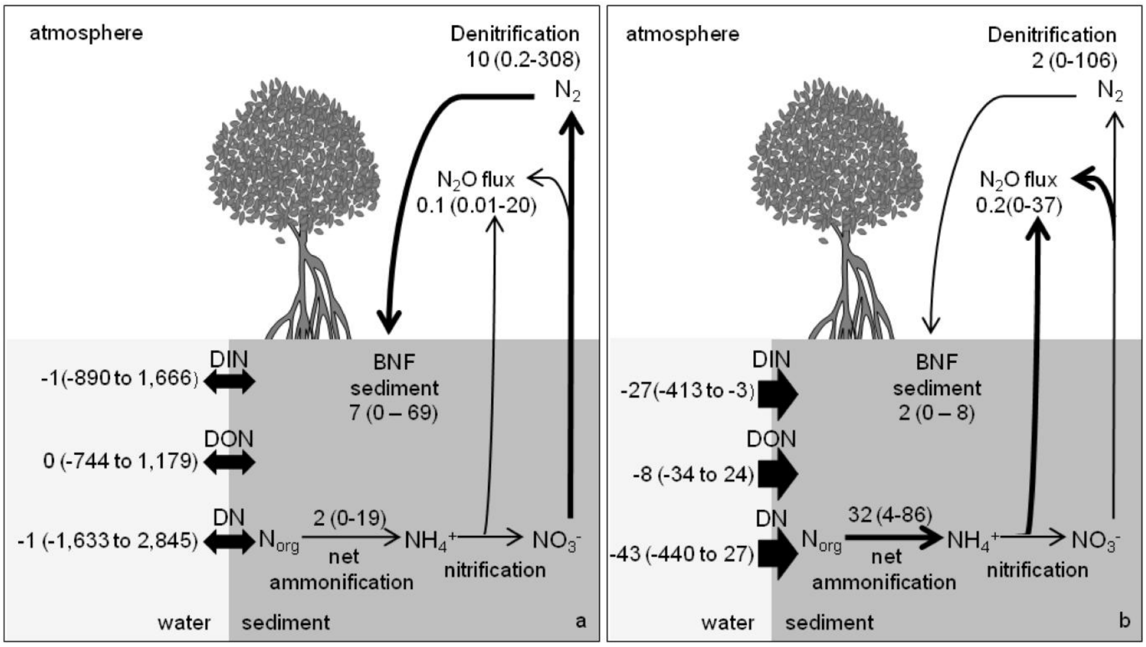

atmosphere
BNF pneumatophores
0.0003(0 - 0.0008)
36(0 - 199)
BNF cyanobacterial crusts
0.1(0 - 0.2)
116
Denitrification
10(0.2-308)
(0-0.5)
N$_2$O flux
0.1(0.01-20)
(0.0006-0.003)
N$_2$
N$_2$O
NO ?
BNF roots
0.02(0.01 - 0.02)
(2 – 10)
BNF senescent leaves*
0.04(0 - 0.26)
0.8
BNF leaf litter
1(0 - 6)
BNF sediment
0.001(0 - 0.0052)
7(0 - 69)
BNF microbial mats
7(0 – 34)
water
NH$_4^+$
-0.5 (-890 to 1,685)
NO$_x^-$
-0.2 (-890 to -1,666)
DIN
-1(-890 to 1,666)
DON
0 (-744 to 1,179)
DN
-1 (-1,633 to 2,845)
ammonification
gross
304(92-575)
net
2(0-19)
0.0003(0-0.005)
Organic-N
NH$_4^+$
nitrification
gross
0.0001(0-0.0004)
net
0.00002(0-0.003)
NO$_3^-$
microbial immobilization
microbial immobilization
sediment
mean or median rates (and ranges) in mgN.g^{-1}.d^{-1} and mgN.m^{-2}.d^{-1}

Figure 2



mean or median rates (and ranges) in mgN.m^{-2}.d^{-1}

Figure 3

Capítulo 2

Isotopic evidence that nitrogen enrichment intensifies nitrogen losses to the atmosphere from subtropical mangroves

Carla Roberta Gonçalves Reis, Sasha Carey Reed, Rafael Silva Oliveira e
Gabriela Bielefeld Nardoto

O presente capítulo foi submetido para apreciação do periódico *Journal of Ecology* em 29 de Janeiro de 2018 e encontra-se em processo de revisão por pares.

CRGR e GBN conceberam o estudo; CRGR, SCR e GBN delinearam a metodologia; CRGR coletou, analisou os dados e liderou a escrita do manuscrito. Todos os autores contribuíram criticamente com a interpretação dos resultados e com a versão final do manuscrito.

Title: Isotopic evidence that nitrogen enrichment intensifies nitrogen losses to the atmosphere from subtropical mangroves

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Abstract

1. Mangrove ecosystems provide critical societal goods and services and although nitrogen (N) enrichment can have large effects on mangroves' capacity to provide these services, our understanding of excess N input effects on N cycling in mangrove ecosystems remains quite limited. To advance our understanding of how N enrichment affects mangrove ecosystems, we evaluated the hypothesis that increasing N inputs decrease biological N fixation (BNF), but intensify N dynamics and N losses to the atmosphere in these systems.
2. To test this hypothesis, we evaluated N concentrations in sediment and vegetation, rates of biological N fixation in sediment and leaf litter, net sediment ammonification and nitrification rates, and overall N dynamics and N losses to the atmosphere using the natural abundance of N stable isotopes ($\delta^{15}N$) in estuarine water and the sediment-plant-leaf litter system. We performed these analyses at non-N-enriched and N-enriched fringe and basin mangroves in the Estuarine Lagunar-Complex of Cananeia-Iguape, southeastern Brazil.
3. The $\delta^{15}N$ of ammonium and nitrate in estuarine water characterized the excess N input from agricultural and/or sewage sources at N-enriched sites. The $\delta^{15}N$ in the sediment-plant system was higher at N-enriched than non-N-enriched sites. However, concentrations of N in sediment and vegetation were similar or lower at N-enriched relative to non-N-enriched sites. Biological N fixation and net ammonification and nitrification rates were also similar between N-enriched and non-N-enriched sites.
4. Synthesis: Excess N inputs intensified N losses to the atmosphere from mangrove sediments, 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 volatilization and/or direct denitrification.

Keywords: ammonification, biological nitrogen fixation, denitrification, global change ecology, nitrification, nitrous oxide, phosphorous, stable isotopes

1. Introduction

Nitrogen (N) availability is one of the main factors regulating the functioning of natural ecosystems (Vitousek and Howarth 1991; Elser et al. 2007). Excess N input via water pollution and N deposition from the atmosphere can have several consequences for natural ecosystems (Galloway et al. 2008): reduced N input via biological N fixation (Niu et al., 2016; Reed, Cleveland, & Townsend 2011), modified N cycling and increased N losses to the atmosphere (Erisman et al. 2013; Niu et al. 2016), altered climate feedbacks via increased nitrous oxide (N_2O) and other greenhouse gas emissions (Erisman et al., 2013; Erisman, Galloway, Seitzinger, Bleeker, & Butterbach-Bahl 2011), and altered provision of societal goods and services (Compton et al., 2011; de Vries, Goodale, Erisman, & Hettelingh 2014). However, quantifying the consequences of increased anthropogenic inputs of N remains an important research challenge. Patterns in N dynamics (i.e., N transformations in soil) and N losses to the atmosphere in terrestrial ecosystems can be evaluated indirectly by the natural abundance of N stable isotopes ($\delta^{15}N$) in the soil-plant system. Because the $\delta^{15}N$ integrates long-term fractionation against the heavier ^{15}N isotope during N transformations such as nitrification, denitrification, and volatilization (Högberg 1997; Robinson 2001), the $\delta^{15}N$ in the soil-plant system can be used as a measurement of the intensity of N dynamics and N losses to the atmosphere from these systems (e.g., Houlton, Sigman, & Hedin 2006; Nardoto et al., 2008). Terrestrial ecosystems with intensified N dynamics and N losses to the atmosphere have higher $\delta^{15}N$ signatures in the soil-plant system (Craine et al. 2009, 2015b). 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 provide important societal goods and services directly and indirectly, including significant carbon (C) sequestration in coastal areas (Alongi, 2014a) and the storage of a larger amount of C per unit area than terrestrial forests and marine ecosystems (Alongi, 2014b; Donato et al., 2011), mangroves' trapping of excess organic matter and nutrients from surrounding waters helping to determine water quality in coastal areas (e.g., Alongi & McKinnon, 2005; Jordan, Stoffer, & Nestlerode 2011), and also the mangroves' support of coastal fisheries (e.g., Aburto-Oropeza et al., 2008; Carrasquilla-Henao & Juanes, 2017). Despite of the importance of N availability in the provision of the critical societal services provided by mangrove ecosystems, data for mangrove N dynamics and N losses to the atmosphere in the face of excess N inputs are quite limited. Taken together, the studies that do exist suggest that biological N fixation can be dramatically reduced by increased N availability (Romero, Jacobson, Fuhrman, Fogel, & Capone 2012; Whigham, Verhoeven, Samarkin, & Megonigal 2009), while N losses to the atmosphere, including of N_2O, seem to be intensified by excess inputs of N (e.g., Corredor, Morell, & Bauza 1999; Fernandes, Loka Bharathi, Bonin, & Michotey 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, Nardoto, & Oliveira 2017). 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 N transformation pathways in different types of mangroves.

To advance our understanding of how N enrichment via water pollution and N deposition from the atmosphere affect mangrove ecosystems and to improve our capacity to make effective decisions that maintain resilience in the provision of societal goods and services by these systems, we 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 biological N fixation in sediment and leaf litter, sediment net ammonification and nitrification rates, and $\delta^{15}N$ in the sediment-plant-leaf litter system and estuarine water at non-N-enriched and N-enriched fringe and basin mangroves in the Estuarine Lagunar-Complex of Cananeia-Iguape, São Paulo, Brazil. This study builds on an earlier study of N dynamics at conserved fringe and basin mangroves in the Cananeia region (Reis, Nardoto, Rochelle, Vieira, & Oliveira 2017), which comprises the most conserved and protected mangrove areas in the São Paulo State (Cunha-Lignon et al. 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.

2. Material and Methods

2.1. Study area

The study was conducted at 6 mangrove sites under a humid subtropical climate in the Estuarine Lagunar-Complex (ELC) of Cananeia-Iguape, southeastern Brazil, in 2016 and 2017 (Fig. 1): a fringe and a basin mangrove in a conserved mangrove area in the Cardoso Island State Park that were control sites (Control); a fringe and a basin mangrove in the Cananeia Island, closer to small urban areas and believed to receive some N input (+N); and a fringe and a basin mangrove strongly affected by N enrichment from the Ribeira de Iguape River watershed in the Iguape region (++N).

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 & Leechm. ex Moldenke (Acanthaceae) (families according to APG III 2009). Because of the influence of the Ribeira de Iguape river discharge, sediment is ~ 70% silt and clay in at +N and ++N sites, while ~ 80% sand in Control sites, under a greater marine influence (Table 1). As a consequence, organic matter concentrations are about two to four times higher in +N and ++N than the Control sites. The sum of exchangeable bases and the cation exchange capacity (CEC) are about two times higher in sediment at fringe sites relative to basin sites. More information for the Control sites can be found in Reis, Nardoto, Rochelle et al. (2017).

2.2. Sampling design

Three transects of 60 m length each and with a distance of 30 m 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, leaf samples were obtained from 3 arboreal individuals with diameter at breast height (DBH) ≥ 4 cm, as well as a composite leaf litter sample, hand-collected from the forest floor below the sampled trees. Leaves were collected to assess N and P concentrations and $\delta^{15}N$ was assessed in leaves and leaf litter (methods below).

Sediment samples at 0-10 cm depth were also obtained from each sampling point to characterize $\delta^{15}N$, total N concentrations, net ammonification and nitrification rates, and the concentrations of available NH_4^+, NO_3^- and P. 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. 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).

Estuarine water samples were collected during flood tide at 3 sampling points in the shoreline of each fringe site, and at 1 sampling point in the Valo Grande canal (VG) to characterize the $\delta^{15}N$ 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.

