

Interactive Effects of Abiotic and Biotic Factors Drive Aquatic Plant Colonization in Subtropical Mangroves

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Research Article

Keywords: aquatic macrophytes, biological invasion, foliar attributes, nitrogen pollution, salinity, anthropogenic alterations

Posted Date: March 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-309998/v1>

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Abstract

The colonization and spread of non-native species are recognized as a critical driver of environmental change in mangrove ecosystems. However, environmental factors that favor non-native plant colonization in mangroves are still poorly understood. To contribute to filling this gap, we investigated the effect of selected abiotic factors associated controlling aquatic macrophytes colonization in mangroves in Southeastern Brazil. Furthermore, we also assessed foliar attributes of native and non-native species to evaluate whether biotic attributes favored the colonization. We selected 18 plots in mangrove forests under different levels of anthropogenic N inputs, both colonized and non-colonized by aquatic macrophytes in the Estuarine-Lagoon Complex of Cananeia-Iguape, southeastern Brazil. We measured interstitial salinity, sediment nitrate and ammonium concentrations, and sediment physicochemical properties. We also measured foliar nitrogen (N) concentrations, foliar C:N, and foliar $\delta^{13}\text{C}$ of both native and non-native species. We found that interstitial salinity at 10 cm depth followed by nitrate concentrations in sediment were the main factors associated with the occurrence of aquatic macrophytes in the studied mangrove areas. Moreover, non-native species had about 2-fold higher foliar N concentrations as well as about a 1.5-fold lower C:N ratios compared to native species. Low salinity and increased N availability in sediment allowed for the success of aquatic macrophytes into mangrove forests, also resulting in high amount of dead mangrove trunks (up 53% of basal area).

1. Introduction

Mangroves are the only forest formations that occur at the interface of terrestrial, freshwater, and marine ecosystems (Alongi 2002). Because of their key location, mangrove forests have important biogeochemical roles that translate into the provisioning of critical ecosystem services (Van Lavieren et al. 2012; Lee et al. 2014). For instance, mangroves usually store large quantities of carbon (C) per unit of area in both above and below ground biomass as well as in soil compared to other upland tropical forests and marine ecosystems (Donato et al. 2011; Alongi 2014).

Although there is wide recognition of the social, economic, and ecological relevance of mangroves, these ecosystems are still under strong human pressure (Valiela et al. 2001). One of the main drivers of ecosystem degradation is biological invasion. Despite the fact biological invasion is worldwide well recognized as one of the main vectors of global change promoting modification in the structure and functioning of ecosystems (Vitousek et al. 1996; Dukes and Mooney 1999; Mack et al. 2000; Vila et al. 2011), such driver has not received enough attention in mangrove ecosystems (Biswas et al. 2018). This is especially true considering the lack of investigations of environmental factors that favor the colonization of non-native species (Ren et al. 2014) and the functional attributes of non-native species that thrive in mangroves (Biswas et al. 2018).

Environmental factors that make an ecological system more susceptible to the colonization of non-native species are widely discussed, though there is a consensus that disturbance plays a key role in this process (Dukes and Mooney 1999; Mack et al. 2000; Biswas et al. 2012; Zhang et al. 2012). At the

regional scale, human interventions such as building canals, roads, and dams promote deep alterations of river flows in the upstream watershed, which in turn modify the freshwater input (and associated nutrient loads) to downstream estuarine ecosystems (Gopal and Chauhan 2006; Röderstein et al. 2013; Sathyanathan et al. 2014; Reis et al. 2019). These disturbances change environmental conditions and, as a consequence, may facilitate the colonization of non-native species into mangroves (Lugo 1998; Dahdouh-Guebas et al. 2005; Biswas et al. 2018). For example, measures to recover streamflow to mangroves have been taken in Colombia. Rivers were dredged to restore the river flow that had been altered for the construction of highways. Nonetheless, the combination of large rain events at the same time of these interventions caused a salinity reduction which, in turn, led to the colonization of aquatic macrophytes (*Typha domingensis* Pers) in these mangroves (Röderstein et al. 2013).

Under non-disturbed conditions, abiotic factors such as (i) low oxygen levels in sediment, (ii) periodic floods, and (iii) large variations in salinity act as barriers to the colonization of non-native species in mangroves (Schaeffer-Novelli et al., 2000; Spalding, Kainuma & Collins, 2010). That works for both terrestrial and freshwater plant species that hardly survive when competing with mangroves (Lugo 1998). Therefore, the success and significance of the impacts of the colonization and spread of non-native species will depend not only on the abiotic factors of the ecosystem under disturbance but also on the functional attributes of non-native species relative to native vegetation (Van Kleunen et al. 2010; Pyšek et al. 2012; Lee et al. 2017). In a context of disturbance, alterations in salinity and nutrient availability (especially nitrogen) may directly favor non-native species by promoting rapid growth and reproduction. This, in turn, leads to changes at the community level through, for example, the increase in mortality rates of native species (Dukes and Mooney 1999; Bradley et al. 2010; Vila et al. 2011; Gufu et al. 2018). Thus, it is important to understand both the abiotic and biotic factors controlling the colonization of non-native species as well as the effects at the native community level to prevent, minimize, and manage its changes (Pyšek et al. 2012; Ren et al. 2014).

The occurrence of non-native species can have significant effects at the species, community, and ecosystem levels (Vila et al. 2011). This might occur especially if native species differ from non-native species in terms of functional attributes (Lee et al. 2017). Such differences may provide competitive advantages for non-native species (e.g., increased resource use efficiency, fast growth rate, and higher fitness) since they generally have a greater performance compared to native species (Van Kleunen et al. 2010; González-Muñoz et al. 2013). Photosynthetic metabolism (e.g., C_3 x C_4) is a functional attribute that may differ between native and non-native species. The stable carbon isotopic signature ($\delta^{13}C$) indicates the type of photosynthetic pathway used by plants (Ehleringer et al. 2000). Moreover, the $\delta^{13}C$ also has the potential to provide insight into plant water use efficiency (i.e., assimilation of C per unit of water lost in transpiration) and consequently on photosynthetic capacity, thus highlighting differing competitive abilities among C_3 plants. Foliar N concentration and carbon: nitrogen ratio (C:N) are commonly used to evaluate differences between native and non-native species, which may be associated with N use and influences ecosystem process (Liao et al. 2008; Lee et al. 2017).

