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Isotopic evidence of subtle nutrient enrichment in mangrove habitats of Golfo Dulce, Costa Rica

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Abstract

Mangroves are of great ecological and socio-economic importance, yet they are under threat from urban development on the southern Pacific coast of Costa Rica. To test for possible nutrient-related impacts, we compared water-column nutrient concentrations, C and N stable isotope values and other environmental variables between mangroves with known sewage loading (three "nutrient loaded" locations) and those without such loading (three "reference" locations). Instantaneous nutrient concentrations were low at all locations, Secchi depth was greater at reference locations, and chlorophyll concentrations were higher at nutrient loaded mangroves. Suspended matter did not vary between reference and nutrient loaded mangroves, and nor did bivalve and algal $\delta^{13}C$ and $\delta^{15}N$ values. Enrichment of $\delta^{15}N$ and $\delta^{13}C$ of red mangrove leaves at the nutrient loaded locations is attributed to pulsed inputs of materials that were not detected in the instantaneous nutrient data. We provide evidence of isotopic enrichment at nutrient loaded locations from mangrove material and recommend that adequate waste water treatment be carried out on all anthropogenic discharges into this vulnerable marine system.

KEYWORDS

Anadara, benthic filter feeders, *Bostrychia*, macroalgae, *Rhizophora*, stable isotopes

1 | INTRODUCTION

Mangrove habitats can act as filters for eutrophication and lessen the impacts of nutrient loading on adjacent coastal habitats such as seagrasses and coral reefs (Bouillon, Moens, & Dehairs, 2004; Kathiresan & Bingham, 2001; Rodelli, Gearing, Gearing, Marshall, & Sasekumar, 1984). Mangrove forests are highly productive tropical coastal ecosystems (Duarte & Cebrian, 1996), which can serve as important nursery habitats, stabilize sediments, buffer against tsunamis and storms, store carbon, and provide food and resources for coastal communities (Donato et al., 2011; Hogarth, 1999; Tomlinson, 1994), but increased human population and catchment development threaten these coastal ecosystems (Duke et al., 2007). Mangrove forests are vulnerable habitats, which endure a multitude of anthropogenic stresses and continue to decline worldwide (Duke et al., 2007; Valiela, Bowen, & York, 2001). Sewage discharge in particular can reduce water quality, generate anoxic conditions, and increase water-column pathogens, and thus impact these habitats (Lapointe & Clark, 1992; Lapointe, O'Connell, & Garrett, 1990).

In Costa Rica, 99.9% of mangroves are located on the Pacific coast, and the most extensive mangrove forests occur in the southern sector (Cortés, 2016; Cortés & Wehrtmann, 2009; Jiménez & Soto, 1985). Golfo Dulce on that coast is an environment with relatively low nutrient concentrations (Córdoba-Muñoz & Vargas-Zamora, 1996; Morales-Ramírez, Acuña-González, Lizano, Alfaro, & Gómez, 2015; Wolff, Hartmann, & Koch, 1996) and abundant unlogged forest (Quesada-Alpízar & Cortés, 2006). Golfo Dulce is known for its beauty, habitats, and marine megafauna (Chacón-Chaverri, Martínez-Cascante, Rojas, & Fonseca, 2015; Morales-Ramírez et al., 2015), and its mangroves are nursery grounds for commercially important shrimp (Jesse, 1996) and mud cockle (*Anadara tuberculosa* and *Anadara similis*) for human consumption (Silva Benavides & Carrión, 2001; Silva & Carrillo, 2004; Stern-Pirlot & Wolff, 2006). However, there is increasing pressure from urban and tourism development, including nutrient input, destructive fishing, aquaculture projects, deforestation, and land run-off (Cortés, 1990; González-Chen, 2009; Loaiza, 2007; Morales-Ramírez et al., 2015; Quesada-Alpízar & Cortés, 2006). Golfo Dulce is particularly sensitive to pollution, given its long water residence times due to its fjord-like bathymetry and limited water circulation (Hebbeln & Cortés, 2001; Morales-Ramírez et al., 2015). Nutrient enrichment and other stress factors may be affecting the Golfo Dulce mangroves; these include high metal concentrations, polychlorinated biphenyl pollution (García-Céspedes, Acuña-González, & Vargas-Zamora, 2004; Spongberg, 2004; Spongberg & Davis, 1998) and coliform contamination from raw sewage discharge in Golfito Bay (García, Acuña-González, Vargas-Zamora, & García-Céspedes, 2006). Timely identification of nutrient enrichment is needed for management of this vulnerable system (Morales-Ramírez et al., 2015); however, the evidence for any impacts is circumstantial; nutrient enrichment in Golfo Dulce is considered to be low or negligible (Morales-Ramírez et al., 2015; Silva & Acuña-González, 2006), and instantaneous measurements may be poor indicators of low level or transitory nutrient inputs.