2.3. 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 & 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 & 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*.

2.4. $\delta^{15}N$ of NH_4^+ and NO_3^- in estuarine water

For the analysis of $\delta^{15}N$ of NH_4^+, samples were filtered through 0.45-µm filter membrane and preserved by decreasing the pH with concentrated H_2SO_4 (Hannon and Böhlke 2008). The $\delta^{15}N$ 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, McClelland, Sigman, Fry, & Peterson 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 $\delta^{15}N$ of NO_3^-, samples were filtered through 0.2-μm filter membrane and preserved by raising the pH with NaOH (Coplen, Qi, Revesz, Casciotti, & Hannon 2012). The $\delta^{15}N$ of NO_3^- was analyzed by the enzymatic conversion of NO_3^- to N_2O by the denitrifying bacteria *Pseudomonas aureofaciens* (Sigman et al., 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 U.S. 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 $\delta^{15}N$ analysis.

2.5. 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, Holsten, Jackson, & Burns 1968). After 2-hours of incubation at ambient temperature, the atmosphere inside the tubes was collected and analyzed for ethylene (C_2H_4) concentrations using a gas chromatograph in the Southwest Biological Science Center of the US Geological Survey. Control incubations were performed with samples without C_2H_2 addition to discount endogenous C_2H_4 production, and with C_2H_2 addition without samples to discount C_2H_4 concentrations in the acetylene gas used.

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), since 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 dry weight and areal basis.

2.6. Net ammonification and nitrification rates

Net ammonification and nitrification rates in sediment were quantified according to the incubation and extraction procedures described by Reis, Nardoto, Rochelle et al.

(2017), modified from Piccolo, Neill, and Cerri (1994). Extracts were analyzed for concentrations of N-NH_4^+ with a Nessler reagent (Greweling and Peech 1960) and N-NO_3^- (Meier, 1991, cited in Sutton et al., 2014) using an UV spectrophotometer in the Laboratório de Ecossistemas of the Universidade de Brasília.

2.7. δ^{15}N in the sediment-plant-leaf litter system and leaf N concentrations

Sediment, leaf, and leaf litter samples were prepared according to procedures described by Reis, Nardoto, Rochelle et al. (2017) and analyzed for total C and N, C:N ratio, and δ^{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.

2.8. Sediment available P and leaf P concentrations

Available 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.

2.9. 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 Jr (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).

3. Results

3.1. Vegetation structure

Aboveground biomass was similar between Control and +N fringe sites, but about two times higher at these sites than ++N fringe (Table 2 and Table S1/ANEXO 1). Aboveground biomass was about two times higher at Control basin than ++N basin, and about five to six times higher than +N basin. Aboveground biomass was also up to 10 times higher at fringe than basin sites. Considering both fringe and basin sites, *Rhizophora mangle* dominated 99% of BA in Control and 94% in +N, while *L. racemosa* dominated 89% of BA in ++N.

3.2. $\delta^{15}N$ 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 -1.2 to 0.1‰ at ++N site (Table 3). The $\delta^{15}N$ of NH_4^+ was also six times higher at +N (3.6‰) than 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. The $\delta^{15}N$ of NO_3^- was below detection limit for Control samples.

3.3. 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 (Fig. 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.

3.4. Net ammonification and nitrification rates and available N and P

Concentrations of N-NO_3^- in sediment were two 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 (1st and 3rd 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. 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 ($P \geq 0.05$).

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 to 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).

3.5. $\delta^{15}N$ in the sediment-plant-leaf litter system

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

Leaf $\delta^{15}N$ values were about two to three times higher in *L. racemosa* and *A. shaueriana* than *R. mangle* (Table S2/ANEXO 2). *Laguncularia racemosa* leaf $\delta^{15}N$ 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 $\delta^{15}N$ values higher at ++N and +N than Control fringe sites, and two orders of magnitude higher at +N than Control basin sites, *F*(4,75) = 3.42, *P* = 0.014 (data not shown).

The $\delta^{15}N$ values from fringe sites were about three times higher in sediment, about two to five times higher in leaves, and three to eleven times higher in leaf litter than basin sites. Leaf $\delta^{15}N$ values of both *R. mangle* and *L. racemosa* were higher at fringe than basin sites ($P \geq 0.05$).

3.6. Leaf N and P concentrations

Leaf N concentrations were higher at +N than ++N fringe sites, and Control fringe sites were not statistically different from either the +N or ++N fringe sites (Fig. 4). Leaf N concentrations were higher at Control and +N than ++N basin sites, and higher at fringe than basin sites. Leaf N concentrations were about two times higher in *A. shaueriana* than *R. mangle* and *L. racemosa,* and higher in *R. mangle* than *L. racemosa* (Table S2/ANEXO 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 Control basin sites. Leaf P concentrations were similar between fringe and basin sites 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 two times higher at Control and +N than ++N basin sites. Leaf N:P ratios were similar between fringe and basin sites. Leaf N:P ratios were similar between *R. mangle* and *A. shaueriana*, but higher in these species than the N:P ratios for *L. racemosa*. Leaf N:P ratios of *R. mangle* were about two times higher at Control and +N sites relative to the ++N fringe sites, 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 Control basin than Control fringe, while higher at +N fringe than basin, $F(4,31) = 4.74$, $P = 0.006$ (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).

3.7. 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 (Fig. 4). Leaf C:N ratios were also higher at basin than fringe in Control and ++N sites, but similar at +N. Leaf C:N ratios were up to two times higher in *L. racemosa* than *R. mangle* and *A. shaueriana*, and two times higher in *R. mangle* than *A. shaueriana* (Table S2/ANEXO 2). Leaf C:N ratios of *L. racemosa* were similar between fringe sites, and between basin sites ($P \geq 0.05$) (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 (1st and 3rd 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 two times higher at basin than fringe sites in +N and ++N, but similar in Control ($P \geq 0.05$).

4. Discussion

Collectively, our results indicate that the excess of N intensified N losses to the atmosphere from mangrove sediment, but that biological N fixation, and net ammonification and nitrification rates were not affected by N enrichment, likely because excess N was quickly lost from the system (Fig. 5). We further argue that a large proportion of excess N was mainly lost as N_2O via incomplete direct denitrification, and by NH_3 volatilization, rather than as N_2 via complete coupled denitrification. Considering 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 characterized the excess of N originated from human activities affecting mangroves in 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 the excess of NO_3^- from sewage and agricultural sources in the upstream watershed reaching mangrove areas 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^+ in +N indicated inputs of NH_4^+ from local sewage sources. The higher $\delta^{15}N$ in the sediment at ++N and in the leaves at ++N and +N than Control sites also indicated the excess N input from human activities in the ++N and +N sites. Previous studies consistently reported higher leaf $\delta^{15}N$ values from N-enriched than 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 heavier isotope (*i.e.*, Mckee, Feller, Popp, & Wanek 2002; Fogel et al., 2008). Nitrogen sources from both sewage and agriculture in the upstream watershed can result in high leaf $\delta^{15}N$ values in mangroves (Costanzo, O'Donohue, & Dennison 2003; Gritcan, Duxbury, Leuzinger, & Alfaro 2016). Despite the fact that inorganic N fertilizers have lower $\delta^{15}N$ signatures than organic matter, excess N input from both sources led to intensified N dynamics and N losses to the atmosphere in the upstream watershed, resulting in ^{15}N-enriched NH_4^+ and NO_3^- pools (Fry, Gace, & McClelland 2003). Both sewage and inorganic N fertilizers from the upstream watershed and local sources contributed with the excess N affecting mangrove areas in the ELC of Cananeia-Iguape.

The high $\delta^{15}N$ values in the sediment-plant system from Iguape and Cananeia Island sites not only showed excess N inputs from human activities to mangroves, but also intensified N losses to the atmosphere from these systems as a consequence of excess N input (e.g., Costanzo, O'Donohue, & Dennison 2004; Fry & Cormier, 2011; Reef, Feller, & Lovelock 2014). Interestingly, the concentrations of NH_4^+ in sediment were similar between N-enriched and non-N-enriched sites, indicating that the excess NH_4^+ reaching N-enriched sites were absorbed by mangrove plants, immobilized in the microbial biomass, converted to other forms of N, and/or lost to the atmosphere (Fig. 5). Because the $\delta^{15}N$ does not change when a N pool is divided but not transformed (Robinson, 2001) such as in N exchange with tidal water or leaching, the higher $\delta^{15}N$ at N-enriched sites indicated higher N losses to the atmosphere. While we did not measure total N content in vegetation, considering the lower aboveground biomass and the dominance by *L. racemosa* in ++N, which has a lower foliar N concentrations and higher C:N ratios in leaves than *R. mangle*, a lower total N content would be expected at N-enriched relative to non-N-enriched sites. This suggests that vegetation was not an important 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 (e.g., Alongi, Christoffersen, & Tirendi 1993; Rivera-monroy & Twilley, 1996; Reis, Nardoto, & Oliveira 2017), and therefore likely an important fate of excess N in N-enriched sites. Nevertheless, despite higher organic matter concentrations and lower C:N ratios in sediment at N-enriched sites, sediment net nitrification rates were similar between N-enriched and non-N-enriched sites, so that 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 high pH range (around 6.8, up to 8.6) recorded in the studied sites. This pathway would help explain the ^{15}N-enriched N pools in the sediment of N-enriched sites. Ammonia volatilization rates were previously reported for mangrove sediment as a function of NH_4^+ availability, with rates up to about 2 $mgN.g^{-1}.d^{-1}$ under N fertilization (Fogel et al. 2008).

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, as discussed above, both NO_3^- immobilization in microbial biomass and denitrification were likely the main sinks of excess NO_3^- in nitrate-enriched sites (Fig. 5). Redox potentials were typically moderately reducing, which is favorable for denitrification in waterlogged conditions (Patrick Jr. & Mahapatra, 1968; Reef, Feller, & Lovelock 2010). Because net nitrification rates were similar between study sites, excess NO_3^- was likely mainly 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, Nardoto, & Oliveira 2017; Rivera-Monroy & Twilley, 1996). In addition, previous studies consistently found increased N_2O fluxes from N-enriched than non-N-enriched mangroves (e.g., Chen, Tam, & Ye 2010; Fernandes, Loka Bharathi, Bonin, & Michotey 2010; reviewed by Reis, Nardoto, & Oliveira 2017). Therefore, direct incomplete denitrification was likely an important pathway of N loss to the atmosphere at nitrate-enriched 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 to 20 $mgN.m^{-2}.d^{-1}$) greatly overlap those reported for terrestrial tropical forests (0.02 to 13.7 $mgN.m^{-2}.d^{-1}$; Silver et al., 2000; Kiese, Hewett, Graham, & Butterbach-Bahl 2003; Fang et al., 2015), while rates reported for N-enriched mangroves can be even higher (up to 37 $mgN.m^{-2}.d^{-1}$) (reviewed by Reis, Nardoto, &

Oliveira 2017) than those reported for N-enriched terrestrial tropical forest soils (up to 12 $mgN.m^{-2}.d^{-1}$) (e.g., Keller, Veldkamp, Weitz, & Reiners 1993).

Biological N fixation rates in mangrove sediment were previously reported to be reduced up to about 50 to 75% upon long-term N-amendment (Romero, Jacobson, Fuhrman, Fogel, & Capone 2012; Whigham, Verhoeven, Samarkin, & Megonigal 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 helps support a framework of N being relatively quickly lost from the system, such that diazotroph communities remain N-limited (Fig. 5). Recent findings also indicate that BNF rates in sediment at highly N-enriched mangroves can be similar or even higher than non-N-enriched mangroves (Ray, Majumder, Das, Chowdhury, & Jana 2014; Shiau, Lin, Tan, Tian, & Chiu 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 to 69 $mgN.m^{2}.d^{-1}$) and N-enriched mangroves (0 to 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 to 6 $mgN.g^{-1}.d^{-1}$), based on the same technique (reviewed by Reis, Nardoto, & Oliveira 2017).