The Estuarine-Lagoon Complex (ELC) of Cananeia-Iguape, São Paulo State, Brazil is a Ramsar site (Ramsar 2017). Mangroves at the southern region of the complex are the widest and best conserved in São Paulo State (Cunha-Lignon et al. 2011). On the other hand, in the northern part of the complex, there was a significant increase in freshwater input due to the building of a canal in 1852 (locally known as “Valo Grande”) to deviate flow from Ribeira de Iguape river (Mahiques et al. 2009, 2013). The Ribeira de Iguape river is one of the largest rivers in the southeastern region of Brazil, draining more than 23,350 km². This deviation, as well as the input of sewage and excess nutrients from agricultural activities in the Ribeira de Iguape river basin, resulted in important changes in the estuary (locally known as “Mar Pequeno”) and associated mangroves. This possibly favored the colonization by aquatic macrophytes in the adjacent mangrove areas (Cunha-Lignon et al. 2011; Cunha-Lignon and Menghini 2016; Reis et al. 2019).

To shed more light on the controls of the colonization of mangrove ecosystems by non-native species, we investigated the effect of selected abiotic factors affecting the colonization of aquatic species in the ELC of Cananeia-Iguape. We also assessed functional foliar attributes of non-native and native species to clarify the role of biotic attributes to the success of non-native species in these systems.

2. Material And Methods

2.1 Study area

The study was conducted in 18 plots inserted in 8 fringe mangrove sites in the ELC of Cananeia-Iguape, southeastern Brazil in 2015 (Fig. 1). This study was carried out by the Integrated Monitoring of Mangroves Research Group recognized by the National Council for Scientific and Technological Development (CNPq, Brazil). The study plots integrate a permanent plot network where mangrove vegetation structure has been monitored since 2001.

The study sites differ mainly in two aspects: (i) levels of anthropogenic N inputs (Reis et al. 2019) and (ii) presence of aquatic macrophytes. We selected two conserved mangrove areas under high marine influence, free of aquatic macrophytes and with no influence of anthropogenic N inputs. These areas were our control sites. We also selected two mangrove sites free of aquatic macrophytes but subject to medium N inputs from sewage discharge of a small urban area in the Cananeia Island (+ N). Lastly, we selected four mangrove sites close to the Valo Grande canal that were strongly affected by excess N inputs from the Ribeira de Iguape river basin. Two of these sites comprised mangroves areas without the presence of aquatic macrophytes and submitted to excess N inputs ranging from medium to high (+ + N). The other two sites included mangrove areas colonized by aquatic macrophytes and subjected to high N inputs (+++NM) (Reis et al. 2019) (Table 1).

Table 1

Mean and maximum concentrations of ammonium nitrogen (NH₄⁺-N), nitrate nitrogen (NO₃⁻-N), and Chlorophyll, and the trophic state of estuarine water in the Estuarine-Lagoon Complex of Cananeia-Iguape from 2010 to 2015

Treatments	NH ₄ ⁺ -N (mg/l)		NO ₃ ⁻ -N (mg/l)		Chlorophyll a (µg/l)		Thophic State
	mean	maximum	mean	maximum	mean	maximum	
Control	0.10	0.13	NA	NA	4.6	8.5	mesotrophic
+N	0.12	0.35	NA	NA	7.9	29.4	mesotrophic to eutrophic
++N	0.14	0.80	NA	NA	15.8	62.6	eutrophic to hypereutrophic
+++NM	0.35	1.43	0.47	3.55	10.4	47.6	eutrophic to hypereutrophic
Data from the monitoring of the quality of coastal waters of the state of São Paulo of Companhia Ambiental do Estado de São Paulo (CETESB); data is available at https://servicos.cetesb.sp.gov.br/infoaguas/ . NA = Not applicable: all values were below detection limit < 0.20							

Mangrove vegetation in the ELC of Cananeia-Iguape is composed by the species *Rhizophora mangle* L. (Rhizophoraceae), *Laguncularia racemosa* (L.) C.F. Gaertn. (Combretaceae), and *Avicennia schaueriana* Stapf & Leechm. ex Moldenke (Acanthaceae) (families according to APG, 2009) (Schaeffer-Novelli et al. 1990). The vegetation in the study plots differ in the dominance of mangrove species, stage of ecological succession, structural development, and in the presence of aquatic macrophytes (Supplementary Table 1). Landsat time series indicated the absence of aquatic macrophytes in the mangrove areas adjacent to the Valo Grande canal in 1997. The occurrence of aquatic macrophytes was first recorded in 2010 (Cunha-Lignon et al. 2011), but the exact year of colonization is unknown.

The dominant aquatic macrophytes species at the +++NM plots were *Hymenachne amplexicaulis* (Rudge) Nees (Poaceae), *Bacopa monnieri* (L.) Wettst. (Plantagenaceae), *Crinum salsum* Ravenna (Amaryllidaceae), and other unidentified species of the families Poaceae and Cyperaceae.

The climate in the ELC of Cananeia-Iguape is classified as subtropical, "Cfa" according to the climatic classification of Köppen-Geiger (Alvares et al. 2014). Mean monthly temperature ranges from 24.5 °C in January to 7.8 °C in July. Mean annual precipitation is about 2,300 mm, without a marked dry season. Tides are semidiurnal with a mean amplitude of 0.82 m to 1.25 m in spring tides (Schaeffer-Novelli et al. 1990). The ELC of Cananeia-Iguape was previously reported to experience sporadic frosts (Schaeffer-Novelli et al. 1990).

2.2 Sampling design

In July 2015, vegetation structure was measured and sediment and foliar samples were obtained in each plot. To avoid trampling and possible interferences in the vegetation structure inside the plots, all foliar and sediment samples were obtained immediately outside each plot. The interstitial salinity was also obtained *in situ* next to each plot.

The plots have varying sizes, according to the stem density (Schaeffer-Novelli and Cintrón 1986; Schaeffer-Novelli et al. 2015). In each plot, all trees taller than 1 m were identified and had their diameter at breast height (DBH) registered with a metric diameter tape, and their height measured using a telemeter or a telescopic pole. The condition of the trunks (alive or dead) was also registered (Schaeffer-Novelli and Cintrón 1986; Schaeffer-Novelli et al. 2015).

A sediment sample (0–10 cm depth) was collected per plot to quantify the concentrations of NH_4^+ -N and NO_3^- -N. Extra sediment samples at 0–10 and 10–20 cm depths were also collected next to each plot to characterize sediment physicochemical properties. The sediment samples for physicochemical analyses from plots in the same site were pooled together, totaling two composite samples by treatment (i.e., Control, + N, ++ N, and +++NM). All sediment samples were kept refrigerated until analysis.