Stable isotope data (notably δ^{13}C and δ^{15}N) can provide useful information on food-web structure (Fry, 2006; Peterson & Fry, 1987; Post, 2002), and δ^{15}N can also indicate anthropogenic nutrient loading (Costanzo, O'Donohue, Dennison, Loneragan, & Thomas, 2001; Costanzo, Udy, Longstaff, & Jones, 2005; Piola, Moore, & Suthers, 2006; Risk & Erdmann, 2000; Rogers, 1999; Teichberg et al., 2010; Udy & Dennison, 1997). The stable isotope signatures of organisms are subject to tissue turnover effects but have the benefit of time integration such that they may help detect chronic low-level impacts, including pulsed nutrient run-off events. Mangrove macroalgae have an isotopic turnover rate of as little as four days (Costanzo et al., 2005; Costanzo, O'Donohue, & Dennison, 2003), whereas marine bivalves can show isotopic variation in weeks to months (Lefebvre, Harma, & Blin, 2009; Paulet, Lorrain, Richard, & Pouvreau, 2006; Piola et al., 2006). In contrast, isotopic turnover rates of mangrove trees occur at yearly timescales, which are linked to their slower growth and thus tissue turnover (Costanzo et al., 2003; Pitt, Connolly, & Maxwell, 2009).

Here, we assess potential anthropogenic nutrient enrichment in mangroves of Golfo Dulce, Costa Rica. We hypothesise that δ^{15}N values in mangrove organisms (trees, bivalves, and algae) are higher in mangroves with sewage inputs ("nutrient-loaded") compared with those without them ("reference") indicating subtle, but long-term nutrient enrichment.

2 | METHODOLOGY

2.1 | Study sites

The study was carried out in Golfo Dulce (southern Pacific coast of Costa Rica; Figure 1), which is ca. 20 km long, with maximum depths of 200 m and a 60 m depth sill at its entrance (Hebbeln & Cortés, 2001; Morales-Ramírez et al., 2015; Quesada-Alpízar & Cortés, 2006; Richards, Anderson, & Cline, 1971). Golfo Dulce is considered to be a fjord-like embayment given its shallow entrance, steep borders, and deep waters; this leads to limited water circulation and anoxic conditions at depth (Morales-Ramírez et al., 2015). The water column of Golfo Dulce is low in nutrient concentrations (Morales-Ramírez et al., 2015; Silva & Acu a-González, 2006); however, the human population around Golfo Dulce increased by 9% and 14% in Osa and Golfito districts, respectively, between the years 2000 and 2009 (data of the Instituto Nacional de Estadística y Censo (INEC) available at http://www.inec.go.cr/estadisticas-vitales). There is no waste water treatment at any location near Golfo Dulce, and raw sewage is directly discharged into the gulf.

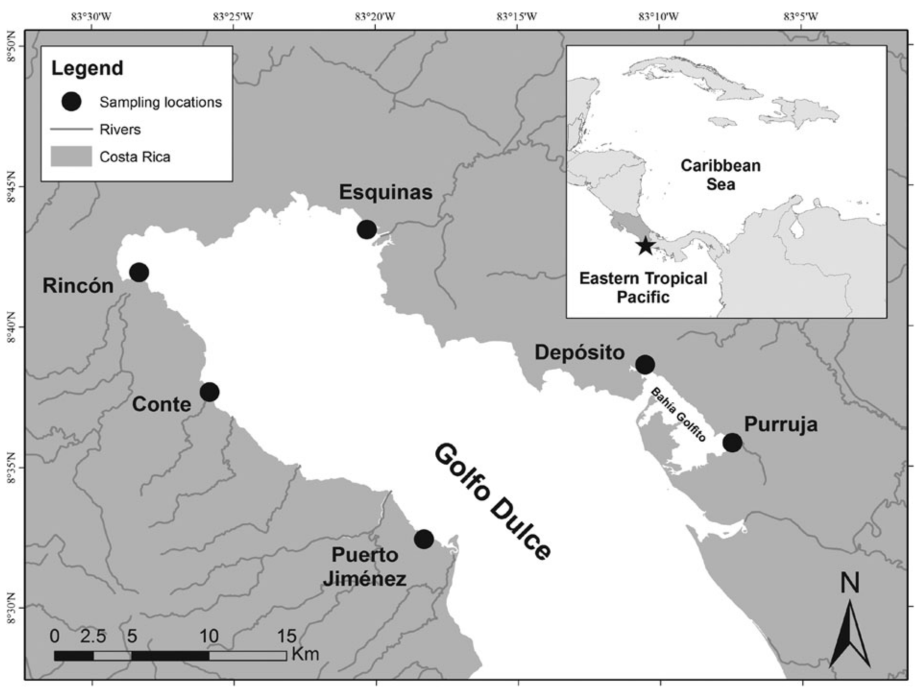

FIGURE 1 Study sites: Reference (Conte, Rincón, and Esquinas) and nutrient loaded mangrove (Jiménez, Depósito, and Purruja) locations in Golfo Dulce, southern Pacific of Costa Rica (star)

To test our hypothesis of point based nutrient loading, six mangrove forest locations were selected based on distance from human settlements with varying population size and nutrient inputs from untreated sewage. Mangroves were considered *a priori* to be (a) minimally impacted (low or negligible sewage input; hereafter "reference" mangroves); or 2 exposed to untreated sewage discharge (hereafter "nutrient loaded"). Three reference mangroves (Rincón, Esquinas, and Conte), and three nutrient loaded mangroves (Puerto Jiménez, Depósito, Purruja) were sampled in 2009 (Table 1). Reference mangroves were associated with longer and wider rivers that pass mostly through pristine rainforest areas, and through some land used for agriculture. Nutrient loaded mangroves were associated with shorter rivers directly adjacent to urban settlements (Table 1). The mangrove at Puerto Jiménez is located adjacent to Puerto Jiménez town and receives untreated effluents that are discharged into the river. The Depósito and Purruja mangroves lie within a small embayment (Golfito Bay) that receives raw sewage discharge (García et al., 2006) and effluents from the towns of Golfito and Purruja, respectively.

