



External and local controls on land-sea coupling assessed by stable isotopic signatures of mangrove producers in estuaries of Pacific Panama

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ARTICLE INFO

Keywords:

Bromeliads
Deforestation
Macroalgae
Orchids
Pelliciera
Rainfall
Rhizophora
Sediments
Upwelling
Within-estuary transformations

ABSTRACT

Foliar stable isotopic signatures of nitrogen, carbon, and sulfur in mangrove vegetation from the Pacific coast of Panama were insensitive to inputs from watersheds with different area of forest land cover, and to seasonal, inter-annual, and global-scale-driven contrasts in rainfall and upwelling. N, C, and S content of mangrove vegetation were not affected by inputs from watersheds with different degrees of deforestation, but showed some influence of down-estuary transformations. While there was substantial variation that remained un-explained, isotopic signatures and nutrient contents were largely determined by species-specific features, and showed substantial small-scale variation reflecting local differences, within-estuary plant-sediment links. The ability of mangrove estuaries to erase effects of deforestation points out that conservation of these wetland ecosystems is important, because, at least in the sites we studied, transformations within mangrove estuaries were strong enough to protect water quality in receiving coastal waters.

1. Introduction

Stable isotopic ratios of nitrogen, carbon, and sulfur have been widely used to discern sources and to detect within-ecosystem transformations (Peterson and Fry, 1987; Fry et al., 2000; Valiela and Bowen, 2002; Bouillon et al., 2004; Bottrell and Newton, 2006; Michener and Lajtha, 2007; Bode et al., 2011). In mangrove forests, stable isotope studies have been applied to delineate external and internal sources of organic matter, use of organic matter by mangrove food webs, assessment of exchanges of dissolved, particulate and organisms between mangrove estuaries and coastal waters, discern inter-specific differences among mangrove plants, and assess influence of properties of sediments on which plants grow (Okada and Sasaki, 1995, 1998; Pardo et al., 2007; Bouillon et al., 2008; Kristensen et al., 2008; Valiela et al., 2018). In the present study, we used stable isotopic signatures and elemental content of nitrogen, carbon, and sulfur in mangrove vegetation to understand couplings among watersheds, mangrove forests, and coastal waters, and transformations down-estuary in a series of watersheds on the Pacific coast of Panama that had undergone

different degrees of deforestation.

In the tropics, watershed deforestation can be a major agent of ecological change (Downing et al., 1999; Sjöling et al., 2005; Valiela et al., 2012). Loss of forest in this region of Panama occurs by artisanal-level burns aimed at creating pastures for livestock; a pattern that is common throughout the tropics (Chen et al., 2010). There were significant biogeochemical transformations in the dissolved nutrients during transit through the estuaries (Valiela et al., 2013a, b), transformations that might alter nutrient supply for mangroves growing along the reaches of the estuaries. Such within-estuary changes might be discerned in isotopic signatures in mangrove vegetation.

The seawater-exposed parts of estuaries in this region support mangrove forests that are dominated by red mangrove, *Rhizophora mangle*, and the piñuelo mangrove, *Pelliciera rhizophorae*. The red mangrove is the most widespread tree species across neotropical coasts (a congener, *R. racemosa* is present in low numbers in restricted locations in our study area; it is difficult to distinguish from *R. mangle* unless fruiting). The piñuelo mangrove was a dominant species of neo-tropical coasts since the Miocene (Jimenez, 1984), but has retreated as red

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<https://doi.org/10.1016/j.marenvres.2018.03.003>

Received 18 January 2018; Received in revised form 5 March 2018; Accepted 8 March 2018

Available online 10 March 2018

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mangrove expanded. Piñuelo mangrove has now been reduced to a geographic range narrowly restricted to northern Ecuador to southern Costa Rica (Duke et al., 1998). Two other tree species, black (*Avicennia germinans*) and white mangrove (*Laguncularia racemosa*) can be found in salty zones within the region, but in this part of Panama are far less abundant than red and piñuelo mangroves.