Next to each plot, mature foliar samples (i.e., green and fully expanded) from three mangrove trees with $\text{DBH} \geq 4$ cm were obtained, as well as foliar samples of the aquatic macrophytes, one sample per species. Foliar samples were analyzed for the $\delta^{13}\text{C}$, and concentrations of C and N. The foliar samples were washed with tap water and then oven dried at 40°C for 48 hours immediately after fieldwork.

2.3 Abiotic factors

2.3.1 Physical and chemical properties of sediment

The sediment samples at 0–20 cm depth were analyzed for concentrations of organic matter, Na^+ , K^+ , Ca^{2+} , Mg^{2+} , and available P, sum of exchangeable bases, and cation exchange capacity according to Embrapa (1997, 2009). Sediment texture was analyzed using the hydrometer method (Bouyoucos 1932) and classified using the U.S. Department of Agriculture textural triangle. Organic matter (OM) was extracted with potassium dichromate in sulfuric medium and quantified by titration using ammonium ferrous sulfate. Available exchangeable bases were extracted with ammonium acetate 1M.

Concentrations of Na^+ were analyzed by flame photometry, K^+ by atomic emission, and Ca^{2+} and Mg^{2+} by atomic absorption spectrophotometry. Available P was extracted with Mehlich 1 solution and quantified with ammonium molybdate using a spectrophotometer. These analyses were carried out in the Department of Soil Science of the Luiz de Queiroz School of Agriculture, University of São Paulo.

Also, in each plot, we used a Napoleon-type auger and an optical refractometer to measure *in situ* the interstitial sediment salinity at 10 cm (Sal10) and at 50 cm (Sal50) depth.

2.3.2 Concentrations of available N in sediment

Concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in sediment were quantified according to the extraction procedures described by Reis et al. (2017a). Extracts were analyzed for concentrations of $\text{NH}_4^+\text{-N}$ with a Nessler reagent (Greweling and Peech 1960) and $\text{NO}_3^-\text{-N}$ (Meier 1991, cited in Sutton et al. 2014) using a UV spectrophotometer in the Laboratório de Ecossistemas of the Universidade de Brasília. Concentrations of mineral N (N_{min}) were calculated by summing the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in sediment.

2.4 Biotic factors

2.4.1 Vegetation structure

From the data obtained in the field, we calculated the mean values of height (H) (m), DBH (cm), and basal area of live trunks (BALT) and dead trunks (BADT) (%) for each plot.

2.4.2 Foliar attributes

Foliar samples were ground with a knife grinder and powdered with liquid nitrogen in a mortar.

The carbon and nitrogen isotope ratios were determined by combustion using an elemental analyzer (Carlo Erba, CHN-1100) coupled to a Thermo Finnigan Delta Plus mass spectrometer at the Laboratory of Isotope Ecology of the Centro de Energia Nuclear na Agricultura (CENA/Universidade de São Paulo), Piracicaba, SP, Brazil. The results were expressed in delta notation (δ), in parts per thousand (‰), based on internationally recognized standard. We used the following equation: $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ (‰) = $(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}} \times 1.000$, where R_{sample} and R_{standard} represent the heavy/light isotope molar ratio of the sample and standard, respectively. The standard used for carbon analysis was Pee Dee Belemnite (Vienna Pee Dee Belemnite - Vienna PDB; $^{13}\text{C}:^{12}\text{C}$ ratio = 0.01118), and the standard used for nitrogen analysis was atmospheric air ($^{15}\text{N}:^{14}\text{N}$ ratio = 0.0036765). Internal standards (tropical soil and sugarcane leaves) are routinely interspersed with target samples during analysis runs. Long-term analytical error for the internal standards are of 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, 1% for organic C, and 0.02% for total N.

2.5 Statistical analyses

The normality of raw data and residues was tested using the Shapiro-Wilk W-test. The homoscedasticity of the variances was verified by the Barlett test.

Multiple comparisons between the treatments (i.e., Control, +N, ++N, and +++NM), regarding the sediment physicochemical properties and biotic and abiotic data, were evaluated using the Permutational Multivariate Analysis of Variance (PERMANOVA), using Euclidean distance matrix with 999 permutations after standardization of the data with the function "scale" (Anderson 2001). A Pearson correlation matrix was used to evaluate the correlation between abiotic and biotic variables in order to eliminate highly correlated variables from the analyses ($r > 0.9$) (Supplementary Fig. 1). Given that BADT and BALT were highly correlated, only BADT was used for PCA and further analyses (Supplementary Fig. 1).

The study plots and the abiotic and biotic variables were ordered through Principal Component Analysis (PCA) to verify the environmental distance between treatments (Legendre and Legendre 2012).

Univariate comparisons of abiotic and biotic variables between treatments were performed using the one-way ANOVA F-test followed by *pos-hoc* Tukey HSD test. Comparisons of foliar attributes between mangrove and aquatic macrophytes at the +++NM plots were tested using Student's t-test or the non-parametric Mann-Whitney U-test.

The variance inflation factor (VIF) values of the predictor variables were evaluated. Variables with VIF > 20 were excluded because they presented high collinearity. We conducted a redundancy analysis (RDA) to evaluate the effect of the selected abiotic factors (Sal10 + NH₄⁺-N + NO₃⁻-N) on the biotic variables that presented significant differences between treatments on univariate comparisons (BADT, foliar C:N and foliar $\delta^{13}\text{C}$) and also foliar N, which is an important foliar attribute. The statistical significance of the RDA axes and groupings were tested by the analysis of similarities (ANOSIM). A partial RDA (pRDA) was performed to evaluate the individual effect of each predictive abiotic variable on the set of response biotic variables (Legendre and Legendre 2012). The significance of the effect of each predictor variable on the response variables was tested by an analysis of variance (ANOVA).

All analysis was performed using the software R (R Core Team 2017) at $p < 0.05$.

3. Results

3.1 Physical and chemical properties of sediment

Sediment texture and the active, potential, and exchangeable pH values were similar among study plots. Concentrations of organic matter (OM), available P, Na⁺, K⁺, Ca²⁺, and Mg²⁺, as well as SEB and CEC in sediment at 0–20 cm depth were also similar between the study plots (PERMANOVA, $F_{(3,4)} = 1.6919$, $p = 0.385$) (Supplementary Table 2).