In addition to differences in N cycling along the gradient of anthropogenic N inputs, the $\delta^{15}N$ 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 $\delta^{15}N$ 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). These results presented here add to this by indicating higher N availability at fringe than basin sites from either higher N input and/or higher N mineralization rates in sediment. Because biological N fixation and net ammonification rates were similar between fringe and basin sites, higher inorganic N input from tidal waters likely contributed with 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 waters and basin mangroves (Rivera-Monroy, Day, Twilley, Vera-Herrera, & Coronado-Molina 1995). Sum of exchangeable bases were also higher at fringe than basin sites as a consequence of higher inundation frequency and nutrient input from tidal waters.

The higher $\delta^{15}N$ 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, Nardoto, Rochelle et al., 2017). Because of higher aboveground biomass and N concentrations in leaves at fringe than basin sites, vegetation was likely an important fate of the higher N input from tidal waters at fringe sites. In turn, the different N dynamics in the fringe sites likely helped sustain a much larger mangrove aboveground biomass. As discussed for N-enriched sites above, because we observed 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 was also likely immobilized in microbial biomass in sediment and/or lost to the atmosphere. NH_3 volatilization and direct denitrification were likely 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 (Reis, Nardoto, Rochelle et al., 2017), and higher direct denitrification rates at fringe than basin sites (Rivera-Monroy et al., 1995). 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, and *R. mangle* N:P ratios were lower at N-enriched than non-N-enriched sites, which characterized the excess P input at N-enriched sites originating from human activities. Because of the higher P availability at N-enriched 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 in sediment and of NH_4^+ and NO_3^- in sediment and estuarine water were not good indicators of the excess P and N reaching mangrove sites in the ++N and +N sites. Nutrient concentrations represent the net balance of several factors as discussed above for sediment, and therefore are less reliable indicators of long-term nutrient status. Long-term indicators, such as mangrove δ^{15}N signature and leaf nutrient concentrations are better indicators of mangrove nutrient status than indicators that integrate over shorter timescales (e.g., Wolters, Gillis, Bouma, van Katwijk, & Ziegler 2016; see Reis, Nardoto, Rochelle et al., 2017).

Mangrove sites in the Control and +N were dominated by *R. mangle*, while mangrove sites in ++N 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* were reported to outcompete *R. mangle* during the seedling stage at high nutrient availability (McKee 1995), and to dominate mangrove stands under low salinity (Estrada, Soares, Chaves, & Cavalcanti 2013). The diversion of freshwater into the estuary dramatically reduced sediment porewater sanility in the Iguape region, with values ranging from 0 to 4, while porewater salinity ranges from 20 to 28 in the Cananeia region (Cunha-Lignon et al. 2015). Whether the Valo Grande opening promoted *L. racemosa* dominance in mangrove stands in the Iguape region is unknown but it likely contributes with 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 N losses to the atmosphere and P availability in mangrove ecosystems, but also promotes the invasion by aquatic macrophytes in mangrove areas in the Iguape region (Cunha-Lignon et al. 2011). In the present study, we selected mangrove sites without a marked presence of aquatic macrophytes, and avoided to collect 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 (C. R. G. Reis et al., unpublished data), and the study of their consequences for carbon dynamics and stocks in mangrove sediment is underway.

In conclusion, the results of the present study indicated that the excess N related to anthropogenic inputs intensified N losses to the atmosphere from mangrove sediment, as indirectly indicated by higher $\delta^{15}N$ values in the sediment-plant system of N-enriched relative to non-N-enriched 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 that excess N was quickly lost from the system. These results also suggest that excess N was likely mainly lost as N_2O via incomplete direct denitrification, and by NH_3 volatilization, rather than as N_2 via complete coupled denitrification. 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 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 they continue to cycle higher inputs of N. More studies on N pollution effects on climate feedbacks of mangrove ecosystems are needed, especially considering a range of temperature regimes. 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 a powerful way to assess dynamic N cycling processes and responses to change (Reed, Cleveland, & Townsend 2008), are promising approaches to provide a detailed description of N pollution effects on N dynamics and N_2O emissions from mangrove ecosystems.

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Authors' contributions

CRGR and GBN conceived the ideas; CRGR, SCR and GBN designed methodology; CRGR collected and analyzed the data. All authors contributed discussing the results and writing the manuscript. All authors gave final approval for publication.

Data accessibility

Data will be made available from the U.S. Geological Survey Repository.

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Table 1. Physical and chemical properties of sediment at 0-20 cm depth from mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil.

	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 from non-N-enriched (Control) fringe (F) and basin (B) mangroves, and those subjected to some N input from small urban areas (+N), and high N input from a large upstream watershed (++N). Data of Control sites are from Reis et al. (2017b). Data of +N and ++N sites are from 5 samples combined into a composite sample for each site and analyzed using the same methods (unpublished data). Sa = sand, Si = silt, Cl = clay, Lo = loam, OM = organic matter, SEB = sum of exchangeable bases, CEC = cation exchange capacity

Table 2. Live aboveground biomass (AGB) and basal area dominance by *Rhizophora mangle* and *Laguncularia racemosa* at the mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil

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

Data from one plot comprising 20 arboreal individuals taller than 1 m per site. Data from non-N-enriched (Control) fringe (F) and basin (B) mangroves, and those subjected to some N input from small urban areas (+N), and high N input from a large upstream watershed (++N)

Table 3. Natural abundance of nitrogen stable isotopes ($\delta^{15}N$) (‰) of ammonium (NH_4^+) and nitrate (NO_3^-) in estuarine water from the mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil

	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 3 samples collected during flood tide at the shoreline of each fringe site, and 1 sample in the Valo Grande (VG) canal. Data from non-N-enriched (Control) mangroves, and those subjected to some N input from small urban areas (+N), and high N input from a large upstream watershed (++N). B.D. = below detection limit

Table 4. Natural abundance of nitrogen stable isotopes ($\delta^{15}N$) (‰) in the sediment-plant-leaf litter system from non-N-enriched and N-enriched mangrove sites around the world. Modified from Reis et al. (2017a)

Sample	Species	Non-N-enriched				N-enriched			P-value	N source	Location
		Fringe	Basin/Dwarf	NR	*P* -value	Fringe	Basin/Dwarf	NR			
sediment	*Rhizophora mangle*	0.1	-0.5[d]		*						BLZ(3)
	-	3.7				5.2			**	shrimp farms	THA(4)
	multispecies	3.6	0.7		***						BRA(13)
leaves	*R. mangle*	2	-5 to 2[d]			7 to 10	-3 to -1[d]			agriculture	USA(1)
	Avicennia marina			1.6 to 2.2				3.9 to 12.2		sewage	AUS(2)
	R. mangle	0.1	-5.4[d]			-3.4	-8.1[d]			urea	BLZ(3)
	multispecies	3.7				5.2			**	shrimp farms	THA(4)
	Laguncularia racemosa	1.5	1.2			11.4 to 12.3				bird guano	BLZ(5)
	R. mangle	0	-10[d]		**						BLZ(6)
	Rhizophora stylosa	2.4 to 4.4				5.1 to 5.6			*	shrimp farms	AUS(7)
	R. mangle	-0.6	-6.8[d]		**						BLZ(8)
	R. mangle	0.2 to 0.8	-7.3 to -3.2[d]			-4.1 to -1	-9.7 to -4.8[d]		**	urea	BLZ(8)
	R. mangle	0.2	-11.1 to -5.5[d]								PRI(9)
	R. mangle			2.6				4.7 to 11.6	*	watershed	USA(10)
	multispecies	-1 to 2				0 to 4			*	bat guano	AUS(11)
	A. marina	1.6 to 4				5			*	kangoroo feces	AUS(11)
	R. mangle	9				15			**	bird guano	MEX(12)
	multispecies	3.6	0.9		***						BRA(13)
leaf litter	*R. mangle*	0.1	-3.3[d]		*						BLZ(6)
	multispecies	3	0.2		***						BRA(13)

Mean or median values or ranges are presented. NR = mangrove type not reported, ^{d} Data for dwarf subtype. P-values are presented when 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). Source: (1) Fry, Bern, Ross, and Meeder (2000); (2) Costanzo, O'Donohue, Dennison, Loneragan, and Thomas (2001); (3) Mckee, Feller, Popp, and Wanek (2002), data for sediment at 0-20 cm depth; (4) Thimdee, Deein, Thimdee, Sangrungruang, and Matsunaga (2002), 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; (5) Wooller, Smallwood, Jacobson, and Fogel (2003); (6) Wooller, Smallwood, Scharler, Jacobson, and Fogel (2003); (7) Costanzo, O'Donohue, and Dennison (2004); (8) Fogel et al. (2008); (9) Medina, Cuevas, and Lugo (2010); (10) Fry and Cormier (2011); (11) Reef, Feller, and Lovelock (2014), data for *R. stylosa*, *Ceriops tagal*, and *Lumnitzera rosea* pooled together; (12) Adame, Fry, Gamboa, and Herrera-Silveira (2015); (13) Reis, Nardoto, Rochelle, Vieira, and Oliveira (2017b), data for sediment at 0-10 cm depth, and for *R. mangle*, *L. racemosa*, and *Avicennia shaueriana* pooled together

Figure legends

Fig 1. Location of the mangrove study sites in the Estuarine Lagunar-Complex of Cananeia-Iguape, southeastern Brazil. “Control” = non-N-enriched mangroves, “+N” = mangroves subjected to some N input from small urban areas, and “++N” = mangroves subjected to high N input from a large upstream watershed. The shapefiles of mangrove forest areas were provided by Marília Cunha-Lignon. Source: Cunha-Lignon et al. (2011)

Fig 2. Biological nitrogen fixation rates (BNF) on dry-weight and areal basis in sediment at 0-10 cm depth and leaf litter from the mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil. Data from non-N-enriched (Control) mangroves, and those subjected to some N input from small urban areas (+N), and high N input from a large upstream watershed (++N). Grey bars indicate fringe mangroves and white bars indicate basin mangroves. Median and 1st and 3rd quartiles values are presented. NS = not significant statistical difference, ** $P \leq 0.01$. Different letters indicate significant statistical difference among study sites (N = 7 for each study site)

Fig 3. Natural abundance of nitrogen stable isotopes ($\delta^{15}N$) in the sediment-plant-leaf litter system from the mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil. Data from non-N-enriched (Control) mangroves, and those subjected to some N input from small urban areas (+N), and high N input from a large upstream watershed (++N). Grey bars indicate fringe mangroves and white bars indicate basin mangroves. Median and 1st and 3rd quartiles values are presented. *** $P \leq 0.001$, * $P \leq 0.05$. Distinct letters indicate significant statistical difference between study sites (N = 9 for sediment and leaf litter samples and 27 for leaf samples for each study site)

Fig 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 the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil. Data from non-N-enriched (Control) mangroves, and those subjected to some N input from small urban areas (+N), and high N input from a large upstream watershed (++N). Grey bars indicate fringe mangroves and white bars indicate basin mangroves. Median and 1st and 3rd quartiles values are presented. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$. Distinct letters indicate significant statistical difference between study sites (N = 27 for N concentrations and C:N ratios, and from 9 to 19 for P concentrations and N:P ratios for each study site)

Fig 5. Schematic view of nitrogen (N) cycling at the non-N-enriched Control (A) and N-enriched ++N (B) fringe sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil. Solid arrows indicate rates measured in the present study. Dashed arrows indicate rates and fluxes not measured in the present study but indirectly evaluated by $\delta^{15}N$ results. The arrows' width indicates the likely intensity of N transformation and flux rates in the studied sites.