A 400 m coastal section along the shoreline was selected at each mangrove, allowing access during both low and high tide. At the shoreline, mangroves were dominated by one species of mangrove tree, the "red mangrove" (*Rhizophora mangle*), with canopy heights commonly of ca. 5 m at the outer edge. Each section was divided into 20 m sectors to randomly select sites for biological and water sampling.

2.2 | Water quality

2.2.1 | Sample collection

Water samples were collected at high tide at all six locations in 2009 on two separate dates: April 16–18 considered to represent dry season conditions, and during May 22–24 considered to represent rainy conditions (IMN, 2009; Morales-Ramírez et al., 2015). Water samples were collected within 50–200 m of the shoreline. Six sites of each coastal section were chosen randomly. At each site we (a) measured Secchi disk depth (m) three times; (b) collected one 1.8 L subsurface water sample at 10 cm depth in a dark plastic bottle for nutrient and suspended sediment determination; and (c) took one 20 ml subsurface water sample for salinity determination using a hand refractometer (ATAGO ATC). Samples were kept on ice until further processing. Each 1.8 L water sample was well mixed before separating into two subsamples for filtration through glass microfiber filters (ALBET FVC 047) using a vacuum pump (Welch Vacuum 2522B-01). One litre was used for chlorophyll a determination and nutrient analysis, and ca. 800 ml for suspended matter using dried preweighed filters. Filtered water samples for nutrient analyses were stored in high-density black polyethylene 1-L bottles. Filters and filtered water samples were kept frozen until processed in the laboratory.

2.2.2 | Total suspended solids and chlorophyll a analyses

Preweighed filters for determination of suspended matter were dried at 60 °C and reweighed (Sartorius 2842). Weight variation per filter was then related to the total volume of water filtered per sample to determine suspended matter (mg L^{-1}). Filters for chlorophyll determination were placed in 10 ml of acetone 90% in plastic laboratory tubes with screw caps and fitted with removable cardboard covers inhibiting light penetration. Filters were macerated with a fine metal rod. Samples were refrigerated for 20 hr, after which 2 ml of acetone, 90% were added and the sample homogenized. Samples were precipitated using a centrifuge for 10 min (IEC Clinical Centrifuge at 5,000 rpm). To calculate chlorophyll concentration, the supernatant of each sample was analysed in an ultraviolet visible spectrophotometer (Shimadzu, UV-1700 Pharma Spec), at 630, 645, 663, 665, and 750-nm wavelengths (Strickland & Parsons, 1972).

2.2.3 | Nutrient analyses

Previously filtered and frozen water samples were thawed overnight prior to nutrient analysis. Concentrations (μmol L^{-1}) of ammonium, nitrite, nitrate, phosphate, and silicate were determined following the methodology modified for 10 ml samples (Strickland & Parsons, 1972). On the basis of three subsamples per filtered sample, the uncertainty of the analysis was estimated (in μmol L^{-1}) as ±2.21 (ammonium), ±0.07 (nitrite), ±0.40 (nitrate), ±0.06 (phosphate), and ±1.78 (silicate). The average of the three replicates per sample was used for statistical analysis.

TABLE 1 General characteristics of six mangrove habitats in Golfo Dulce, southern Pacific coast of Costa Rica

Condition	Mangrove	Nearest town	Population	Characteristics	River	River length (km)
Reference	Rincón	Rincón	145	Untreated sewage effluent minimal, forest, and agriculture	Rincón	27.3
Reference	Conte	Barrigones	~100[a]	Untreated sewage effluent minimal, forest, and agriculture	Conte	11.1
Reference	Esquinas	Riyito	66	Untreated sewage effluent minimal and forest	Esquinas	23.0
Nutrient loaded	Jiménez	Puerto Jiménez	7,238	Untreated sewage effluent high	Town estuary	0.8
Nutrient loaded	Depósito	Golfito	13,056	Untreated sewage effluent high	Ca aza	3.4
Nutrient loaded	Purruja	Purruja	436	Untreated sewage effluent high	Purruja	2.4

Notes. Population source: Instituto Nacional de Estadística y Censo (INEC), Costa Rica (2000 and 2008). Vegetation and river length source: Instituto Geográfico Nacional de Costa Rica map of the area.

[a]Barrigones population was estimated from village size given lack of available data.