3.2 Abiotic and biotic factors related to the colonization of non-native species

The abiotic variables (Sal10, Sal50, NH₄⁺-N, NO₃⁻-N, and Nmin) were significantly different between treatments (PERMANOVA, $F_{(3,14)} = 10.088$, $p = 0.001$). Similarly, biotic variables including foliar attributes (i.e., N concentrations, C:N ratios, and $\delta^{13}\text{C}$) and vegetation structure data (i.e., DBH, H, BALT, and BADT) also differed among treatments (PERMANOVA, $F_{(3,14)} = 5.5376$, $p = 0.012$). These abiotic and biotic variables were also consistently different among treatments on univariate comparisons, except for DBH, H and foliar N concentrations, which were similar among study plots (see Supplementary Table 3).

The PCA axes of abiotic variables explained 89.9% of the total variance in the data set (Fig. 2A). Ordination axis 1 was negatively correlated to NO₃⁻-N concentrations and interstitial Sal10 and Sal50,

which grouped Control and + N plots. The ordinations of +++NM plots was negatively correlated with the variables Sal10, Sal50, and NO_3^- -N. Ordination axis 2 was negatively correlated with the Nmin and NH_4^+ -N that grouped the ++ N 2–4 plots, which presented the highest concentrations of these variables (Fig. 2A). The PCA axes of biotic variables accounted for 69.1% of the variance (Fig. 2B). Ordination axis 1 indicated that the invaded areas subjected to high N inputs (+++NM plots) were positively correlated with the foliar $\delta^{13}\text{C}$ and N concentrations and with BADT, while were negatively correlated with foliar C:N ratios. The ordination of plots of Control, +N, and ++ N was positively correlated with C:N and by the variables DAP and H (Fig. 2B).

3.3 Influence of abiotic factors on the colonization of non-native species

Axes 1 and 2 of the RDA, accounting for the influence of abiotic on biotic variables, explained 37.57% of the total variance ($F_{(3,14)} = 4.6744$, $p = 0.001$) (Fig. 3). Axis 1 of the RDA explained 33.53% of the variance and the clusters were statistically significant ($F_{(1,14)} = 11.9557$, $p = 0.002$). RDA axis 1 was negatively correlated with interstitial Sal10 and foliar C:N ratios and positively correlated with BADT and foliar $\delta^{13}\text{C}$ and N concentrations. However, interstitial Sal10 was the only predictor variable that had significant influence on axis 1. RDA axis 2, on the other hand, was not significant ($F_{(1,14)} = 1.4414$, $p = 0.481$), explaining only 4.04% of the variance.

Considering the individual influence of each predictor abiotic variable on the biotic variables related to the colonization of aquatic macrophytes (pRDA), Sal10 and NO_3^- -N exhibited the highest coefficients of determination (adjusted R^2) and significant p-values (Sal10: $R^2_{(\text{adj})} = 0.422$, $F = 11.443$, $p = 0.001$; NO_3^- -N: $R^2_{(\text{adj})} = 0.101$, $F = 6.3702$, $p = 0.002$). The influence of NH_4^+ -N was not significant ($R^2_{(\text{adj})} = 0.010$, $F = 1.2704$, $p = 0.306$).

3.4 Foliar attributes of native and non-native species

3.4.1 Foliar C:N ratios and N concentrations

Foliar C:N ratios of aquatic macrophytes (mean \pm standard deviation) (15.4 ± 3.9) were lower compared to mangroves (22.5 ± 4.4) ($t_{(19)} = 3.8489$, $p = 0.001$) (Fig. 4A). Foliar N concentrations of the aquatic macrophytes species were higher (26.0 ± 5.3 g/kg) relative to mangrove species (15.0 ± 3.9 g/kg) ($t_{(19)} = -5.4938$, $p < 0.001$) (Fig. 4B).

3.4.1 Foliar $\delta^{13}\text{C}$

The foliar $\delta^{13}\text{C}$ of mangrove species ($-29.5 \pm 1.2\text{‰}$) was similar to C_3 aquatic macrophytes ($-29.7 \pm 1.0\text{‰}$). The foliar $\delta^{13}\text{C}$ of mangroves and C_3 aquatic macrophytes were both significantly lower than C_4 aquatic macrophytes ($-12.7 \pm 0.9\text{‰}$) ($F = 201.2$, $P < 0,001$) (Fig. 4C).

The foliar $\delta^{13}\text{C}$ of the dominant aquatic macrophytes (C_3 and C_4 plants pooled together, $n = 6$) (median, and first and third quartiles) (-28.5, -29.9 and - 16.9‰) was similar to mangrove species (29.3, -30.1 and 28.6‰) ($U = 48$, $p = 0.290$) (Fig. 4D).

4. Discussion

Many studies have addressed how human alterations of hydrological fluxes in the upstream watershed can modify environmental conditions in downstream mangrove forests (Dahdouh-Guebas et al. 2005; Röderstein et al. 2013). However, most of these studies reported alterations following the reduction of freshwater input into mangrove ecosystems, leading to increased salinity, degradation of the mangroves and dominance by tolerant mangrove species (Gopal and Chauhan 2006; Sathyanathan et al. 2014). Here, we evaluated impacts following the increase of freshwater inputs into mangrove forests. We found large alterations on the structure of native vegetation in the presence of aquatic macrophytes, in agreement with previous studies showing the marked sensibility of mangrove ecosystems to changes in the hydric regime (Lugo 1998; Zhang et al. 2012; Röderstein et al. 2013; Biswas et al. 2018).

The PCA analysis indicated the association between the mangrove areas subjected to high N inputs and colonized by aquatic macrophytes (+++NM plots) with the concentrations of NO_3^- -N in the sediment, interstitial Sal10 and Sal50 and by the biotic variables BADT, foliar $\delta^{13}\text{C}$, N foliar and foliar C:N. The RDA and pRDA analysis indicated that the biotic variables BADT, foliar N concentrations, C:N ratios and foliar $\delta^{13}\text{C}$ were significantly influenced by interstitial salinity at 10 cm depth followed by NO_3^- -N concentrations in mangrove sediment. This suggests that colonization by aquatic plants is mainly controlled by these variables at our study sites. The Valo Grande canal opening has facilitated the entrance of propagules and banks of aquatic macrophytes into the estuary and mangrove areas in the Iguape region. Our results suggest that a salinity reduction to 1.7 PSU following excess freshwater inputs was the main factor contributing to the colonization and subsequent establishment of aquatic macrophytes in these mangrove forests. These results add to previous studies that indicated high salinity as the main environmental filter preventing non-native plant colonization into mangroves (Lugo 1998). The plants that invade mangrove forests are usually able to tolerate high salinity to some extent (Biswas et al. 2018). For this reason, species like aquatic macrophytes would not be able to colonize mangroves unless salinity was significantly reduced in these systems (Lugo 1998; Dahdouh-Guebas et al. 2005; Biswas et al. 2012; Ren et al. 2014). Accordingly, Röderstein et al. (2013) reported that a salinity reduction to 1.0–2.0 PSU was the main factor leading to the establishment of the aquatic macrophyte *Typha domingensis* Pers in mangroves on the Caribbean coast of Colombia.