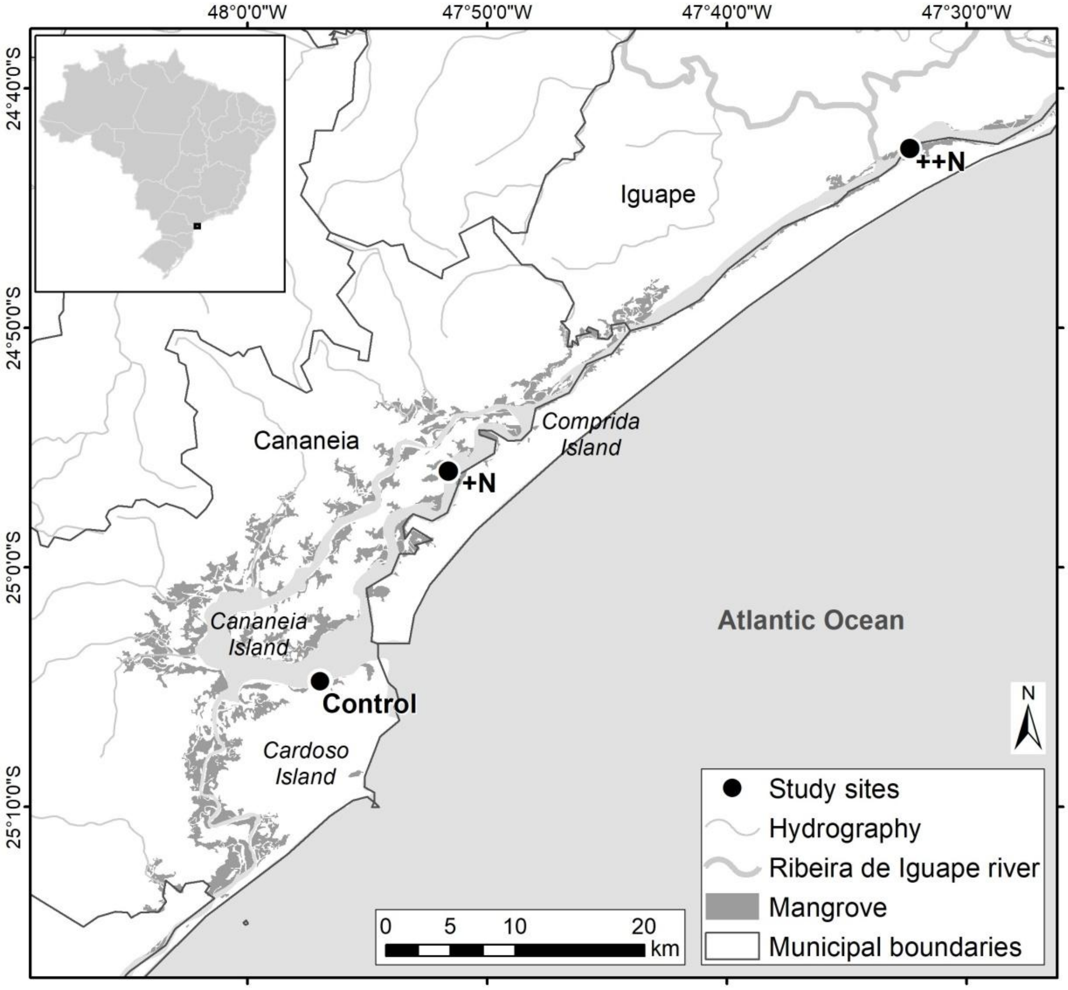
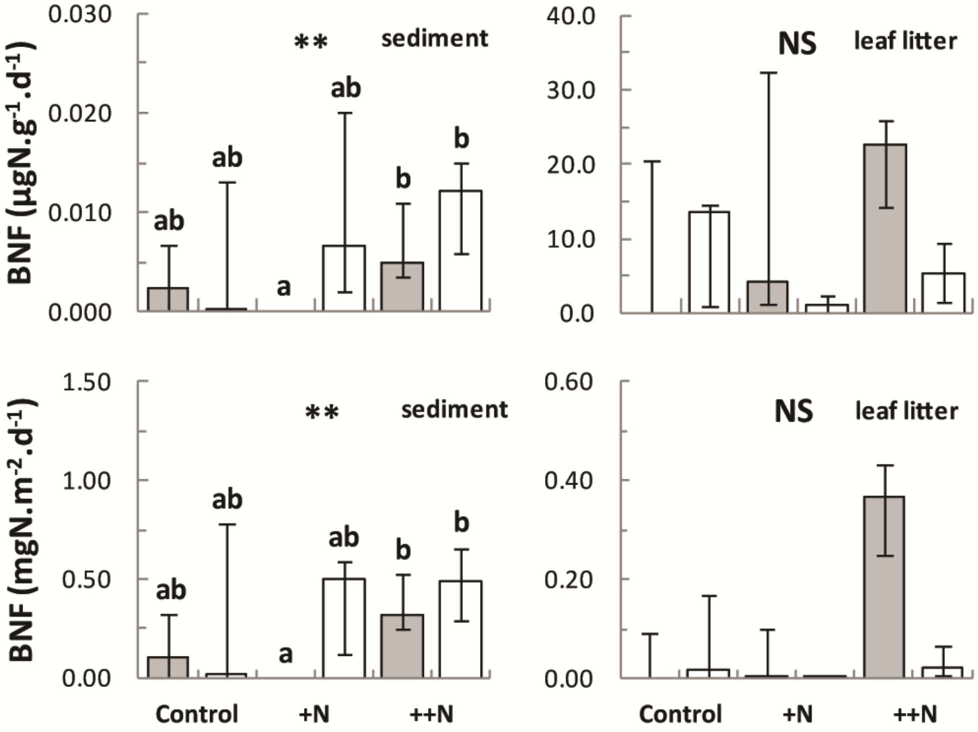
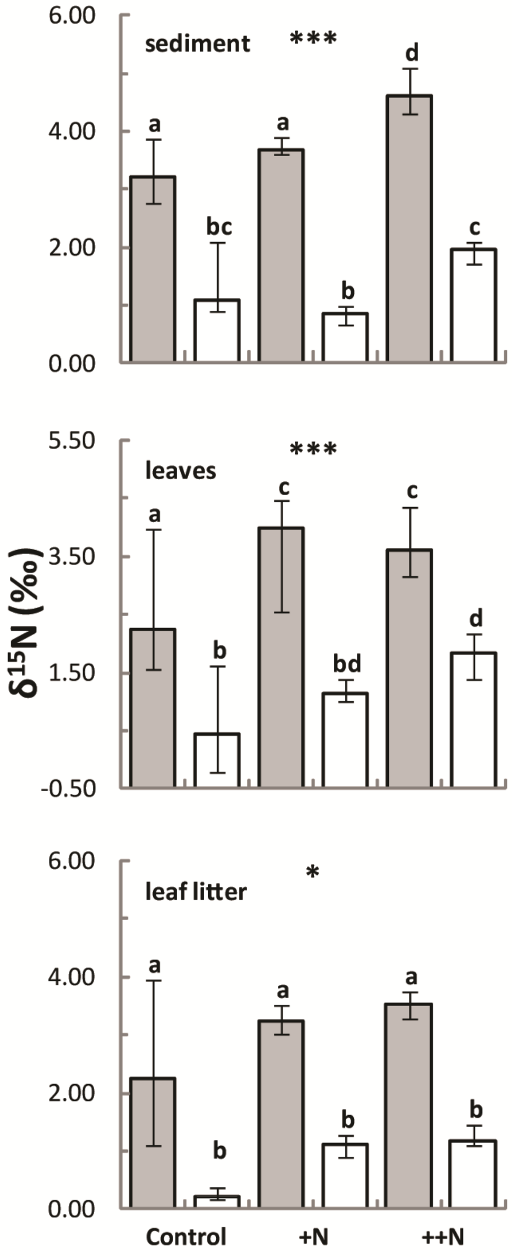

48°0'0"W
47°50'0"W
47°40'0"W
47°30'0"W
24°40'0"S
24°50'0"S
25°0'0"S
25°10'0"S
Iguape
++N
Cananeia
Comprida Island
+N
Cananeia Island
Control
Cardoso Island
Atlantic Ocean
N
Study sites
Hydrography
Ribeira de Iguape river
Mangrove
Municipal boundaries
0
5
10
20
km

Figure 1



BNF (µgN.g^{-1}.d^{-1})
0.030
0.020
0.010
0.000
**
sediment
ab
ab
a
ab
b
b
40.0
30.0
20.0
10.0
0.0
NS
leaf litter
BNF (mgN.m^{-2}.d^{-1})
1.50
1.00
0.50
0.00
**
sediment
ab
ab
a
ab
b
b
0.60
0.40
0.20
0.00
NS
leaf litter
Control
+N
++N
Control
+N
++N

Figure 2


sediment
***
6.00
4.00
2.00
0.00
a
bc
a
b
d
c
leaves
***
5.50
3.50
1.50
-0.50
a
b
c
bd
c
d
δ15N (‰)
leaf litter
*
6.00
4.00
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a
b
a
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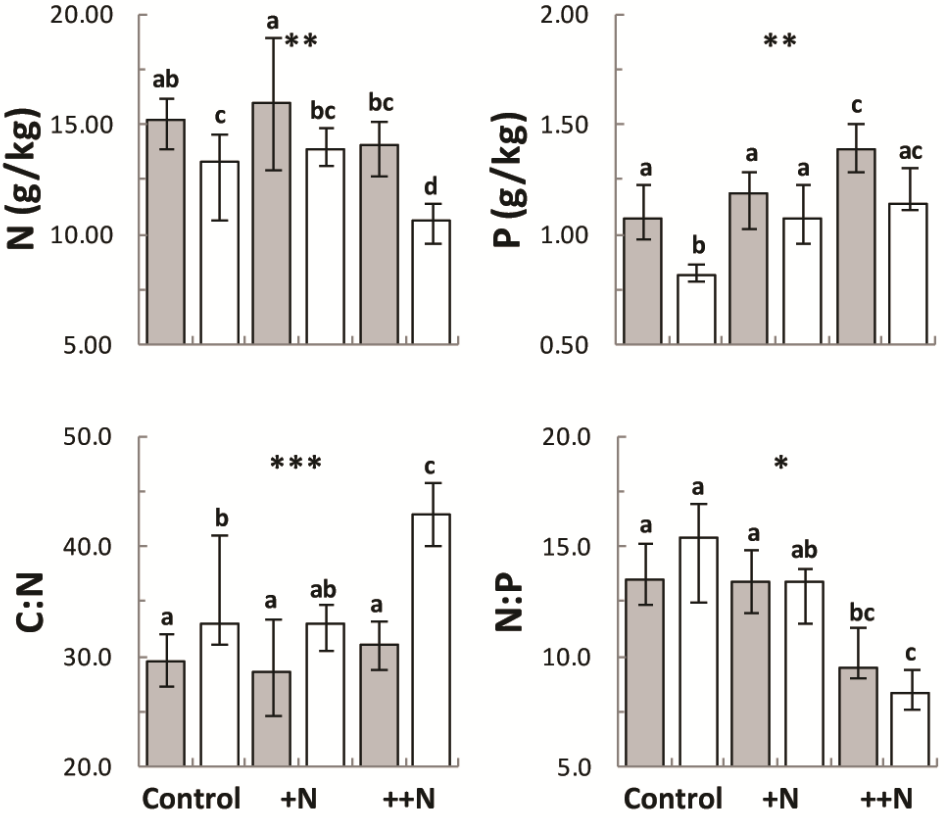
b
Control
+N
++N

Figure 3


N (g/kg)
20.00
15.00
10.00
5.00
ab
c
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bc
bc
d
**
P (g/kg)
2.00
1.50
1.00
0.50
a
b
a
a
c
ac
**
C:N
50.0
40.0
30.0
20.0
a
b
a
ab
a
c
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N:P
20.0
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a
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Control
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Control
+N
++N