2.3 | Biological sampling

Samples were collected between April 28 and May 1, 2009. Because the relevant biota was not found in all six mangroves, biological samples were collected at only four mangrove locations (two reference mangroves: Rincón and Esquinas; and two nutrient loaded mangroves: Puerto Jiménez and Depósito). Puerto Jiménez mangrove has a narrow marine border due to increased urban development, and to maintain comparability among mangroves, samples were collected in random sectors in the 400-m area near the entrance of the mangrove channel. At each mangrove shoreline five sites were selected randomly from the 20-m sectors; except for Puerto Jiménez where only four sites were selected due to mangrove characteristics. Samples of mangrove leaves (*R. mangle*), epiphytic macroalgae growing on *R. mangle* prop roots (*Bostrychia calliptera*), and bivalves (*A. tuberculosa*) were collected for carbon and nitrogen stable isotope analysis.

2.3.1 | Algae

Various samples of the consortium of macroalgal species that grow epiphytic on *R. mangle* mangrove prop roots were collected at each site. A macroalgal sample refers to algae collected from one mangrove prop root. Samples were placed in airtight plastic containers to maintain moisture, kept on ice in the field, and kept frozen until final processing (2–4 weeks). Each sample was thawed at room temperature and cleansed of sediment (using freshwater and a plastic mesh coffee filter). The red macroalga *B. calliptera* was the most abundant alga at the greatest number of sites; therefore, material of this species was separated from individual samples for stable isotope analysis. Algal epiphytes on *B. calliptera* thalli, associated organisms, and point of attachment to the mangrove root were carefully removed with forceps under a dissecting microscope. Samples were then placed in 20 ml plastic containers filled with freshwater at room temperature and placed in an ultrasonic bath (Fisher Scientific FS-14) at 5-min intervals to cleanse adhered sediment and rinsed repeatedly until the water came out clear. Samples were then dried at 60 °C for 72 hr.

2.3.2 | Bivalves

Benthic bivalves (*A. tuberculosa*) were collected among *R. mangle* prop root sediment at each site within each mangrove, within 5 m of the shoreline. Samples were kept on ice in the field. Maximum length of shells was measured with a steel vernier calliper. The adductor muscle of each bivalve was dissected and kept frozen until drying at 60 °C for 72 hr. At Rincón, bivalves were uncommon at the randomly selected sites; therefore, a nearby site within this mangrove with abundant bivalves was sampled on May 2, 2009.

2.3.3 | Mangroves

At each of the five sites within each mangrove three leaves from adult *R. mangle* trees were collected. Care was taken in the field to select healthy leaves of similar size that were fully developed, and without noticeable parasites, discolouration, or herbivory. Samples were kept inside plastic bags on ice for transport. Leaf blades were cleaned with a damp cotton cloth and petioles removed, then dried at 60 °C for 72 hr. Maximum length and width of each leaf were measured.

2.4 | Stable isotope analysis

At each of the four mangrove locations, 10 samples of leaves and bivalve adductor muscle were analysed, for a total of 40 samples of each. Seven samples of algae were analysed per mangrove, for a total of 28 algal samples. Mangrove leaves were ground in an agate mortar and pestle prior to weighing. Dried biological material was weighed in silver capsules (mangrove leaves: 3.2 ± 0.1 mg; algae: 1.5 ± 0.1 mg; and bivalves: 0.5 ± 0.0 mg). To ensure removal of any carbonate material remaining in algal samples prior to stable isotope analysis, 5% HCl was added to the capsule in which each algal sample was weighed, then allowed to evaporate at 70 °C on a hotplate inside a fume cupboard. Carbon and nitrogen elemental analysis was performed using an elemental analyser (CE Instruments NA2500) and an isotope ratio mass spectrometer (VG Isogas Prism III; both instruments interfaced with a VG Dual Reference Gas Box and a VG Diluter). Samples were analysed against the marine sediment standard PACS-2 by the National Research Council of Canada with values of: 5.213‰ $\delta^{15}N$ and −22.227‰ $\delta^{13}C$. Elemental compositions were standardized by comparison with acetanilide standard (C 71.09%, N 10.36%). The standard deviation was ±0.11‰ for $\delta^{13}C$ and ±0.10‰ for $\delta^{15}N$ (n = 17 PACS-2 standard analyses). Stable isotope analysis was carried out in the Grant Institute at Edinburgh University. Elemental analysis error (PACS-2 elemental ratio standard deviation as a percentage of the mean elemental ratios) was 1.6% for C and 5.4% for N.

2.5 | Statistical analysis

One way analysis of variance, nested when appropriate, and correlations between variables were carried out in the statistical program R (v2.13.0) (R Development Core Team, 2012). Mangrove and corresponding treatment (reference and nutrient loaded) were factors, with response variables being water quality parameters (two visits) and biological data. Data were tested for normality and heteroscedasticity. Outliers were removed from the analysis only when necessary due to lack of effectiveness from transformations, yet results proved to be the same with or without outliers. Nonparametric Kruskal Wallis analyses were carried out when transformation of data or removal of outliers were unsuccessful.