Concentrations of NO_3^- -N in sediment were the second factor that mostly affected the colonization by aquatic macrophytes at our study sites. It can be explained by the fact that nutrient concentrations in sediment affect the growth of non-native species (Ren et al. 2014). For instance, while the occurrence of non-native species in mangroves and other coastal areas in south China was negatively correlated with sediment salinity, their biomass was positively correlated with total N content in sediments (Ren et al.

2014), underscoring the importance of N availability for the growth of non-native species. The Valo Grande canal also carries excess N inputs from human activities resulting in high N availability in mangrove sediment at the +++NM and ++ N plots. Despite the ++ N plots exhibited higher NO_3^- -N and mineral N concentrations in sediment relative to the +++NM plots, aquatic macrophytes were not recorded inside the mangrove stands where the ++ N plots were located. Because these stands were located closer to an open ocean inlet, salinity ranged from 13.0 to 20.0 PSU at the ++ N plots. Thus, the high salinity likely prevented the establishment of aquatic macrophytes species at these stands, despite propagules and floating banks of aquatic macrophytes being found in the surrounding estuarine waters. This highlights that salinity is the main factor controlling the establishment of non-native species at our study sites.

The co-occurrence of aquatic plants and mangroves has been found in ecotonal zones and bordering mangrove forests (Tomlinson 1986; Lugo 1998). The establishment and dominance of aquatic plants inside mangrove stands, however, is a consequence of disturbance, and can result in negative effects on the native vegetation (Biswas et al. 2007, 2018). We observed large alterations on mangrove vegetation structure at the plots colonized by aquatic macrophytes (+++NM) (Cunha-Lignon et al. 2011; Cunha-Lignon and Menghini 2016). The BADT data indicated the higher amount of dead mangrove trunks at the +++NM plots (up 53% of basal area) compared to the other plots (up to 20%). In fact, the gradual and progressive increase in dead mangrove trees in areas close to the Valo Grande channel has been reported by long-term monitoring (e.g., Cunha-Lignon et al. 2015). This can be related to a higher competitive advantage of aquatic macrophytes versus mangroves under low salinity and high N availability allowing the fast growth of non-native species (Dukes and Mooney 1999; Vilà and Weiner 2004; Bradley et al. 2010; Biswas et al. 2012, 2018; González-Muñoz et al. 2013; Gufu et al. 2018). Further studies are needed to understand the mangrove mortality process in these areas.

The higher foliar N concentrations and lower C:N ratios of aquatic macrophytes relative to mangroves suggested a higher N demand by the non-native species. Accordingly, we have also found that aquatic macrophytes exhibit higher foliar $\delta^{15}\text{N}$ than mangroves at the +++NM plots (Reis et al., unpublished data). Moreover, the lower NO_3^- -N concentrations in mangrove sediment at the +++NM plots compared to the others likely reflected a higher absorption of N by the species of aquatic macrophytes, along with higher N losses to the atmosphere via denitrification as a consequence of excess inorganic N inputs and intensified N cycling (Reis et al. 2017b, 2019). The differences in foliar N and C:N ratios between native and non-native species can also modify the quality of the organic matter in the sediment, which could also potentially affect N and C pools and cycling in the system (Liao et al. 2008; Lu et al. 2014; Lee et al. 2017). Further studies are needed to evaluate the consequences of the establishment of non-native species to the functioning of these mangrove forests.

While mangrove trees have C_3 photosynthesis pathways, the species of aquatic macrophytes studied here exhibited predominantly C_3 but also a few C_4 photosynthesis pathways, thus reflecting a change in the acquisition of C. For this reason, we observed a greater variability in foliar $\delta^{13}\text{C}$ of non-native than

native species at the +++NM plots. Due to the occurrence of C₃ and C₄ non-native photosynthetic plants and the similarity between the foliar δ¹³C values of the C₃ non-native and native species, this attribute was not a good indicator for differentiating mangroves and aquatic macrophytes in the case of this study.

In summary, the abiotic factors interstitial Sal₁₀ and NO₃⁻-N concentrations in sediment facilitated the colonization by aquatic macrophytes in the studied mangrove areas. We also verified the higher foliar N concentrations and lower foliar C:N ratios of non-native relative to native plants, possibly reflecting a greater N demand and assimilation of N by non-native plants. The combination of these abiotic and biotic factors and the competitive interactions of the native and non-native communities have resulted in the dominance of aquatic macrophytes in the mangrove understory and the high amount of mangrove dead trunks (BADT) in the plots colonized by aquatic macrophytes (+++NM). Additionally, we did not observe the occurrence of young mangrove individuals at the +++NM plots, likely as a consequence of the dense biomass of non-native plants that forms a physical barrier to the dispersion of mangrove propagules, thus affecting the recruitment and succession of mangroves (Cunha-Lignon et al. 2011; Biswas et al. 2012, 2018).

Considering that most non-native plant colonization and establishment in mangroves result from changes in abiotic conditions as a result of disturbances (Biswas et al. 2018) and that these changes may be difficult to reverse (Bradley et al. 2010), management efforts should be focused on the major disturbance factors that control the colonization of non-native plants by promoting restoration of abiotic conditions and recolonization of native species (Röderstein et al. 2013). In the case of this study, the rehabilitation of regional hydrological conditions coupled with the reduction of excess nutrient inputs, especially of N, should be the first step in the management of aquatic macrophytes in mangroves of the ELC of Cananeia-Iguape. Therefore, the rehabilitation of mangrove forests near the mouth of the Valo Grande is a relevant topic due to the alarming amount of dead trunks observed in these areas and the many important ecosystem services that mangroves provide.