Figure 4

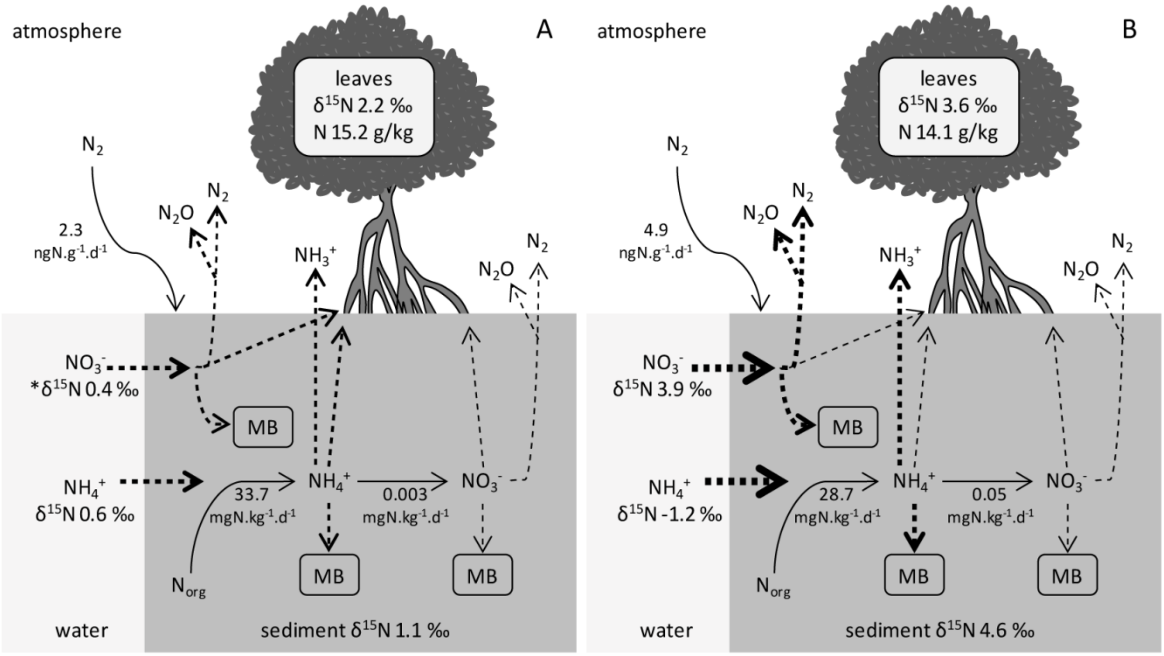
A
atmosphere
leaves
δ15N 2.2 ‰
N 15.2 g/kg
N2
2.3
ngN.g-1.d-1
N2O
N2
NH3+
N2O
N2
NO3-
*δ15N 0.4 ‰
MB
NH4+
δ15N 0.6 ‰
33.7
mgN.kg-1.d-1
NH4+
0.003
mgN.kg-1.d-1
NO3-
Norg
MB
MB
water
sediment δ15N 1.1 ‰
B
atmosphere
leaves
δ15N 3.6 ‰
N 14.1 g/kg
N2
4.9
ngN.g-1.d-1
N2O
N2
NH3+
N2O
N2
NO3-
δ15N 3.9 ‰
MB
NH4+
δ15N -1.2 ‰
28.7
mgN.kg-1.d-1
NH4+
0.05
mgN.kg-1.d-1
NO3-
Norg
MB
MB
water
sediment δ15N 4.6 ‰

Figure 5

Capítulo 3

Assessment of indicators of nitrogen dynamics in subtropical mangroves

Carla Roberta Gonçalves Reis, Marília Cunha-Lignon, Jéssica Airisse Guimarães Sampaio, Rafael Silva Oliveira e Gabriela Bielefeld Nardoto

CRGR, GBN e MCL conceberam o estudo e delinearam a metodologia; CRGR, MCL e JAGS coletam os dados. CRGR e JAGS conduziram análises laboratoriais. CRGR analisou os resultados e liderou a escrita do manuscrito. Todos os autores contribuíram criticamente com a interpretação dos resultados e com a presente versão do manuscrito.

Title: Assessment of indicators of nitrogen dynamics in subtropical mangroves

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Abstract

Mangroves play a substantial role in nitrogen (N) cycling at the terrestrial-aquatic interface in coastal areas worldwide. However, although excess N input and biological invasions can have large consequences on N cycling in mangroves, and to the critical societal goods and services these ecosystems provide, our capacity to readily assess and monitor N dynamics in mangrove ecosystems remains limited. Our main goal was to evaluate the applicability of leaf $\delta^{15}N$ and N concentrations as indicators of N dynamics in mangrove ecosystems for monitoring purposes. We evaluated N concentrations in sediment and vegetation, and leaf $\delta^{15}N$ at 18 study plots of fringe forests differing in species dominance, stage of ecological succession, and structural development in conserved mangroves, and those subjected to excess N input and invasion by aquatic macrophytes in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil. Concentrations of nitrate in sediment were lower at invaded N-enriched than non-N-enriched mangroves. While leaf N concentrations and C:N and N:P ratios were mostly similar among study sites, leaf $\delta^{15}N$ was higher at N-enriched than non-N-enriched mangroves, and at invaded than non-invaded N-enriched mangroves. Invasive aquatic macrophytes had higher leaf N concentrations and $\delta^{15}N$, and lower C:N ratios than mangroves. Despite the large variability on vegetation structure of fringe forests, leaf $\delta^{15}N$ was a consistent indicator of alterations in N dynamics following N pollution and biological invasion in mangrove ecosystems.

Keywords: biological invasion, nitrogen pollution, nitrogen stable isotopes, nitrous oxide, phosphorous

1. Introduction

Increasing nitrogen (N) input and biological invasions are key drivers of global change with potentially large consequences for the conservation of natural ecosystems and the societal goods and services they provide (e.g., Vilà et al. 2011; Pyšek et al. 2012; Erisman et al. 2013; de Vries et al. 2014). Mangroves are coastal woody wetlands that greatly overlap N dynamics and flux rates of terrestrial tropical forests, playing a substantial role in N cycling at the terrestrial-aquatic interface in coastal areas worldwide (reviewed by Reis et al. 2017a). Excess N input has been shown to have large effects on N cycling in mangrove ecosystems (e.g., Chen et al. 2010; Fernandes et al. 2010; Romero et al. 2012; reviewed by Reis et al. 2017a). Biological invasions also have the potential to largely affect N dynamics in natural ecosystems (e.g., Liao et al. 2008; Lee et al. 2017). Mangroves provide important societal goods and services which can be affected by excess N input and biological invasions, including trapping of excess nutrients from tidal waters helping to determine the water quality in coastal systems (e.g., Alongi and McKinnon 2005; Jordan et al. 2011), and the support of fisheries (e.g., Aburto-Oropeza et al. 2008) and sustainable tourism (e.g., Thiagarajah et al. 2015). Yet, our capacity to readily assess and monitor N dynamics in mangrove ecosystems remains limited.

The Estuarine-Lagunar Complex (ELC) of Cananeia-Iguape in southeastern Brazil, UNESCO World Heritage and Ramsar sites (Fig 1), is an example that disturbed mangrove areas no longer provide important goods and services to society. One one hand, the southern part of the system in the Cananeia region containing the best conserved mangroves (Cunha-Lignon et al. 2011), there is a greater diversity of fishes of economic importance (Cunha-Lignon et al. 2017) and a population of the Guiana dolphin *Sotalia guianensis* (Ferro de Godoy et al. 2015), which support sustainable tourism in the region (Barcellini et al. 2013; Monteiro-Filho et al. 2018). On the other hand, the northern part of the system in the Iguape region, is strongly influenced by the diversion of freshwater from the Ribeira de Iguape river through the Valo Grande canal into the estuary (Mahiques et al. 2009, 2013). Because of the freshwater input into the estuary, aquatic macrophytes invaded mangrove areas and mangrove mortality is high (Cunha-Lignon et al. 2011; Cunha-Lignon and Menguini 2016), fisheries diversity is lower compared to the southern part of the system (Cunha-Lignon et al. 2017), while *S. guianensis* is not found in this part of the estuary (Ferro de Godoy 2016).

In a previous study in the ELC of Cananeia-Iguape, non-N-enriched (i.e., unpolluted) mangroves in the Cananeia region were more efficient conserving N within the system, while mangroves receiving excess N input from sewage and agriculture in the upstream watershed in the Iguape region had intensified N losses to the atmosphere, likely including nitrous oxide (N_2O) emissions, a potent greenhouse gas (Reis et al., under review). In that study, we assessed effects of increased N input on N dynamics in mangroves of similar vegetation structure and without the presence of invasive aquatic macrophytes to avoid confounding effects. From the measurements taken, leaf $\delta^{15}N$ and nutrient concentrations were the best integrators of mangrove nutrient status and dynamics. In the present study, our main goal was to evaluate the applicability of leaf $\delta^{15}N$ and N concentrations as indicators of N dynamics in mangrove ecosystems for monitoring purposes. To achieve this goal, we evaluated leaf $\delta^{15}N$ and nutrient concentrations in mangroves of different vegetation structures, and with and without the presence of invasive aquatic macrophytes over larger spatial and temporal scales in the ELC of Cananeia-Iguape.

2. Material and Methods

2.1. Study area

The study was conducted at 18 mangrove plots contained in eight mangrove sites under a humid subtropical climate in the Estuarine Lagunar-Complex (ELC) of Cananeia-Iguape, São Paulo, Brazil, from 2015 to 2017 (Fig.1). These study plots integrate a permanent plot network established in January 2001 where mangrove vegetation structure has been monitored about once a year (Cunha-Lignon et al. 2011; Cunha-Lignon and Menguini 2016). The ELC of Cananeia-Iguape comprise mangrove areas strongly affected by excess N input from sewage and agriculture in the Ribeira de Iguape River watershed (VG and ICA sites), that likely receive some N input from small urban areas in the Cananeia Island (NO and BA sites), and conserved areas under a greater marine influence and virtually no excess N input (SG and CN sites) (Reis et al., under review). Mangrove vegetation is composed of *Rhizophora mangle* L. (Rhizophoraceae), *Laguncularia racemosa* (L.) C.F. Gaernt. (Combretaceae), and *Avicennia schaueriana* Stapf & Leechm. ex Moldenke (Acanthaceae) (families according to APG III 2009). The study plots comprise fringe forests that differ in species dominance, stage of ecological succession, structural development, and in the presence of invasive aquatic macrophytes (Table 1). The four study plots located closer to the Valo Grande canal in the Iguape region were invaded by aquatic macrophytes. Dominant invasive aquatic macrophytes in these study plots included *Urochloa arrecta* and *Paspalum repens* (Cunha-Lignon and Menguini 2016), and species of the genera *Scirpus* and *Commelina*.

2.2. Sampling design

To avoid interferences on vegetation structure inside the plots, samples and data were taken immediately outside the plots. Next to each plot, leaf samples were obtained from three arboreal individuals with diameter at breast height (DBH) $\geq$ 4 cm annually from July 2015 to 2017 to characterize the $\delta^{15}N$ and concentrations of N and P in leaves. Leaf samples of nine dominant species of invasive aquatic macrophytes were also sampled in July 2015, one sample per species, to characterize the $\delta^{15}N$ and concentrations of N.

Sediment samples at 0-10 cm depth were obtained next to each plot once a year from July 2015 to 2017 to characterize concentrations of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$. Extra sediment samples at 0-20 cm depth were obtained in July 2015 to characterize physical (texture) and chemical properties (concentrations of organic matter, Na^+, K^+, Ca^{2+}, Mg^{2+}, and available P, sum of exchangeable bases, and cation exchange capacity). Available P concentrations were also measured in 0-10 cm depth sediment samples obtained in 2016.

Redox potentials (Eh) of sediment interstitial water at 0-5 cm depth were measured *in situ* with a portable meter once a year from July 2015 to 2017. Sediment interstitial water pH values at 0-5 cm depth were also measured *in situ* with a portable meter in July 2017.

Sediment samples were kept refrigerated until analysis and leaf samples were rinsed with tap water and dried at 40°C over 48h immediately after fieldwork.

2.3 Sediment physical and chemical properties

Concentrations of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ in sediment were quantified according to procedures described by (Reis et al. 2017b). Extracts were analyzed for concentrations of $N\text{-}NH_4^+$ with a Nessler reagent (Greweling and Peech 1960) and $N\text{-}NO_3^-$ (Meier 1991, cited in Sutton et al. 2014) using an UV spectrophotometer in the Laboratório de Ecossistemas of the Universidade de Brasília.

Sediment samples at 0-20 cm depth were combined into a composite sample for each mangrove site and analyzed for texture, concentrations of organic matter (OM), Na^+, K^+, Ca^{2+}, and Mg^{2+}, sum of exchangeable bases (SEB), and cation exchange capacity (CEC) according to Embrapa (1997, 1999). Available P was extracted with Mehlich 1 solution and quantified with ammonium molybdate using a spectrophotometer (Embrapa 1999). These analyzes were performed in the Departamento de Ciências do Solo of the Escola Superior de Agricultura Luiz de Queiroz at Universidade de São Paulo.