3 | RESULTS

3.1 | Water quality

3.1.1 | Water transparency

Chlorophyll concentration was higher in nutrient loaded mangroves ($p < .001$; Figure 2a). Secchi depth was greater in reference mangroves than in nutrient loaded mangroves ($p < .001$), which did not vary seasonally ($p > .05$; Figure 2b). Suspended matter did not vary between reference and nutrient loaded mangroves ($p > .05$; Figure 2 c). Both chlorophyll and suspended matter varied between sampling dates ($p < .001$), where Jiménez and reference mangroves showed a similar trend of lower chlorophyll and suspended matter in the rainy season (Figure 2a,c).

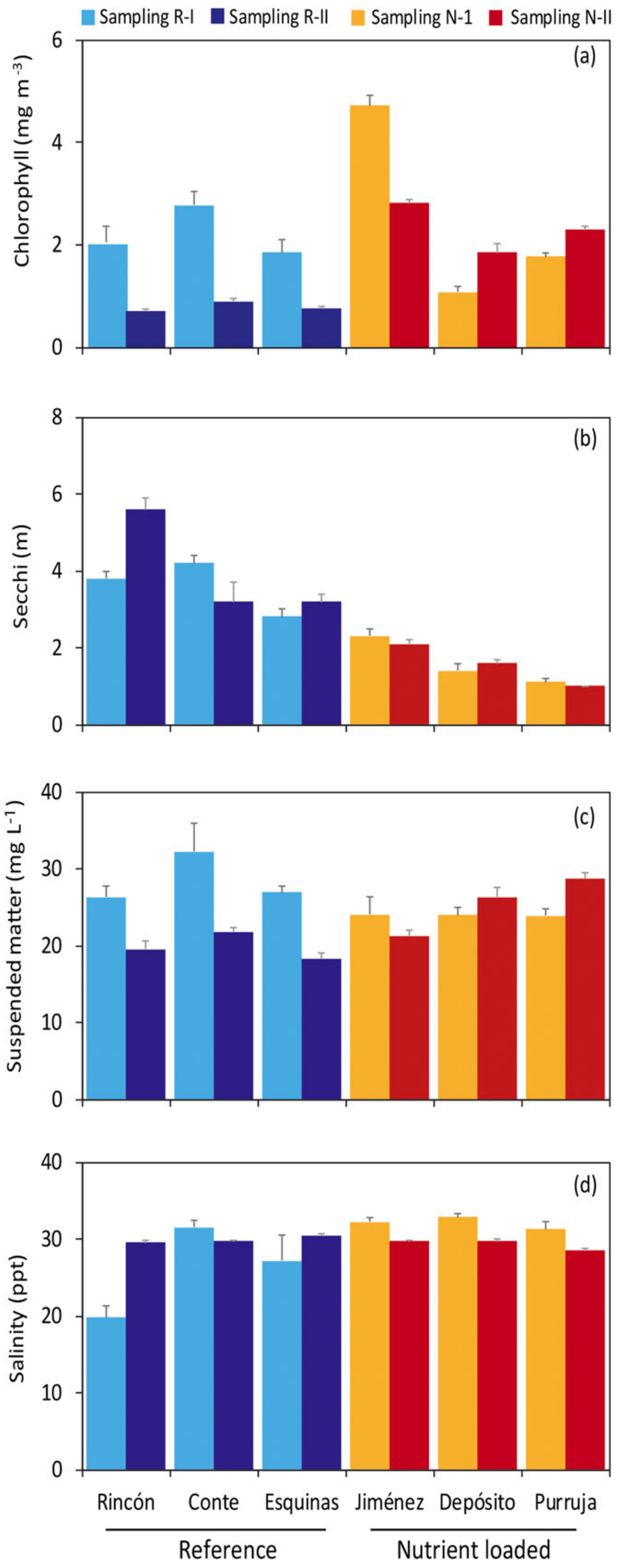

FIGURE 2 Water column suspended sediment (a), chlorophyll concentration (b), Secchi depth (c), and salinity (d; mean ± SE) at three reference mangroves (R) and three nutrient loaded mangroves (N) in Golfo Dulce, southern Pacific of Costa Rica. Sampling I = April 2009, sampling II = May 2009

3.1.2 | Nutrient concentrations and salinity

Salinity did not vary between reference and nutrient loaded mangroves ($p > .05$) or by sampling date ($p > .05$; Figure 2d). Nitrite concentrations at reference mangroves did not differ from those at nutrient loaded locations ($p > .05$). Ammonium and nitrate were not included in the statistical analysis as detected concentrations were at the limits of reliable detection (ammonium = 1.18; nitrate = 0.26). Phosphate and silicate concentrations varied between reference and nutrient loaded mangroves ($p < .05$). Neither one showed variation within mangroves between sampling dates (both $p > .05$; Table 2).

3.2 | $\delta^{15}N$ and $\delta^{13}C$ isotopic values

3.2.1 | Macroalgae

There was no variation in stable isotope composition or C:N of algae between reference and nutrient loaded mangroves ($p > .05$; Figure 3), although algal C:N was slightly lower in more $\delta^{15}N$ enriched algal samples ($p < .05$; Table 3).