5. Conclusions

The increased freshwater flow and associated abiotic changes verified in the north sector of the ELC of Cananeia-Iguape were determinant for the species of aquatic macrophytes overcoming the geographic and environmental barriers, allowing their expansion and dominance in mangrove ecosystems. The reduction of salinity and increased N availability in the sediment allied to the functional attributes of the non-native plants related to a high N demand allowed the occurrence and success of the colonization aquatic macrophytes in the studied mangroves.

Declarations

Acknowledgments

We would like to thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio - Brazil) and the Instituto Florestal (Secretaria do Meio Ambiente, São Paulo, Brazil) for the permissions to conduct this study in the Cananeia-Iguape-Peruibe Protection Area [Processes 37339 and 47365] and the Cardoso Island State Park [Processes 260108-003.838/2010 and 260108-012.547/2014], respectively. We are also thankful to Marcos Bornschein for identifying the species of aquatic macrophytes, and to João Paulo Sena Souza for providing the map of the study area.

Competing Interests

The authors declare that there are no competing interests

Funding Info

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior CAPES – Brazil [001 to J.A.G.S. and 1422671 to C.R.G.R]; the Conselho Nacional de Desenvolvimento Científico e Tecnológico CNPq– Brazil [445418/2014-1 to M.C-L]; the Fundação Grupo O Boticário, edital Bio&Clima Lagamar [BL0006_2012_1 to M.C-L.]; Fundação de Amparo à Pesquisa no Distrito Federal (FAPDF) [0193.000344/2017]; Rufford Foundation [grant 20243-1].

Author contribution

JAGS – Sampling, writing the manuscript, statistical analysis

CRGR - Writing the manuscript, statistical analysis, sampling design

MCL – Sampling design, sampling, writing the manuscript

GBN - Sampling design, writing the manuscript, funding

LFS - Writing the manuscript, funding

Data Availability

The authors may provide the data used in the manuscript upon request

Animal Research (Ethics)

No animals have been involved in the present research.

Consent to Participate (Ethics)

There was no humans used in the present research.

Consent to Publish (Ethics)

The authors allow the data be published in case the manuscript is accepted.

Plant Reproducibility

The present research did not make use of plant reproducibility techniques.

Clinical Trials Registration

The present research did not make use of clinical trials.

Gels and Blots/ Image Manipulation

The present research did not make use of gels and blots and image manipulation.

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Figures

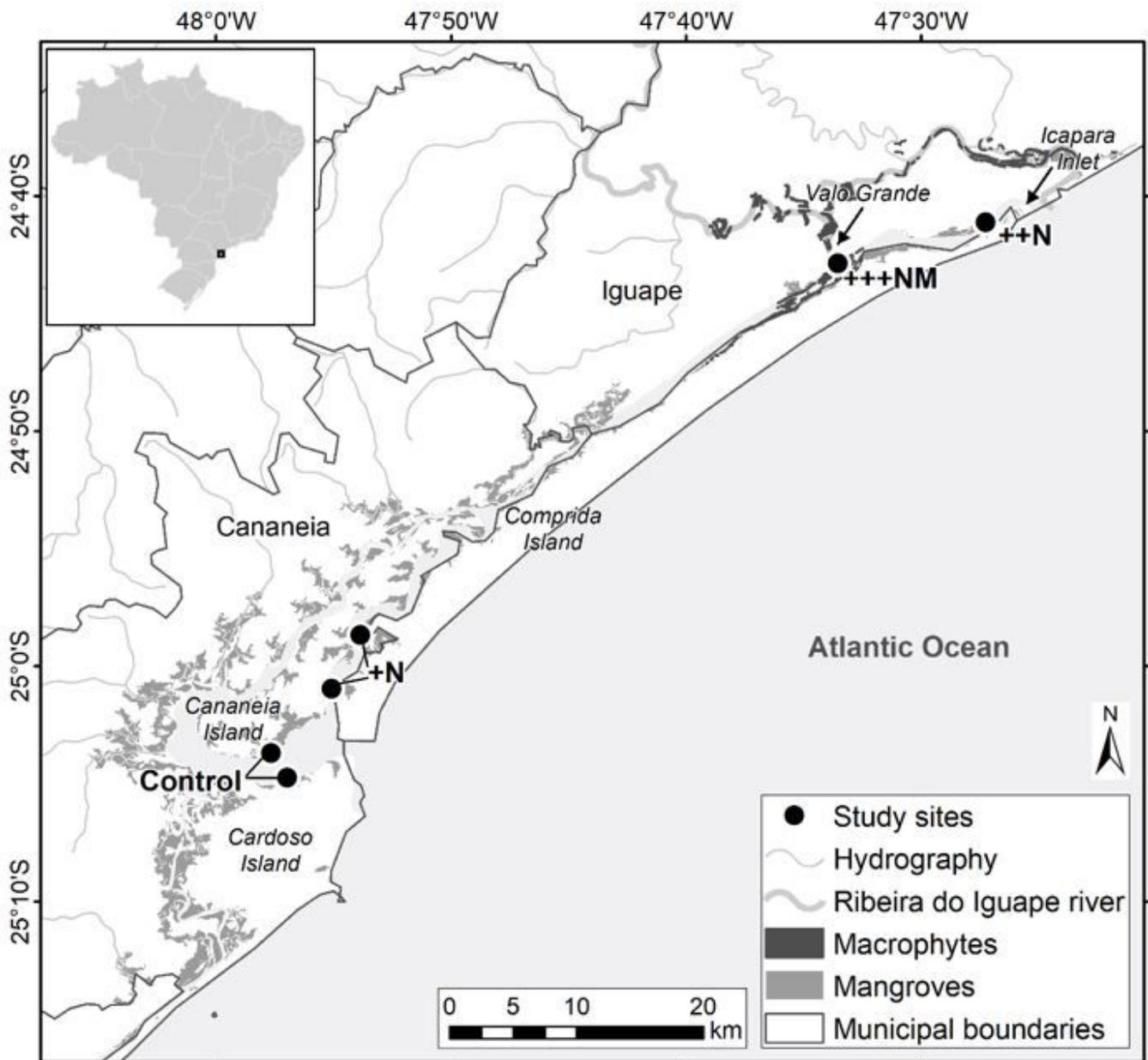


Figure 1

Location of mangrove study sites in the Estuarine Lagoon-Complex of Cananeia-Iguape, southeastern Brazil. Study sites included two non-N-enriched and free of aquatic macrophytes reference sites (Control); two sites free of aquatic macrophytes and subjected to medium N inputs (+N); two sites without the presence of aquatic macrophytes and subjected to medium to high N inputs (++N), and two sites colonized by aquatic macrophytes and subjected to high N inputs (+++NM). Because of the map scale, the two study sites at +++NM and at ++N were overlaid. Source of the shapefiles of mangrove forests and aquatic macrophytes areas: Cunha-Lignon et al. (2011) Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the