The fresher upper reaches of mangrove-lined estuaries in the region have some distinctive vegetation elements, including the marica mangrove, *Tabebuia palustris*, a rare large shrub of complex taxonomy (Grose and Olmstead, 2007), restricted to mangrove ecosystems of the Eastern Tropical Pacific (Gross et al., 2014), and assessed to be a vulnerable species (<http://www.iucnredlist.org/apps/redlist/details/178801/0>). The large-leaved, tropically widespread golden leather fern, *Acrostichum aureum*, can be found along shorelines of brackish-to-fresh reaches of the estuaries. Many mangrove trees support a rich variety of epiphyte taxa, including orchids and bromeliads, which grow above tidal submergence. Filamentous green and brown macroalgae commonly grow on mangrove prop roots within the intertidal range.

The western region of Panama, the Pacific coast of Veraguas Province, is subject to pronounced seasonal and inter-annual contrasts in meteorological conditions. Seasonal rainfall changes from dry (100 mm/mo) to wet (338.5 mm/mo) seasons (Valiela et al., 2013b); global-scale processes add strong inter-annual shifts in weather. La Niña episodes of the ENSO cycle affect rainfall as well as frequency of upwelling of deeper waters that deliver nutrients into mangrove estuaries (Valiela et al., 2012). Upwelling affects estuaries on the southern areas of our study region, which fall within the region of the Gulf of Panama to the Southwest, where coastal upwelling is common (D'Croz and O'Dea, 2007). The more northern estuaries on the Gulf of Chiriquí are not exposed to upwelling (Valiela et al., 2012), consistent with results from oceanographic surveys (D'Croz and O'Dea, 2007). There is a further globally driven, inter-annual effect, also associated with La Niña: rainfall has increased by 17% during the last decade (Valiela et al., 2012). Such increases freshen near-surface layers of the coastal ocean, which likely decreases potential upwelling, a change that could affect biota of mangrove estuaries and near-shore areas.

In this paper we first compare nitrogen, carbon, and sulfur stable isotopic signatures and N, C, and S contents in mangrove forest vegetation, to document inter-specific contrasts, and to identify sources of nutrients (sediment, atmosphere, estuary water) in trees, ferns, orchids, bromeliads, and macroalgae growing within mangrove forests. Second, we examine whether isotopic signatures and element content in primary producers were affected by external forcing (inputs from watersheds with different forest cover, seasonal weather, or global-scale changes in rainfall and upwelling) or, in contrast, might mainly be determined by within-estuary biogeochemical transformations or species-specific differences in nitrogen physiology and plant-sediment interactions. The information presented in this study represents the first of its kind for mangrove in the Eastern Pacific Ocean.

2. Material and methods

We sampled vegetation in mangrove ecosystems receiving inputs from eight Panamanian watersheds which differed in the degree of conversion from forests to pastures (Fig. 1, Table 1). Rain falling onto the watersheds mostly percolates into the highly fractured igneous basement, and flows as groundwater, which then discharges into freshwater streams. Smaller and intermittent surface flow occurs. Both groundwater and surface flow then discharge into estuaries lined by mangrove forests. Down-estuary flow and tidal exchanges transport nutrients in solution and as particulate matter through the mangroves that in turn empty into the Pacific Ocean.

To determine N, C, and S isotopic signatures of primary producers, we collected leaves from the dominant taxa. Leaves were selected for sampling because these had sufficient element content, made up much of the biomass of the vegetation, and could potentially show transient

differences in isotopic signatures. Samples of 3–5 leaves from at least three individual plants were combined to make one composite sample from each sampling site. Samples were rinsed with doubly distilled water, dried at 60 °C, ground to a fine powder, and stored at room temperature in glass vessels until analysis.

To determine whether there were seasonal and inter-annual contrasts in stable isotopic signatures in the vegetation, we sampled at the end of wet and dry seasons during three years (2009–2011). The sampling included three periods of relatively un-eventful conditions, one period of La Niña-induced upwelling, and one during La-Niña-related increased rainfall; further details on these features were given in Valiela et al. (2012, 2013b).