3.2.2 | Bivalves

Bivalve $\delta^{13}C$ was slightly depleted in reference than in nutrient loaded mangroves ($p < .001$; Figure 3), however, bivalve $\delta^{15}N$ and C:N did not vary between reference and nutrient loaded mangroves ($p > .05$; Figure 3; Table 3). Bivalve isotopic data varied among mangrove locations ($\delta^{15}N$ & $\delta^{13}C$ $p < .001$; and C:N $p < .05$). Length of benthic bivalves varied among all locations ($p < .001$) but showed no variation between reference and nutrient loaded locations ($p > .05$). Longer bivalves were found at Escondido (5.47 ± 0.17 cm) and Depósito (5.04 ± 0.06 cm), and the bivalves were shorter at Puerto Jiménez (4.18 ± 0.28 cm) and Rincón (3.79 ± 0.48 cm). No correlations were found for bivalve isotopic results or with bivalve length.

3.2.3 | Mangroves

Leaf stable isotope composition varied between reference and nutrient loaded mangroves. $\delta^{15}N$ values were enriched by up to 3‰ at nutrient loaded mangroves ($p < .001$). These mangroves also had enriched $\delta^{13}C$ values ($p < .001$) and a narrower range in C:N ($p < .001$; Figure 3; Table 3).

4 | DISCUSSION

Here, we provide isotopic evidence of subtle nutrient enrichment in mangrove habitats of Golfo Dulce (southern Pacific coast of Costa Rica), despite limited evidence of nutrient loading from water quality analyses. These findings are suggestive of chronic low-level nutrient loading in this gulf. This isotopic information is important for understanding the state of the mangrove ecosystem, and at the same time providing mangrove, bivalve, and algal stable-isotope data for future studies.

Despite the lack of waste-water treatment and the fact that raw sewage flows directly into Golfo Dulce, previous water quality measurements in this gulf have not found evidence of eutrophication, and this was the case in the present study. The nutrient concentrations in the water column at Golfo Dulce can be considered to be

TABLE 2 Nutrient concentration (mean ± SE) at six mangrove habitats in Golfo Dulce, southern Pacific coast of Costa Rica

			Nutrient concentration (μmol L^{-1})				
Condition	Mangrove	Sampling	Phosphate (PO_4^{3-})	Silicate (SiO_4^{2-})	Ammonium (NH_4^+)	Nitrite (NO_2^-)	Nitrate (NO_3^-)
Reference	Rincón	SI	0.28 ± 0.07	53.91 ± 11.04	<1.27	0.052 ± 0.02	<0.26
		SII	0.19 ± 0.02	16.76 ± 2.26	<1.18	<0.03	<0.26
Reference	Conte	SI	0.40 ± 0.11	13.69 ± 11.59	<1.27	0.052 ± 0.01	<0.26
		SII	0.09 ± 0.01	6.42 ± 0.49	<1.18	<0.03	<0.26
Reference	Esquinas	SI	0.10 ± 0.02	8.51 ± 5.00	<1.27	0.053 ± 0.01	<0.26
		SII	0.17 ± 0.02	2.29 ± 0.16	<1.18	<0.03	<0.26
Nutrient loaded	Jiménez	SI	0.10 ± 0.02	3.42 ± 0.89	<1.27	0.063 ± 0.02	<0.26
		SII	0.09 ± 0.02	4.70 ± 1.00	<1.18	<0.03	<0.26
Nutrient loaded	Depósito	SI	0.22 ± 0.04	23.67 ± 5.45	<1.27	0.045 ± 0.01	<0.26
		SII	0.28 ± 0.03	14.21 ± 3.62	<1.18	<0.03	<0.26
Nutrient loaded	Purruja	SI	0.28 ± 0.05	33.40 ± 8.10	<1.27	0.054 ± 0.01	<0.26
		SII	0.15 ± 0.01	20.53 ± 0.52	<1.18	<0.03	<0.26

Notes. Sampling SI = April 2009; SII = May 2009; n = 6. Minimum detection levels (μmol L^{-1}): phosphate = 0.03; silicate = 0.78; ammonium = 1.18; nitrite = 0.03; and nitrate = 0.26.

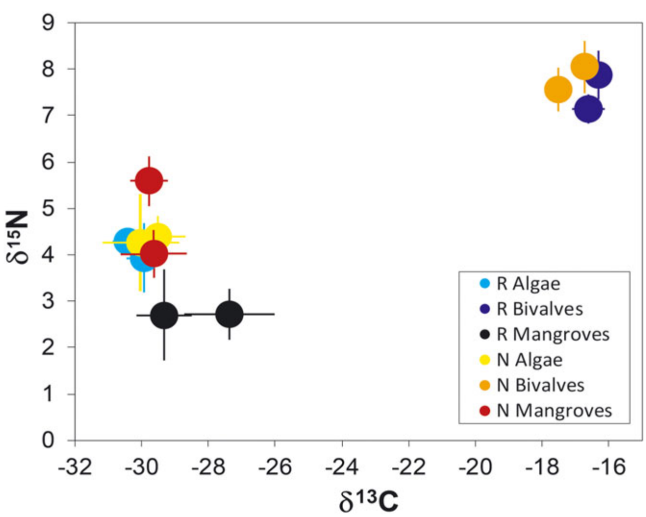

FIGURE 3 δ^{13}C and δ^{15}N values of algae (*Bostrychia calliptera*), bivalves (*Anadara tuberculosa*), and mangroves (*Rhizophora mangle*) at three reference mangroves (R) and three nutrient loaded mangroves (N) in Golfo Dulce, southern Pacific of Costa Rica