2.4. Leaf $\delta^{15}N$ and leaf N and P concentrations

Leaf samples were prepared according to procedures described by Reis et al. (2017b) and analyzed for total C and N, C:N ratio, and $\delta^{15}N$ using an EA (Carlo Erba) coupled with an IRMS (ThermoQuest-Finnigan Delta Plus) (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.

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.

2.5. Statistical analysis

Normality of both raw data and residuals were tested using the Shapiro-Wilk *W*-test. For sediment data analysis, spatial autocorrelation and comparisons between data groups were tested according to the R code proposed by Eisenlohr (2014) with improvements, using Student's *t*-test or one-way ANOVA *F*-test, discounting the effects of spatial autocorrelation.

For leaf data analysis, comparisons between two data groups were tested using Student's *t*-test or by nonparametric Mann–Whitney *U*-test. Comparisons between three or more data groups were assessed by one-way ANOVA *F*-test followed by *post hoc* Tukey HSD or by nonparametric Kruskal-Wallis *H*-test, followed by *post hoc* pairwise comparisons described by Siegel and Castellan Jr (1988).

Similarities between study sites regarding sediment physical and chemical properties were evaluated by principal coordinates analysis (PCoA) ordination (Legendre and Legendre 2012), using Euclidean distances after data standardization to mean zero and unit variance. Statistical significance of PCoA groups were tested by analysis of similarities (ANOSIM) *R*-test, using 9,999 permutations (Clarke 1993). All analyses were performed using R software (R Core Team 2014).

3. Results

3.1. Sediment physical and chemical properties

Ordination axis 1 was negatively correlated to N-NO_3^- concentrations, which grouped VG1, VG2, and ICA1 apart from ICA2 and the study sites in the Cananeia region, $R = 0.97$, $p = 0.01$ (Fig. 2 and Table 2). Concentrations of N-NO_3^- were about three times lower at VG1, VG2, and ICA1, with median (1st and 3rd quartiles) values of 0.07 (0.05 and 0.11) mg/kg, compared to the other study sites, 0.20 (0.12 and 0.24) mg/kg, $t(48) = 6.27$, p = 0.027.

Ordination axis 1 was also positively correlated to SEB, CEC (Table S1/ANEXO 3), concentrations of N-NH_4^+ and mineral N, N-NH_4^+: N-NO_3^- ratios and Eh. These parameters, however, were not statistically different between the ordination resulting groups (N-NH_4^+: $t(48) = 0.02$, p = 0.887; mineral N: $t(48) = 0.01$, p = 0.927; N-NH_4^+: N-NO_3^- ratios: $t(50) = 1.95$, p = 0.132; Eh: $t(51) = 0.74$, p = 0.394). Median (1st and 3rd quartiles) values of N-NH_4^+ and mineral N concentrations in sediment were 6.6 (2.9 and 18.2) mg/kg and 6.8 (3.0 and 18.4) mg/kg, respectively. Median (1st and 3rd quartiles) values of N-NH_4^+: N-NO_3^- ratios were 52 (22 and 151), and of Eh were -128 (-254 and 30).

Ordination axis 2 was highly correlated to percentages of sand, silt and clay in sediment, yet the resulting grouping was not statistically significant, $R = 0.27$, $p = 0.07$.

Available P concentrations at 0-10 cm depth and pH values were also similar among study sites, with median (1st and 3rd quartiles) values of 43.1 (36.6 and 49.5) mg/kg and 6.9 (6.6 and 7.3), respectively (Available P: $F(8,10) = 2.27$, p = 0.113; pH: $F(8,8) = 1.68$, p = 0.236).

3.2. Leaf $\delta^{15}N$

Leaf $\delta^{15}N$ was similar between *L. racemosa* and *A. shaueriana*, and about two times higher in these species than *R. mangle* (Table 3). *Laguncularia racemosa* and *A. shaueriana* leaf $\delta^{15}N$ values were higher in the Iguape than Cananeia region (Fig. 3A). *Laguncularia racemosa* and *A. shaueriana* leaf $\delta^{15}N$ values were also about two times higher at VG than ICA sites in the Iguape region (Fig. 3B). Because of *L. racemosa* dominance, only one individual of *R. mangle* was sampled at VG sites. Considering the other study sites, *R. mangle* leaf $\delta^{15}N$ values were similar between ICA and the study sites in the Cananeia region, $p \geq 0.05$. Leaf $\delta^{15}N$ of invasive aquatic macrophytes was higher than mangroves in the VG study sites (Table 4).

3.3. Leaf N and P concentrations

Leaf N concentrations were higher in *A. shaueriana* than *R. mangle* and *L. racemosa*, and higher in *R. mangle* than *L. racemosa* (Table 4). *R. mangle* leaf N concentrations were higher at ICA sites in the Iguape region, with median (1st and 3rd quartiles) values of 19.0 (17.7 and 20.7) g/kg, relative to the Cananeia region, 17.4 (16.1 and 18.9) g/kg, $t(26) = -2.70$, $p = 0.012$. Leaf N concentrations of *A. shaueriana* and *L. racemosa* were similar among study sites (*A. shaueriana*: $U(16,4) = 26$, $p = 0.618$; *L. racemosa*: $U(24, 28) = 442$, $p = 0.053$). Leaf N concentrations per species were also similar between VG and ICA sites in the Iguape region ($p \geq 0.05$). Leaf N concentrations were about two times higher in invasive aquatic macrophytes than mangroves in the VG study sites (Table 4).

Leaf P concentrations were similar between mangrove species. Leaf P concentrations were higher in the Iguape region, 1.4 (1.2 and 1.5) g/kg, relative to the Cananeia region, 1.2 (1.1 and 1.4) g/kg, $U(28,27) = 264$, $p = 0.027$. Leaf P concentrations were similar among VG and ICA sites in the Iguape region ($p \geq 0.05$).

Leaf N:P ratios were higher in *A. shaueriana* than *R. mangle* and *L. racemosa*, and higher in *R. mangle* than *L. racemosa*. Leaf N:P ratios per species were similar among study sites (*A. shaueriana*: $t(4) = 0.14$, $p = 0.899$; *R. mangle*: $t(13) = 1.62$, $p = 0.128$; *L. racemosa*: $t(21) = 21.34$, $p = 0.221$). Leaf N:P ratios per species were also similar between VG and ICA sites in the Iguape region ($p \geq 0.05$).

3.4. Leaf C:N ratios

Leaf C:N ratios were similar between *R. mangle* and *L. racemosa*, and about two times higher in these species than *A. shaueriana* (Table 3). Leaf C:N ratios of *R. mangle* and *L. racemosa* were similar among study sites, $t(66) = 0.14$, $p = 0.885$. Leaf C:N ratios of *A. shaueriana* were about two times higher in the Iguape than Cananeia region, with median (1st and 3rd quartiles) values of 34 (33 and 38) and 17 (17 and 18), respectively, $U(16,4) = 3$, $p = 0.007$. Leaf C:N ratios per species were also similar between VG and ICA sites in the Iguape region ($p \geq 0.05$). Leaf C:N ratios were about two times lower in invasive aquatic macrophytes than mangroves (Table 4).

4. Discussion

Taken together, our results indicated that despite the large variability on vegetation structure that fringe forests usually exhibit, leaf $\delta^{15}N$ is a consistent indicator of N dynamics in mangrove ecosystems. In addition to excess N input, leaf $\delta^{15}N$ results indicated that invasive aquatic macrophytes further intensified N dynamics and losses to the atmosphere by introducing organic matter of high N concentrations into the sediment.

The higher leaf $\delta^{15}N$ values in the Iguape relative to the Cananeia region indicated that despite the large variability on vegetation structure of fringe forests in the ELC of Cananeia-Iguape, leaf $\delta^{15}N$ was a good indicator of the excess N input originating from human activities affecting mangrove areas in the system. Previous studies also verified that mangrove leaf $\delta^{15}N$ is a reliable tool for detecting and also mapping N pollution in estuarine and coastal systems (Costanzo et al. 2001; Gritcan et al. 2016; Reis et al. under review). The lower leaf $\delta^{15}N$ at study sites in the Icapara inlet relative to study sites closer to the Valo Grande canal also indicated lower N pollution in the Icapara inlet, likely because of a greater marine influence. This was also supported by the similar leaf $\delta^{15}N$ of *R. mangle* between study sites in the Icapara inlet and Cananeia region. The lower influence of Valo Grande discharge waters into the Icapara inlet is also verified by the higher estuarine water salinity (24 to 28) and lower presence of invasive aquatic macrophytes in the Icapara inlet compared to the low estuarine water salinity (0 to 2) and marked presence of invasive aquatic macrophytes in mangrove areas closer to the Valo Grande (Barrera-Alba et al. 2009; Cunha-Lignon and Menguini 2016).

Mangrove leaf $\delta^{15}N$ not only indicated the extent of N input (e.g., Costanzo et al. 2001, 2004; Fry and Cormier 2011), but also the intensity of N dynamics and losses to the atmosphere in mangrove sediment (Reis et al. 2017a, b). For example, fringe mangroves which have higher N inputs from tidal waters and intensified N dynamics and losses to the atmosphere compared to basin and dwarf mangroves, usually have higher leaf $\delta^{15}N$ values in both non-N-enriched (e.g., Wooller et al. 2003; Medina et al. 2010; Reis et al. 2017a, b) and N-enriched mangroves (e.g., Fry et al. 2000; Mckee et al. 2002). In a previous study in the ELC of Cananeia-Iguape, excess N input intensified N losses to the atmosphere, likely including N_2O emissions, from fringe and basin mangroves in the Iguape region, which contributed with the higher leaf $\delta^{15}N$ at these N-enriched mangroves compared to non-N-enriched mangroves in the Cananeia region (Reis et al. under review). In that study, we showed that mangrove leaf $\delta^{15}N$ indicate differences in N input and intensity of N losses to the atmosphere from mangroves of similar vegetation structure. In the present study, we move forward and show that mangrove leaf $\delta^{15}N$ is also a good indicator of the extent of N input and N dynamics and losses to the atmosphere among fringe mangroves of differing vegetation structure.

In addition to excess N input, invasive aquatic macrophytes further intensified N dynamics and losses to the atmosphere in mangrove sediment in the Iguape region. The high leaf N concentrations and $\delta^{15}N$ of invasive aquatic macrophytes relative to mangroves in the study sites closer to the Valo Grande indicated the high demand for N of the invasive species. The lower $N\text{-}NO_3^-$ concentrations in sediment at the study sites closer to the Valo Grande compared to the Cananeia region likely reflected a high N uptake by invasive aquatic macrophytes along with higher losses of N to the atmosphere due to excess N input (Reis et al., under review). As a consequence of a high N demand, invasive aquatic macrophytes introduce organic matter of lower C:N ratios into the sediment relative to mangroves, likely supporting higher microbial activity, organic matter decomposition, and N mineralization rates in sediment (e.g., Vilà et al. 2011; Lee et al. 2017). Higher N availability and microbial activity would also lead to higher N losses to the atmosphere, likely including N_2O emissions (e.g., Chen et al. 2010; Fernandes et al. 2010; reviewed by Reis et al. 2017a), and contribute to the higher mangrove leaf $\delta^{15}N$ values at the invaded study sites. Both excess N input via water pollution and invasion by aquatic macrophytes seem to intensify N dynamics and losses in mangrove sediment, and likely also affect C dynamics and storage in mangrove sediment. The study of the interactive effects of N enrichment and invasion by aquatic macrophytes on C dynamics and stocks in mangrove sediment in the ELC of Cananeia-Iguape is underway.