To see whether inputs from watersheds with different degrees of deforestation affected isotopic signatures in mangrove vegetation, we compared samples collected within the watershed-estuary systems included in Table 1.

To test whether isotopic ratios were affected by within-estuary transformations as water traveled down-estuary to sea, sampling was repeated at six stations spaced down-estuary. The position of the stations differed in each campaign, as we used salinity as a scalar to span the mixing of fresh and sea water. These transects extended from the fresh reaches and upper limit of mangrove plant distribution, to the mouth of each estuary (Valiela et al., 2013b).

To assess whether plant and sediment isotopic ratios were related, we sampled mangrove forest sediments by taking 5-cm deep, 1 cm diameter cores in the same stations down-estuary as mangroves. Stable signature and contents of N, C, and S were measured in sediment samples in the same way as producer samples.

N, C, and S stable isotope and elemental analyses were performed for all samples by mass spectrometry at The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA. Results were calculated based on comparisons with known laboratory standards that had been calibrated against NIST Standard Reference Materials. Stable isotope ratios were reported as per mil (‰) using delta notation determined as $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ or $\delta^{34}\text{S}$ (‰) = $[(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 10^3$, where R was $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$, or $^{34}\text{S}/^{32}\text{S}$. Duplicate determinations on the same sample differed by $\leq 0.2\text{‰}$ in N and C samples, and $\leq 0.5\text{‰}$ in sulfur samples. To aid interpretation of the isotope signatures we also determined N, C, and S contents of all sample types.

In the present study, we first compare nitrogen, carbon, and sulfur isotopic signatures for the various taxa sampled, evaluating species-specific differences. Then we assess whether isotopic signatures were affected by season (variations in rainfall), watershed deforestation, upwelling, and down-estuary transformations. We follow with a comparison of plant versus sediment signatures, and end with a consideration of N, C, and S content of mangrove plants, epiphytes, and algae, relative to vegetation from tropical and temperate forests to place the isotopic signatures in a larger nutritional and global context.

To assess the coupling of the isotopic signatures ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$), nutrient content (%N, %C, %S) and C/N and the salinity (as the relative position down estuary) and the % forest cover on contributing watershed, we regressed these variables (type I regression). To examine if the $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ in mangrove trees and sediments were related, runs test were applied to test whether or not differences from 1:1 line were random or not. Differences in isotopic signatures ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$) and nutrient content (%N, %C, %S) between seasons and upwelling events (based on different sampling years) were tested with a *t*-test for independent samples. Tests made were only considered significant when *P*-values were < 0.05 .

3. Results and discussion

3.1. Isotopic signatures in species of mangrove plants, epiphytes, and algae

The isotopic signatures of the more common mangrove tree species were similar (Fig. 2 left top). The mean and standard error of $\delta^{15}\text{N}$