low (Morales-Ramírez et al., 2015; Silva & Acu a-González, 2006) and similar to those of the Gulf of Chiriquí (Pacific coast of Panama) where the highest values of nitrate were 0.75 μmol L^{-1} and of phosphate were 0.24 μmol L^{-1} (D'Croz & O'Dea, 2007). The Gulf of Panama (Pacific coast of Panama, an upwelling region) had slightly higher nutrient concentrations with phosphate reported up to 1.2 μmol L^{-1} and nitrate up to 14.4 μmol L^{-1}. Nutrient concentrations in Golfo de Nicoya, further north on the Pacific coast of Costa Rica, are higher than in Golfo Dulce, with nitrate reported as high as 10.3 μmol L^{-1}, nitrite up to 2.7 μmol L^{-1} and phosphate as high as 3.6 μmol L^{-1} (Palter, León Coto, & Ballestero, 2007). However, nutrient loading is thought to be occurring in Golfo Dulce given increasing anthropogenic pressure (Cortés, 1990; González-Chen, 2009; Loaiza, 2007; Morales-Ramírez et al., 2015; Quesada-Alpízar & Cortés, 2006), and there is evidence of higher coliform bacterial concentrations in the Golfito embayment (García et al., 2006). The Purruja estuary, the location of one of our nutrient loaded mangroves, is not considered to be highly contaminated despite the presence of polluted areas near sewage input (García et al., 2006); this is attributed to dilution and biological uptake of increased nutrients (Silva & Acu a-González, 2006). Nutrient concentration as indicated by traditional chemical analyses may therefore not have the sensitivity needed to identify nutrient loading at low concentrations or where inputs are pulsed, with the potential dilution of nutrient inputs at current loading rates.

Traditional chemical analyses of nutrient concentration have limited capacity for the detection of biologically significant pulsed nutrient inputs to the system (Costanzo et al., 2001; Costanzo et al., 2005; Gartner, Lavery, & Smit, 2002). There is high precipitation

TABLE 3 Carbon (%), nitrogen (%), and carbon to nitrogen ratios (mean ± SD) for mangrove leaves (n = 10), bivalves (n = 10), and mangrove macroalgae (n = 7) at four mangrove locations in Golfo Dulce, southern Pacific of Costa Rica

	Mangrove leaves			Bivalves			Algae[a]		
	C%	N%	C:N	C%	N%	C:N	C%	N%	C:N
Rincón (R)	44.1 ± 1.2	1.1 ± 0.2	40.3 ± 6.1	46.1 ± 2.4	15.1 ± 1.1	3.1 ± 0.2	33.6 ± 2.9	3.6 ± 0.6	9.4 ± 1.1
Esquinas (R)	45.1 ± 0.6	1.3 ± 0.1	34.5 ± 3.4	41.7 ± 4.0	13.5 ± 1.5	3.1 ± 0.1	31.9 ± 3.9	3.4 ± 0.5	9.5 ± 1.2
Jiménez (N)	43.4 ± 1.6	1.1 ± 0.2	39.0 ± 5.7	43.1 ± 5.0	13.8 ± 1.8	3.1 ± 0.1	30.1 ± 3.5	3.3 ± 0.4	9.2 ± 0.8
Depósito (N)	43.4 ± 1.3	1.2 ± 0.1	36.3 ± 3.3	43.6 ± 1.6	15.6 ± 1.3	2.8 ± 0.2	29.1 ± 2.6	3.4 ± 0.4	8.6 ± 1.0

Notes. R = reference mangroves; N = nutrient loaded mangroves.

[a]Algae were acidified for inorganic carbon removal.

during the rainy season in the area, with approximately 5,000 mm year $^{-1}$ (IMN, 2009), which could reduce measured nutrient concentrations and affect perception of the actual nutrient inputs at the mangrove locations during the rainier periods. Nutrient concentrations may be further diluted when sampling at high tide. Water column nutrient concentrations were sampled on only two occasions at high tide, and may therefore not be representative of the predominant concentrations in the water column throughout the year; however, studies over longer time periods in the gulf have come up with similar findings (Morales-Ramírez et al., 2015). It is also possible that other marine primary producers are consuming nutrients from the water column that were not sampled as part of this study, such as benthic macroalgae which can be seasonal on the Pacific coast of Costa Rica (Cortés, Samper-Villarreal, & Bernecker, 2014) or seagrasses (Samper-Villarreal, Bourg, Sibaja-Cordero, & Cortés, 2014; Samper-Villarreal, Van Tussenbroek, & Cortés, 2018).