part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

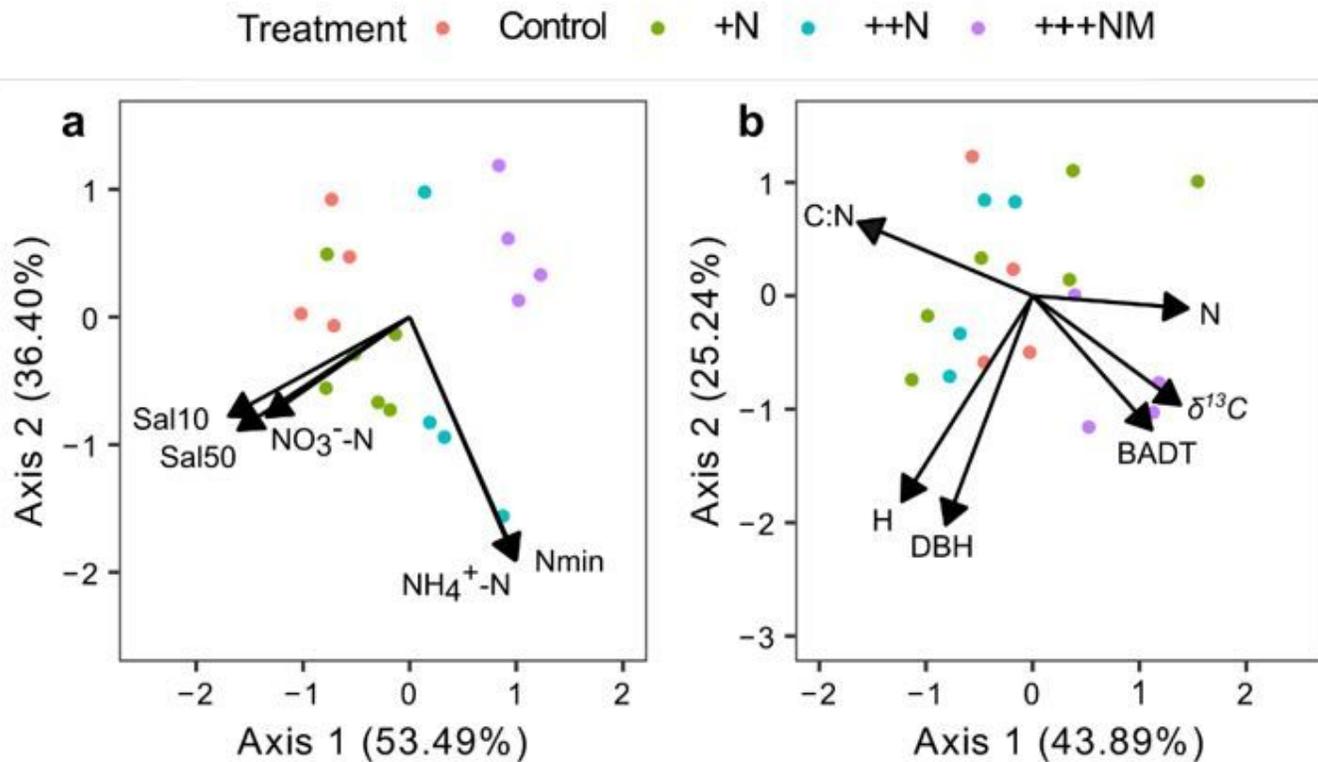


Figure 2

Principal component analysis (PCA) of mangrove study plots according to abiotic (A) and biotic variables (B). Dots of different colors identify the treatments: non-N-enriched and free of aquatic macrophytes plots (Control), plots free of aquatic macrophytes and subjected to medium (+N) and medium to high (++) N inputs, and plots colonized by aquatic macrophytes and subjected to high N inputs (+++NM).