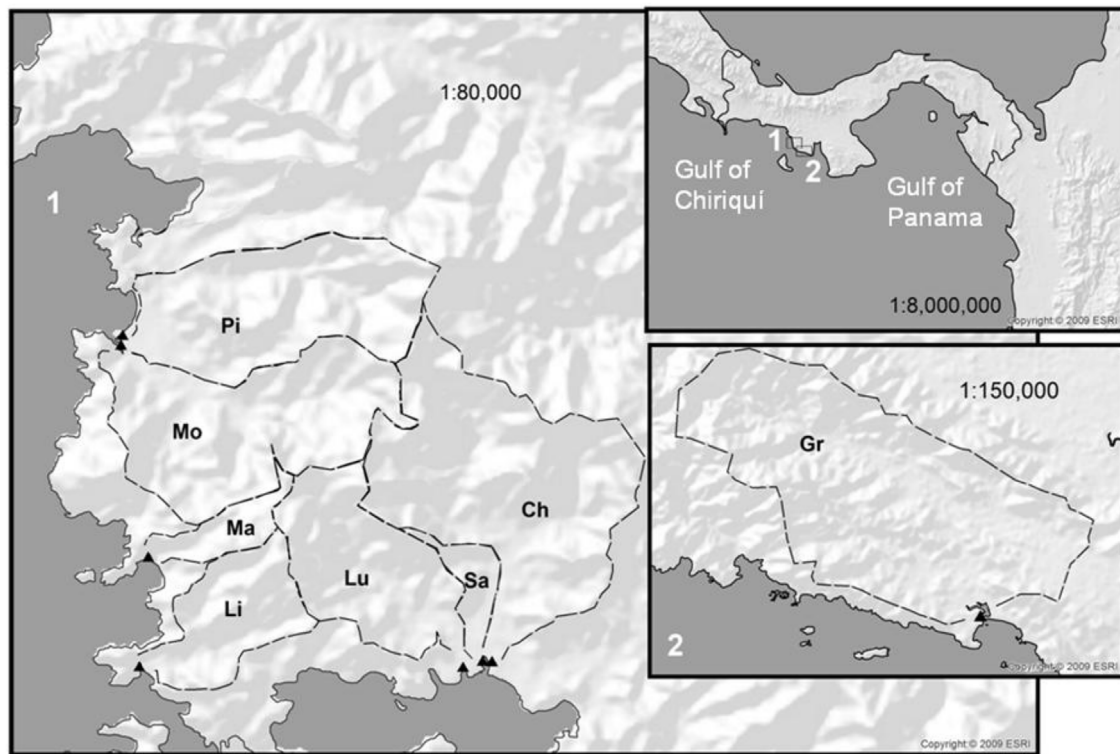


Fig. 1. Map of study areas. Inset, top right: map of Panama with boxes 1 and 2 showing location of study areas. Enlarged Box 1 shows location of seven watershed-estuary systems (Pi: Rio Pixvae, Mo: Rio de la Mona, Ma: Rio Manglarito, Li: Rio Limon, Lu: Rio Luis, Sa: Rio Salmonete, and Ch: Rio Chamuscado). Box 2 shows location of Rio Grande (Gr) watershed. Watershed bounds shown with dashed lines; triangles show location of estuary mouth at each of the watershed-estuary systems.

Table 1

Selected properties of the watershed-estuary systems included in this study (Fig. 1), including area and percentage of land covers. Values derived from the remote sensing sources cited in text and Valiela et al. (2013a).

Watershed-estuary	Area of watershed (ha)	Land cover (% of area)		
		Forest	Pasture	Other
Pixvae	1429	73	23	4
De la Mona	1575	47	47	6
Manglarito	239	91	6	3
Limón	665	92	5	3
Luis	1007	73	18	9
Salmonete	195	29	52	19
Chamuscado	2229	66	28	6
Grande	9639	23	43	34

values of *A. germinans*, *R. mangle*, *P. rhizophorae*, *L. racemosa* and *T. palustris* overlapped broadly in the range of -1 to 5.5‰ (Fig. 2 left top). The variability of $\delta^{13}\text{C}$ in leaves of the different mangrove tree species was quite narrow (Fig. 2 left top), with values of $\delta^{13}\text{C}$ characteristic of the C_3 photosynthetic pathway, which is found in mangrove trees (Andrews and Muller, 1985). The isotopic values we measured in mangrove leaves in Panama were similar to those measured elsewhere in the tropics (Bouillon et al., 2008; Prasad and Ramanathan, 2009; Tue et al., 2012). We should add that the values for *P. rhizophorae* are the first reported; very little is known about this species.

N and C isotopic values for ferns (*A. aureum*) and *T. palustris* (Fig. 2 right top) were somewhat lighter, but overlapped those of *R. mangle*, *P. rhizophorae*, *L. racemosa*, and *A. germinans* (Fig. 2 left top). While bromeliad $\delta^{13}\text{C}$ fell within the same narrow range we found in the mangrove trees, orchid $\delta^{13}\text{C}$ were much enriched, reaching values characteristic of C_4 plants (Fig. 2 top right). This suggests that there must be a mechanism in orchids that leads to further carbon fractionation.