Among the potential leaf indicators of N dynamics evaluated in the present study, leaf $\delta^{15}N$ was the best indicator of alterations on N dynamics following N enrichment and invasion by aquatic macrophytes in mangrove ecosystems. Although we did not measure total N content in vegetation, considering the dominance by *L. racemosa* which had the lowest leaf N concentrations among the mangrove species, and the higher mortality of tree trunks (up to 60% of basal area) at the study sites closer to the Valo Grande relative to Cananeia region (up to 20%) (Cunha-Lignon and Menguini 2016), a lower total N content in aboveground biomass would be expected at the study sites closer to the Valo Grande. This suggests that mangrove vegetation was not an important sink of excess N, which was rather mainly lost to the atmosphere (Reis et al., under review) and assimilated by invasive aquatic macrophytes, as seen by the lower N-NO_3^- concentrations in the sediment of N-enriched mangroves invaded by aquatic macrophytes. As a consequence, mangrove leaf N concentrations and ratios were not good indicators of N dynamics in mangrove sediment. Previous studies have shown that leaf N concentrations and ratios at N-enriched mangroves can be higher, similar, or lower compared to non-N-enriched mangroves (e.g., Feller 1995; Feller et al. 2003, 2007; Lovelock and Feller 2003). Foliar P concentrations, however, were good indicators of P availability in the studied system. Despite the fact that available P concentrations in sediment were similar among study sites, excess P input from sewage, agricultural, and fertilizer production sources are expected in the estuary in the Iguape region (e.g., Barrera-Alba et al. 2009). However, previous studies have shown that leaf P concentrations at P-enriched mangroves can be either higher or similar than non-P-enriched mangroves (e.g., Feller 1995; Feller et al. 2003, 2007). More studies on the use of foliar P concentrations for monitoring P pollution in mangrove ecosystems are needed.

In conclusion, the results of the present study indicated that despite the large variability on vegetation structure of fringe forests, leaf $\delta^{15}N$ is a consistent indicator of N dynamics in mangrove ecosystems. In addition to excess N input, invasive aquatic macrophytes further intensified N dynamics and losses to the atmosphere, likely including N_2O emissions, by introducing organic matter of high N concentrations into mangrove sediment. For monitoring N dynamics in mangroves, we recommend assessing leaf $\delta^{15}N$ of dominant species in the system at a minimum frequency of the leaf lifespan of the target species. Because fringe and basin (or dwarf) mangroves already differ in N dynamics and leaf $\delta^{15}N$, we also recommend standardizing sampling in the most common mangrove type in the system. In addition, differences among species, site and temporal comparisons per species should be evaluated.

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Table 1. Description of vegetation in the mangrove study plots in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil, July 2015

Sítio Grande river (SG)	
SGP1	young fringe dominated by *Rhizophora mangle*
SGP2	mature fringe of greater structural development dominated by *R. mangle*
Cananeia Island (CN)	
CNP1	early successional fringe dominated by *Laguncularia racemosa*
CNP2	mature fringe of greater structural development dominated by *Avicennia schaueriana*
Nóbrega (NO)	
NOP1	young fringe dominated by *L. racemosa*
NOP2	mature fringe of greater structural development dominated by *R. mangle*
Baguaçu (BA)	
BAP1	young fringe dominated by *L. racemosa*
BAP2	young fringe dominated by *L. racemosa*
BAP3	early successional fringe dominated by *A. schaueriana*
BAP4	mature fringe of greater structural development dominated by *A. schaueriana*
Valo Grande canal (VG)	
VG1P1	mature fringe dominated by *L. racemosa*, invaded by aquatic macrophytes
VG1P2	mature fringe dominated by *L. racemosa*, invaded by aquatic macrophytes
VG2P1	mature fringe dominated by *L. racemosa*, invaded by aquatic macrophytes
VG2P2	mature fringe dominated by *L. racemosa*, invaded by aquatic

	macrophytes
Icapara inlet (ICA)	
ICA1P1	young fringe dominated by *L. racemosa*
ICA1P2	mature fringe of greater structural development dominated by *L. racemosa* and *A. schaueriana*
ICA2P1	Young fringe dominated by *R. mangle*
ICA2P2	mature fringe of greater structural development dominated by *R. mangle* and *A. schaueriana*

Early successional stands had mean diameter at the breast height (DBH) of about 2 cm, and mean height (H) between 1 and 2 m; young stands had mean DBH of about 3 cm, and mean H between 3 and 5 m; mature stands of greater structural development have mean DBH ≥ 10 cm, and mean H of about 10 m

Table 2. Pearson's correlation coefficients between sediment physical and chemical properties and PCoA scores

	Axis 1	Axis 2
sand (%)	-0.12	**0.84**
silt (%)	-0.00	**-0.74**
clay (%)	0.35	**-0.82**
OM (g/kg)	-0.28	-0.56
N-NH_4^+ (mg/kg)	**0.79**	0.49
N-NO_3^- (mg/kg)	**-0.67**	0.17
N-NH_4^+:N-NO_3^-	**0.81**	0.49
N mineral (mg/kg)	**0.77**	0.50
available $P_{0\text{-}10\ cm}$ (mg/kg)	-0.12	0.62
available $P_{0\text{-}20\ cm}$ (mg/kg)	-0.31	-0.45
Na^+ ($mmol_c$/kg)	**0.97**	-0.16
K^+ ($mmol_c$/kg)	**0.89**	-0.30
Ca^{2+} ($mmol_c$/kg)	**0.90**	-0.24
Mg^{2+} ($mmol_c$/kg)	**0.91**	-0.30
SEB ($mmol_c$/kg)	**0.96**	-0.19
CEC ($mmol_c$/kg)	**0.95**	-0.25
pH	-0.51	-0.42
Eh (mV)	**0.78**	0.27

Coefficients highly correlated to axes are in bold. OM = organic matter, SEB = sum of exchangeable bases, CEC = cation exchange capacity

Table 3. Natural abundance of nitrogen stable isotopes (δ^{15}N), and concentrations of nitrogen (N) and phosphorous (P) in leaves of *Rhizophora mangle*, *Laguncularia racemosa*, and *Avicennia shaueriana* from mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil, 2015 to 2017

	R. mangle	*L. racemosa*	*A. shaueriana*	Statistics
δ^{15}N (‰)	3.2 (1.3 - 4.3)**a**	4.9 (4.3 - 5.8)**b**	4.7 (4.1 - 5.3)**b**	*F*(2,111) = 29.11, p ≤ 0.0001
	N = 36	N = 56	N = 22	
N (g/kg)	18.4 (16.4 - 19.4)**a**	15.4 (13.6 - 16.6)**b**	23.2 (21.6 - 25.1)**c**	*F*(2,111) = 63.84, p ≤ 0.0001
	N = 36	N = 56	N = 22	
P (g/kg)	1.3 (1.1 - 1.5)**a**	1.3 (1.2 - 1.5)**a**	1.4 (1.2 - 1.5)**a**	*F*(2,87) = 1.15, p = 0.5615
	N = 27	N = 42	N = 21	
N:P	15.4 (14.4 - 17.7)**a**	12.1 (11.3 - 13.3)**b**	18.1 (16.8 - 19.8)**c**	*F*(2,57) = 32.41, p ≤ 0.0001
	N = 18	N = 14	N = 14	
C:N	27.3 (25.2 - 30.4)**a**	25.6 (22.5 - 28.4)**b**	17.9 (16.8 - 21.1)**c**	*H*(2,111) = 18.46, p ≤ 0.0001
	N = 36	N = 56	N = 22	

Median values and 1st and 3rd quartiles are presented. Distinct letters indicate significant statistical difference between species

Table 4. Natural abundance of nitrogen stable isotopes ($\delta^{15}N$), concentrations of nitrogen (N) and carbon/nitrogen ratios (C:N) in leaves of mangroves and invasive aquatic macrophytes from the Valo Grande study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil, 2015 and 2016

	mangroves	macrophytes	Statistics
$\delta^{15}N$ (‰)	5.8 (5.2 - 6.2)**a**	6.8 (6.4 - 7.1)**b**	*t*(16) = 2.45, p = 0.0262
N (g/kg)	13.9 (12.8 - 16.3)**a**	25.4 (24.0 - 31.1)**b**	*t*(10) = 6.03, $p \leq 0.0001$
C:N	23.5 (20.7 - 25.4)**a**	14.9 (12.3 - 16.5)**a**	*t*(15) = -4.95, $p \leq 0.0001$

Median values and 1st and 3rd quartiles are presented. Distinct letters indicate significant statistical difference (N = 24 for mangroves and 9 for aquatic macrophytes)

Figure legends

Fig 1. Location of the mangrove study sites and occurrence areas of invasive aquatic macrophytes in the Estuarine Lagunar-Complex of Cananeia-Iguape, southeastern Brazil. Source of the shapefiles of mangrove forests and aquatic macrophytes areas: Cunha-Lignon et al. (2011)

Fig 2. Ordination of mangrove study sites according to sediment physical and chemical properties from 2015 to 2017, based on principal coordinates analysis. Open circles = study sites in the Cananeia region, closed circles = study sites in the Iguape region, SG = Sítio Grande, CN = Cananeia Island, NO = Nóbrega, BA = Baguaçu, VG1 = Valo Grande 1, VG2 = Valo Grande 2, ICA1 = Icapara 1, ICA2 = Icapara 2, N-NO_3^- = nitrate nitrogen concentration

Fig 3. Natural abundance of nitrogen stable isotopes ($\delta^{15}N$) in leaves of *Laguncularia racemosa* and *Avicennia shaueriana* from mangrove study sites A) in the Cananeia and Iguape regions, and B) at Valo Grande (VG) and Icapara (ICA) study sites in the Iguape region, in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil, 2015 and 2016. Median and 1st and 3rd quartiles values are presented. **** $P \leq 0.0001$, * $P \leq 0.05$

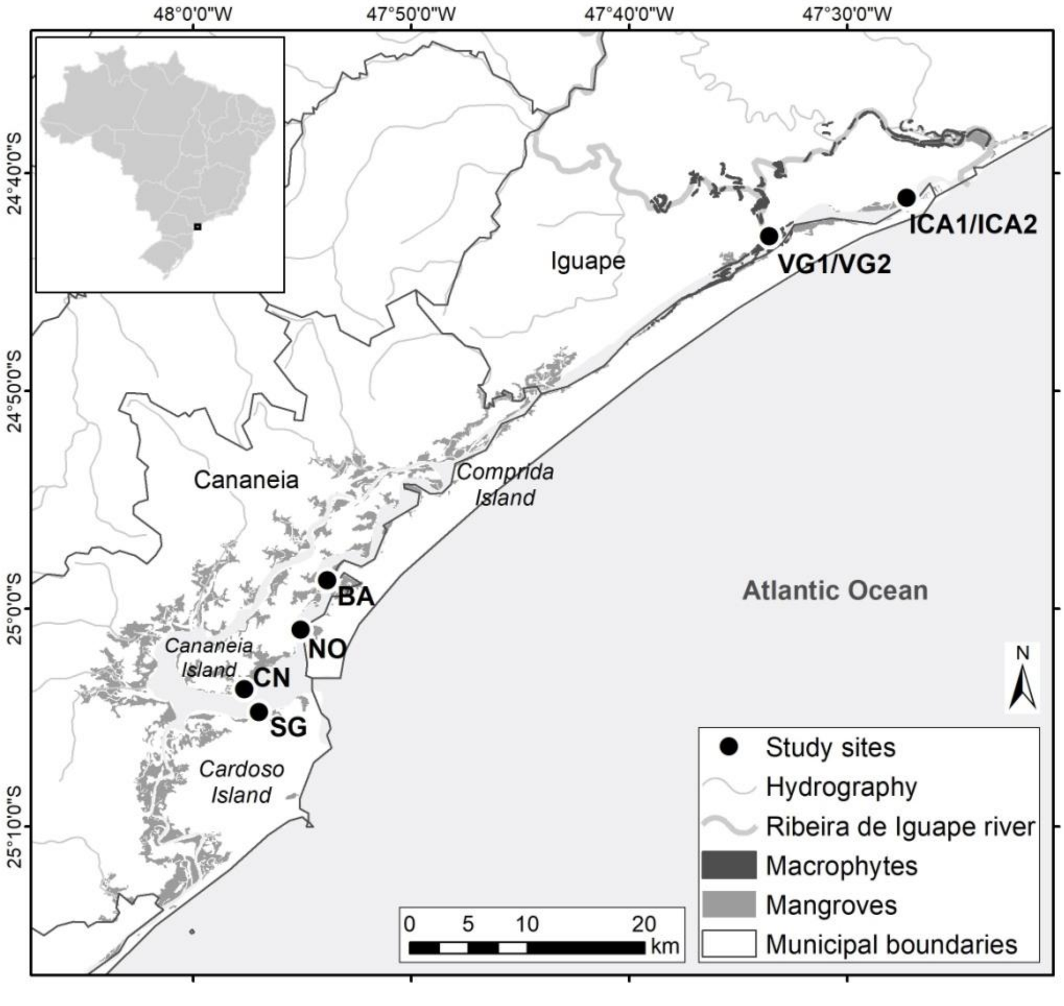
48°0'0"W
47°50'0"W
47°40'0"W
47°30'0"W
24°40'0"S
24°50'0"S
25°0'0"S
25°10'0"S
Iguape
ICA1/ICA2
VG1/VG2
Cananeia
Comprida Island
Atlantic Ocean
BA
NO
Cananeia Island
CN
SG
Cardoso Island
N
Study sites
Hydrography
Ribeira de Iguape river
Macrophytes
Mangroves
Municipal boundaries
0 5 10 20 km