In contrast to low nutrient concentrations, water transparency and chlorophyll concentration both revealed diminished water quality at nutrient loaded mangroves. Nutrient loaded mangroves had higher chlorophyll concentrations and lower Secchi depths than reference mangroves. As phytoplankton is nutrient (primarily nitrogen) limited in the Eastern Tropical Pacific (Pennington et al., 2006), an increase in chlorophyll concentrations at nutrient loaded locations may indicate an increase in phytoplankton productivity, which may be depleting nutrients from the water column. At nutrient loaded locations Secchi depth was similar to the limited water transparency found in Golfo de Nicoya (Palter et al., 2007). At Golfo Dulce, the input of nutrients from raw sewage may therefore be either sufficiently diluted, as to not be detectable by chemical analyses, or be at a level that it is biologically assimilated by the biota leading to low nutrient concentrations in the water column. Studies on phytoplankton nutrient uptake and growth rate should be carried out in Golfo Dulce.

Mangrove leaf isotopic values showed enriched δ^{15}N at nutrient loaded mangroves, whereas algae and bivalves showed no variation between reference and nutrient loaded mangroves. The most likely explanation for this is the difference in the tissue turnover rate among these three organisms. Mangroves are considered to be good long-term (years) indicators of nutrient loading (Costanzo et al., 2003; Pitt et al., 2009), obtaining their nutrients from interstitial water which can accumulate pulsed nutrient inputs (Hogarth, 1999; Kathiresan & Bingham, 2001; Tomlinson, 1994). *Rhizophora* trees produce a new leaf only approximately every 100 days (Farnsworth, Ellison, & Gong, 1996). Algae have high turnover rates because they obtain their nutrients directly from the water column (Costanzo et al., 2003; Costanzo, O'Donohue, & Dennison, 2000; Fertig et al., 2009; Gartner et al., 2002) and can show enriched δ^{15}N values within days of exposure to greater nutrient concentrations (Costanzo et al., 2005; Gartner et al., 2002; Savage & Elmgren, 2004). Bivalves obtain their nutrients indirectly from the water column and have a turnover rate of several weeks to months (Fertig et al., 2009; Piola et al., 2006). Lack of variation of bivalve δ^{15}N between reference and nutrient loaded mangroves could have resulted from the selection of muscle tissue for this analysis, as some tissues of benthic invertebrates are more sensitive indicators of sewage effluent than others (Piola et al., 2006). Lack of variation in algal and bivalve isotopic data between reference and nutrient loaded locations may actually be indicative of prevailing water column conditions during the dry season prior to sample collection. Isotopic analysis of bivalves and algae at different seasons may provide greater clarity on this topic. However, algae δ^{15}N at low nutrient concentrations can be very low (Costanzo et al., 2001; Costanzo et al., 2005), and on this occasion all algae stable isotope values were similar to those of mangroves at nutrient loaded mangrove locations. This lack of isotopic variation in the bivalve and alga between nutrient loaded and reference locations might indicate that the algae and bivalves have been subject to nutrient loaded conditions at all locations in recent times.

Other possible causes of isotopic variation in mangrove leaves between reference and nutrient loaded mangroves include potential isotopic variation in soil enrichment factors and nutrient sources, which were not sampled as part of this study. Deposition of bloom phytoplankton or suspended matter can occur due to land use in river catchments in Golfo Dulce (Hebbeln & Cortés, 2001). δ^{15}N from sewage derived organic matter in California goes from 1.8‰ (Van Dover, Grassle, Fry, Garritt, & Starczak, 1992) to 5.6‰ (Kwak & Zedler, 1997), with sewage sources in Golfo Dulce potentially at the higher level of δ^{15}N from this range. Limited water circulation between mangroves and seasonal deep-water upwelling (Morales-Ramírez et al., 2015; Quesada-Alpízar & Morales-Ramírez, 2004; Svendsen et al., 2006) may also affect isotopic values in Golfo Dulce, which is anoxic in its deepest areas, as upwelling is thought to alter marine δ^{15}N (Risk & Erdmann, 2000; R. Sweeney, Kalil, & Kaplan, 1980; R. E. Sweeney & Kaplan, 1980). Overall knowledge of water circulation in Golfo Dulce is currently limited, and the assumption that there are no cumulative impacts at mangrove locations should be reassessed once more detailed information is available.

4.1 | Summary and conclusion

Nutrient concentrations determined by traditional chemical water analysis have not been able to identify nutrient loading in Golfo Dulce, despite evidence of punctuated increased coliforms and the overall knowledge that untreated sewage is being delivered to the gulf at increasing rates. Our findings support the notion that nutrient concentrations in the water column are low, however, chlorophyll concentration and water transparency reveal diminished water quality at nutrient loaded mangroves. δ^{15}N of mangrove leaves was enriched at nutrient loaded locations, although algal and bivalve isotopic values did not reveal variations between reference and nutrient loaded mangroves. Nevertheless, the algal isotope values were similar to those of the mangrove leaves at nutrient loaded locations, potentially indicating increased nutrient conditions at all mangroves at the time of sampling. Future evidence of nutrient loading should focus on both isotopic and traditional chemical water quality assessments. At the time of this study, nutrient inputs into Golfo Dulce are considered to be at levels that were readily diluted or consumed by the biota, and thus undetectable with traditional water quality techniques. We provide clear evidence however, of isotopic enrichment at nutrient loaded locations from mangrove material and recommend that adequate waste water treatment be carried out on all anthropogenic discharges into this vulnerable marine system.

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