$\delta^{15}\text{N}$ in brown macroalgae were relatively heavy, on a par to those

in mangrove trees, and heavier than those in green algae (Fig. 2 top right). Macroalgae obtain dissolved nutrients from water, but differences in isotopic signatures in macroalgae may be a function of physiological contrasts rather than differences in sources; slow internal turnover of N in brown algae leads to heavier $\delta^{15}\text{N}$ than green algae (Cohen and Fong, 2004; Umezawa et al., 2007), in part because characteristically large releases of dissolved organic nitrogen lead to heavier nitrogen signatures left in fronds. The isotopic values of brown and green macroalgae in Fig. 2 fall within ranges reported elsewhere (Cole et al., 2004; Prasad and Ramanathan, 2009; Dailer et al., 2010; Teichberg et al., 2010). While $\delta^{13}\text{C}$ values in macroalgae from mangrove ecosystems vary between -35 and -13‰ (Bouillon et al., 2008), the mean values observed in this study fell within the most depleted values (Fig. 2 top right) reported in this literature review. As an explanation, the photoassimilation of respiratory CO_2 originating from the mineralization of mangrove detritus (usually having $\delta^{13}\text{C}$ values of about -26 to -30‰) superseded any possible effect of reduced water motion on isotopic fractionation in macroalgae growing in protected areas (France and Holmquist, 1997); resulting in more depleted $\delta^{13}\text{C}$ values as observed in the Panamanian macroalgae.

Epiphytic orchids and bromeliads showed foliar $\delta^{15}\text{N}$ that clearly differed from signatures in their host trees (Fig. 2 top right, Table 2). The strongly lower values of bromeliads and orchids agree with previous reports (Stewart et al., 1995; Hietz et al., 2002; Wania et al., 2002; Mardegan et al., 2011), and suggest that orchids and bromeliads growing epiphytically take up atmospheric nitrogen with substantial isotopic fractionation. $\delta^{15}\text{N}$ values of nitrate in precipitation, for example, can range from 0.5 to 8‰ (Olsen et al., 2011); in tropical areas; while Wania et al. (2002) reported values of $\delta^{15}\text{N}$ of total soluble N in bulk precipitation from -2.3 to 12.2‰ . $\delta^{15}\text{N}$ values for epiphytes (Table 2) were considerably lower than these values, so some mechanism of uptake involving significant isotopic fractionation must occur. Orchids may host symbiotic mycorrhiza that deliver isotopically depleted N compounds to the plants (Lesica and Antibus, 1990;