Figure 1

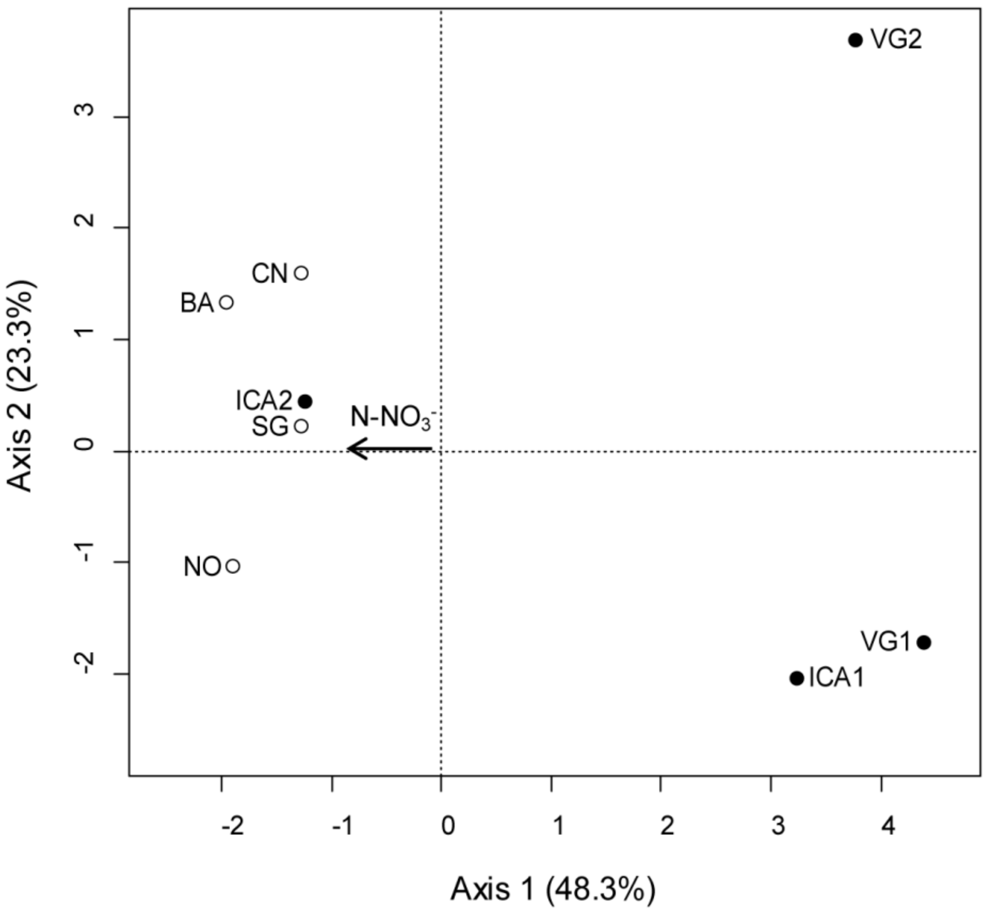
VG2
CN
BA
ICA2
SG
N-NO$_3^-$
NO
VG1
ICA1
Axis 2 (23.3%)
3
2
1
0
-1
-2
-2
-1
0
1
2
3
4
Axis 1 (48.3%)

Figure 2

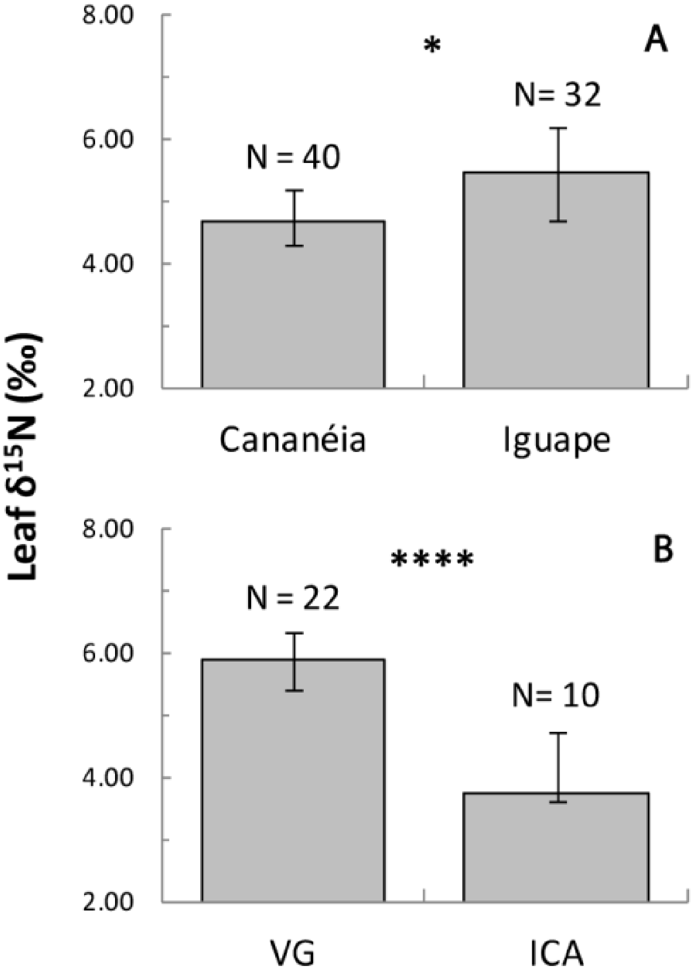
Leaf δ15N (‰)
8.00
6.00
4.00
2.00
*
A
N = 40
N= 32
Cananéia
Iguape
8.00
6.00
4.00
2.00
****
B
N = 22
N= 10
VG
ICA

Figure 3

ANEXO I

Title: Isotopic evidence that nitrogen enrichment intensifies nitrogen losses to the atmosphere from subtropical mangroves

Authors: Carla Roberta Gonçalves Reis’*, Sasha Carey Reed, Rafael Silva Oliveira, and Gabriela Bielefeld Nardoto
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Table S1. Vegetation structure at the mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, State of São Paulo, Brazil

	CoF	CoB	+N1F	+N1B	+N2F	+N2B
Height (m)	5.5 (3.4-6.6)	2.8 (2.0-3.4)	5.8 (4.4-9.2)	1.8 (1.5-2.1)	6.0 (5.0-8.0)	3.0 (2.5-3.5)
DBH (cm)	9.7	3.6	7.4	4.2	8.6	4.0
Density (ind/ha)	3,084	24,691	5,161	4,409	4,222	30,303
BA (m^2/ha)	22.7	25 .1	22.0	6.2	24.4	37.3

Control fringe (CoF) and basin (CoB); N-enriched fringe (+N1F) and basin (+N1B) receiving sewage from small urban areas; and N-enriched fringe (+N2F) and basin (+N2B) receiving sewage and fertilizers from a large upstream watershed. DBH = diameter at breast height, BA = basal area, AGB = aboveground biomass. Height median values and 1st and 3rd quartiles are presented

ANEXO II

Title: Isotopic evidence that nitrogen enrichment intensifies nitrogen losses to the atmosphere from subtropical mangroves

Authors: Carla Roberta Gonçalves Reis*, Sasha Carey Reed, Rafael Silva Oliveira, and Gabriela Bielefeld Nardoto
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Table S2. Natural abundance of nitrogen stable isotopes (δ^{15}N), nitrogen (N) and phosphorous (P) concentrations, and nitrogen/phosphorous (N:P) and carbon/nitrogen (C:N) ratios in leaves of *Rhizophora mangle*, *Laguncularia racemosa*, and *Avicennia shaueriana* from mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil

	R. mangle	*L. racemosa*	*A. shaueriana*	Statistics
δ^{15}N (‰)	1.5 (1.0 - 2.7)**a**	2.4 (1.8 - 4.1)**b**	3.8 (3.3 - 4.3)**b**	F (2,143) = 11.60, $P \leq 0.001$
	N = 91	N = 67	N = 3	
N (g/kg)	14.9 (13.7 - 16.5)**a**	10.9 (10.0 - 12.7)**b**	23.1 (22.6 - 24.0)**c**	F (2,146) = 58.12, $P \leq 0.001$
	N = 91	N = 67	N = 3	
P (g/kg)	1.1 (1.0 - 1.3)**a**	1.0 (0.9 - 1.3)**a**	1.4 (1.4 - 1.5)**a**	F (2,76) = 2.74, $P = 0.071$
	N = 43	N = 40	N = 3	
N:P	14.1 (12.6 - 15.7)**a**	10.8 (9.1 - 12.1)**b**	16.6 (15.3 - 17.7)**a**	F (2,82) = 27.96, $P \leq 0.001$
	N = 43	N = 40	N = 3	

C:N	30.4 (27.6 - 33.0)**a**	39.9 (33.1 - 43.9)**b**	16.9 (16.7 - 17.8)**c**	*F* (2,143) = 35.71, $P \leq 0.001$
	N = 91	N = 67	N = 3	

Median values and 1st and 3rd quartiles are presented. Distinct letters indicate significant statistical difference between species

ANEXO III

Title: Assessment of indicators of nitrogen dynamics in subtropical mangroves

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Table 3. Physical and chemical properties of sediment at 0-20 cm depth from mangrove study sites in the Estuarine-Lagunar Complex of Cananeia-Iguape, southeastern Brazil, 2015

	Cananeia				Iguape			
	SG	CN	NO	BA	VG1	VG2	ICA1	ICA2
sand (%)	77	85	35	76	25	87	60	74
silt (%)	10	13	50	21	46	10	22	11
clay (%)	13	2	15	3	29	3	18	15
texture class	SaLo	LoSa	SiLo	LoSa	ClLo	Sa	SaLo	SaLo
OM (g/kg)	82	127	139	170	124	21	150	75
available P (mg/kg)	52	19	37	25	31	15	34	41
Na$^+$ (mmol$_c$/kg)	164	120	19	51	806	110	839	559
K$^+$ (mmol$_c$/kg)	9	5	3	4	51	6	38	23

Ca^{2+} (mmol$_c$/kg)	40	43	52	48	133	18	113	108
Mg^{2+} (mmol$_c$/kg)	75	75	58	72	236	54	231	139
Al^{3+} (mmol$_c$/kg)	1	< 0.1	20	5	2	< 0.1	26	< 0.1
SEB (mmol$_c$/kg)	288	243	132	175	1226	188	1221	829
CEC (mmol$_c$/kg)	325	282	264	256	1313	198	1363	861

SG = Sítio Grande, CN = Cananeia Island, NO = Nóbrega, BA = Baguaçu, VG1 = Valo Grande 1, VG2 = Valo Grande 2, ICA1 = Icapara 1, ICA2 = Icapara 2, SaLo = sandy loam, LoFSa = loamy fine sand, SiLo = silt loam, ClLo = clay loam, FSa = fine sand, OM = organic matter, SEB = sum of exchangeable bases, CEC = cation exchange capacity