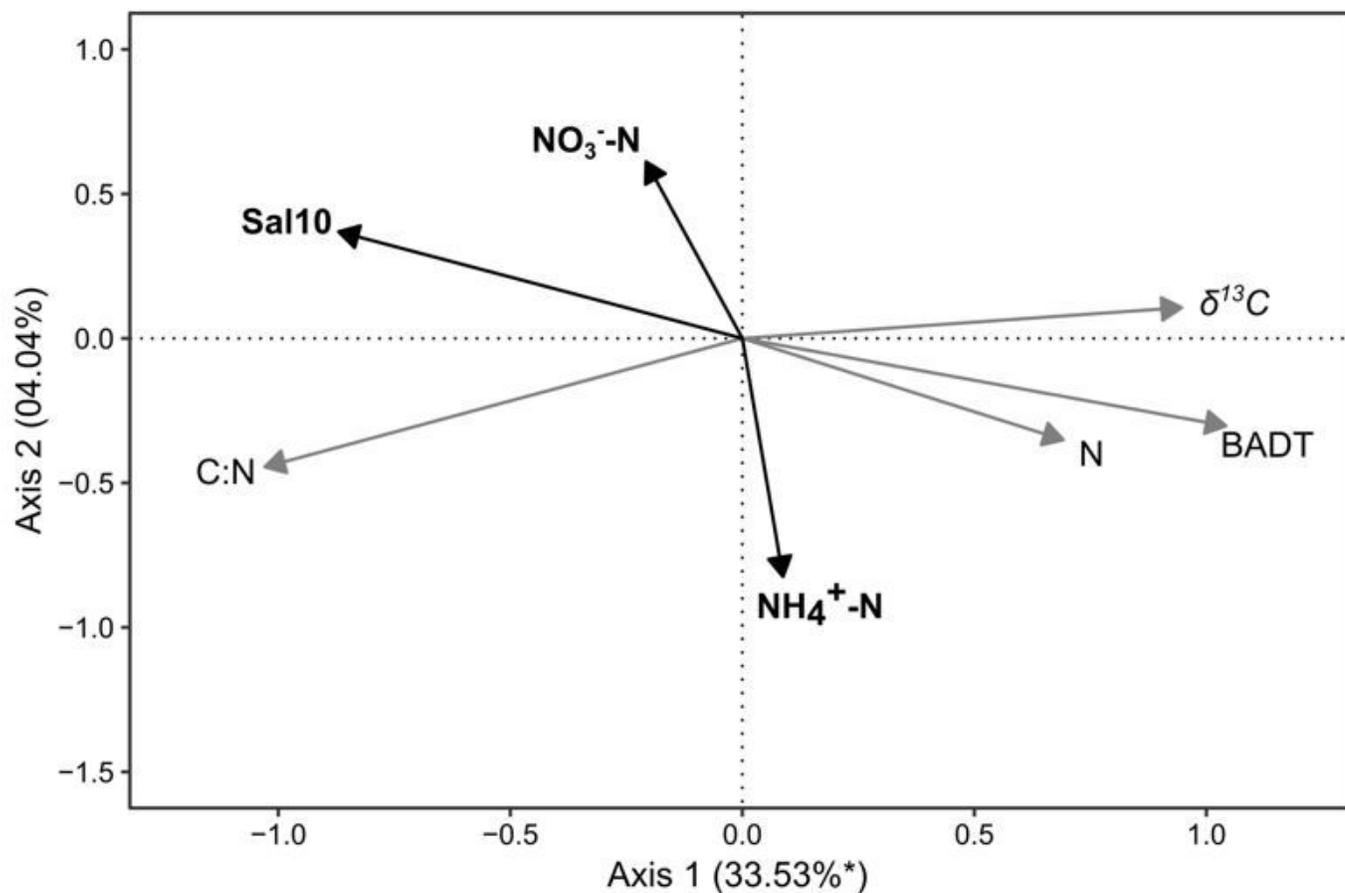


Figure 3

Redundancy analysis (RDA) of the influence of abiotic on biotic factors associated with the colonization of aquatic macrophytes in mangrove areas in the Estuarine Lagoon-Complex of Cananeia-Iguape, southeastern Brazil. Black arrows indicate predictor variables and gray arrows indicate response variables.

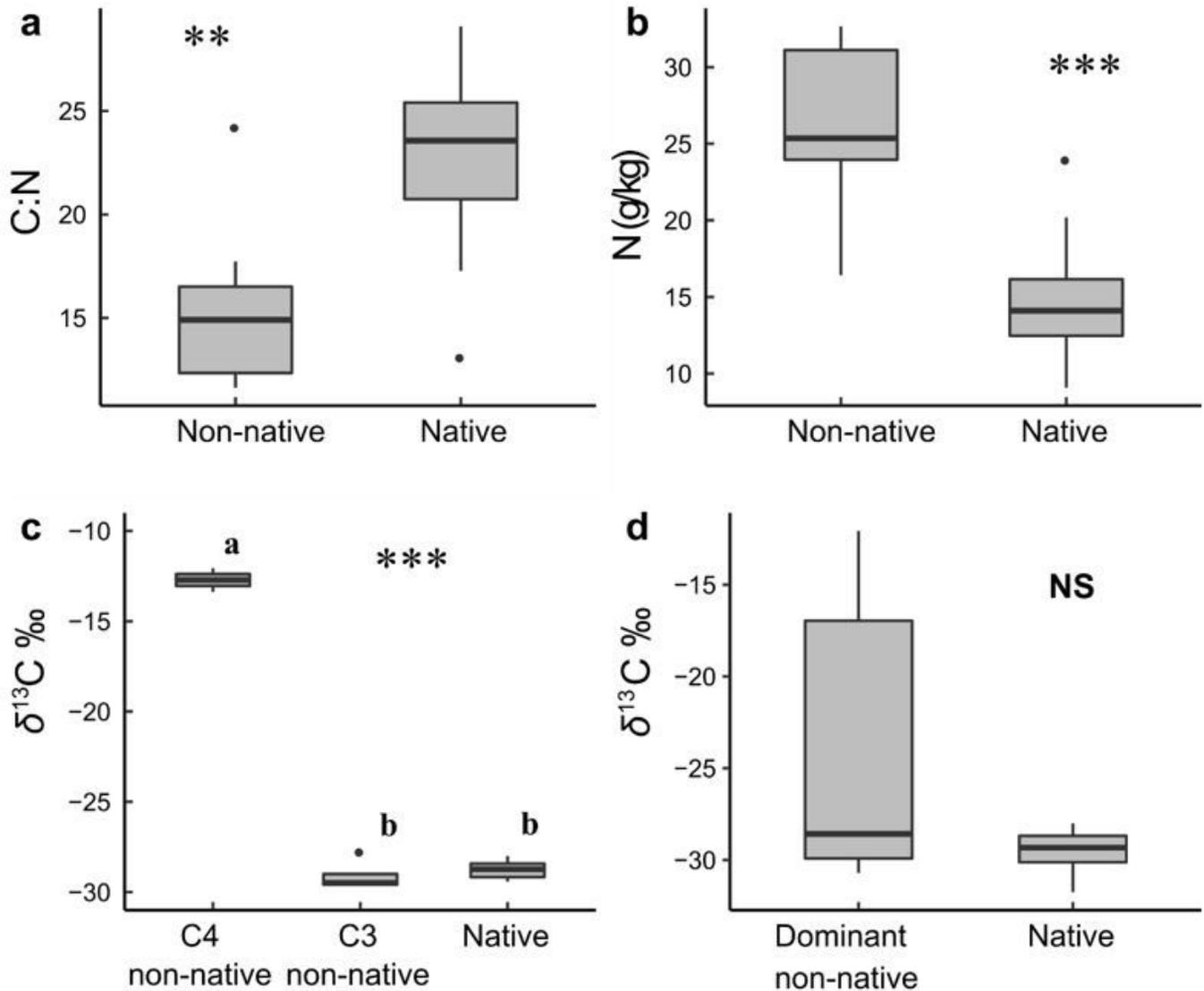


Figure 4

Foliar attributes of native (mangroves) and non-native (aquatic macrophytes) vegetation at the study plots subjected to high N inputs (+++NM plots) in the Estuarine Lagoon-Complex of Cananeia-Iguape, southeastern Brazil, in July 2015. Panels include comparisons of foliar C:N (A), foliar N concentrations (g/kg) (B), and foliar $\delta^{13}\text{C}$ (‰) (C) between native (n=12) and non-native plants (n=9). In panel D, the foliar $\delta^{13}\text{C}$ (‰) of native (n=12) and the dominant non-native plants (n=6) (C3 and C4 plants pooled together). Significance codes: p < 0.05 ‘*’, 0 ‘***’, 0.001 ‘**’, >0.05 ‘NS’. NS = no significant statistical difference. Different letters indicate significant statistical difference between native and non-native species.

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