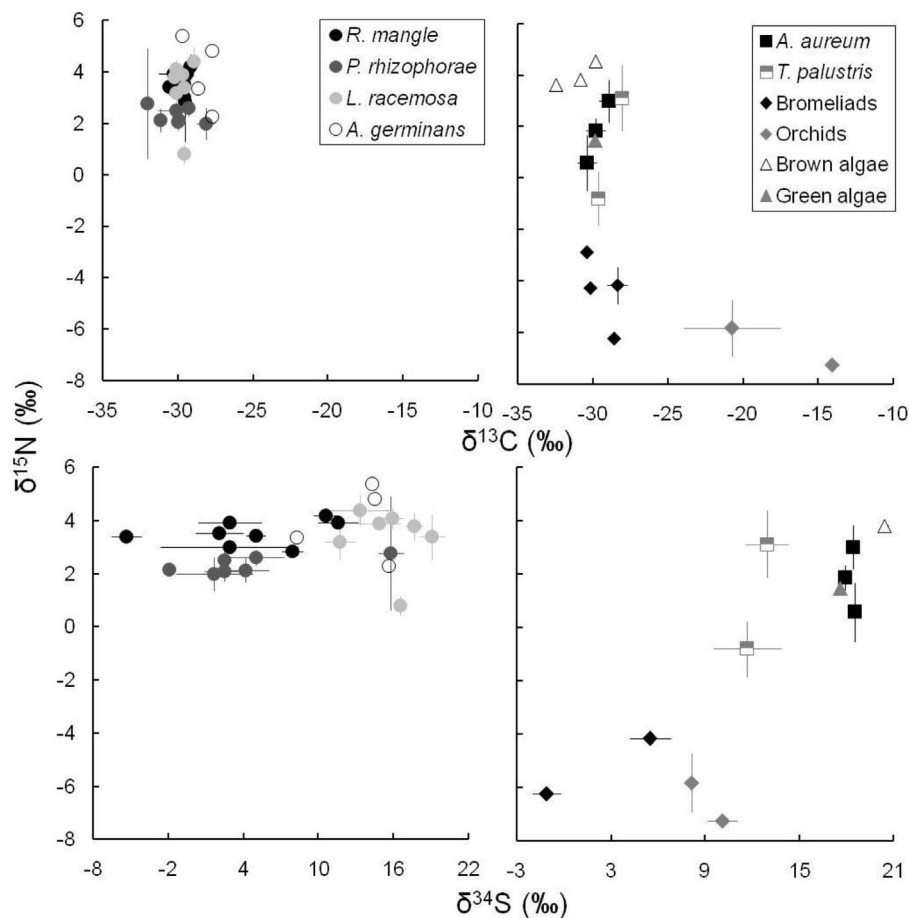


Fig. 2. $\delta^{15}\text{N}$ versus $\delta^{13}\text{C}$ (two top panels) and versus $\delta^{34}\text{S}$ (two lower panels) (mean \pm se of each estuary) of all the taxa studied. The two dominant and two subdominant mangrove species are included in the left panels, and other species in right panels. Note that the Y-axis of the two lower panels differ.

Högberg, 1997). In addition, symbiotic N_2 -fixers might be present, whose fractionation of 0–3‰ (Shearer and Kohl, 1986, 1993), leads to $\delta^{15}\text{N}$ values in the hosts of –3 to 0‰ (Fry, 1991). The light $\delta^{15}\text{N}$ observed in these epiphytes suggest that the heavier isotopic signatures found in mangroves are not likely due to a potential foliar uptake of heavy marine-derived nitrogen borne in aerosols from sea spray, since both epiphytes and mangroves are exposed to the same atmospheric inputs.

$\delta^{34}\text{S}$ of the mangrove species spanned a broad range, mean values varied from –5.3 to a maximum of 19.1‰ (Fig. 2 bottom left). The very large range observed is probably a result of contrasting sources and processing. These ranges are comparable to those reported for other mangroves, –15.6–9.8‰ (Okada and Sasaki, 1995, 1998) and –14.1–14.7‰ (Fry and Smith, 2002). Although $\delta^{34}\text{S}$ in the different mangrove taxa in our data, and the literature, spanned and even exceeded these source values [which could vary from 20‰ in seawater sulfate, to 6‰ in atmospheric sulfur (Fry et al., 1982)]. *R. mangle* and *P. rhizophorae* showed lowest, and *L. racemosa* mangrove highest $\delta^{34}\text{S}$ values. Such variation in $\delta^{34}\text{S}$ needs investigation, as it probably indicates significant local and species-specific differences in physiology

and ecology. Variation in $\delta^{34}\text{S}$ was more pronounced than in N and C stable isotopes, as found by Okada and Sasaki (1998). This variability may be associated to high variation of sulfur biogeochemical processes in water and sediment, and to different fractionation of sulfur isotopes by different plants (Okada and Sasaki, 1998; Bouillon et al., 2008).

Sulfur isotopic values in macroalgae and epiphytes do suggest direct uptake from seawater and rain, respectively. $\delta^{34}\text{S}$ in the macroalgae were about 18–21‰ (Fig. 2 bottom right), which could result from uptake of seawater sulfate with little fractionation. $\delta^{34}\text{S}$ in epiphytic species ranged –1 to 10‰ (Fig. 2 bottom right), a range that might be expected in taxa that depend on atmospheric deposition as the nutrient supply. The values of sulfur signatures in the epiphytes imply that aerosols from marine sulfate sources made minor contributions to airborne delivery of sulfur.

3.2. Factors affecting isotopic signatures

Isotopic values included in Fig. 2 were means calculated from many data sets that subsumed substantial variation. The following sections focus on that variability, to assess the degree to which variation in

Table 2

N, C, and S (mean \pm se) isotopic signatures, nutrient content (%N, %C, and %S) and C/N of epiphytic bromeliads and orchids, and brown and green macroalgae.

	n	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{34}\text{S}$	%N	%C	C/N	%S
Bromeliads	10	–4.3 \pm 06	–28.8 \pm 0.5	0.2 \pm 0.03	0.7 \pm 0.06	42.9 \pm 0.9	53.7 \pm 7.1	0.07 \pm 0.004
Orchids	7	–6.0 \pm 0.9	–19.8 \pm 2.9	4.2 \pm 3.9	0.5 \pm 0.1	43.9 \pm 1.4	86.2 \pm 16.6	0.1 \pm 0.04
Brown algae	9	3.9 \pm 0.1	–31.04 \pm 0.4	20.4 \pm 0.01	2.83 \pm 0.1	25.87 \pm 0.5	9.2 \pm 0.4	1.9 \pm 0.2
Green algae	3	1.5 \pm 0.4	–29.9 \pm 0.3	17.6	2.6 \pm 0.3	24.2 \pm 2.5	9.3 \pm 0.2	0.8

isotopic signature could be associated with variation in rainfall, degree of deforestation of the watersheds delivering materials to the mangrove estuaries, upwelling, within-estuary transformations during transit from fresh to marine reaches or plant-sediment interactions.

3.2.1. Effects of variation in rainfall

In spite of the strong seasonality of rain in the region (Valiela et al., 2012), we found no significant difference in isotopic signatures (t -test for independent samples, $P > 0.05$) between samples collected at the end of the wet and at the end of the dry seasons of 2009–2011 (data not shown). The lack of seasonal or inter-annual contrasts in isotopic ratios suggests either that mangrove trees integrated temporal differences over periods longer than a few months, or that differences in external inputs associated with season do not prompt isotopic effects. The lack of seasonal differences in mangrove plant isotopic signatures was also found elsewhere (Saunders, 2006), and parallel findings of no clear seasonal differences in nutrient concentrations in these estuaries (Valiela et al., 2013b).

3.2.2. Effects of discharges from watersheds with different degrees of deforestation

Degree of deforestation on contributing watersheds had no evident effect on stable isotopic values of N, C, or S (Fig. 3). The lack of effect of watershed deforestation was true for all species that we sampled, in spite of watersheds with a wide range of forest cover (Table 1).

The lack of response of mangrove isotopic signatures to watershed influences (Fig. 3) parallels our earlier findings that down-estuary biogeochemical transformations erased watershed imprints that were detectable upstream in the fresh reaches (Valiela et al., 2013b, 2014). Nutrient acquisition by mangrove vegetation in the systems we studied, and as reflected by their foliar isotopic signatures, depended less on external terrestrial-derived inputs than on within-estuary mechanisms; at least in watersheds with minimal agriculture, and where human and domestic animal inputs are small.

3.2.3. Effects of upwelling

If upwelling was an important source of dissolved nitrogen, we might find mangrove plants bearing heavier signatures, as denitrification in subsurface layers would leave heavier isotopic signatures of dissolved inorganic nitrogen (Sigman et al., 2009). Isotopically enriched nitrogen could then be taken up by the plants, as reported in

other upwelling areas with other coastal biota (Hill et al., 2006; Hill and McQuaid, 2008; Viana and Bode, 2013). Our conjecture was based on the supposition that upwelling contributed nitrate made heavier by microbial action. We found, however, that upwelling mostly delivered hypoxic water containing ammonium (Valiela et al., 2014), which may not necessarily have borne heavier nitrogen signatures.

We examined the possible influence of upwelling in two ways, and found no compelling evidence that changes in supply of marine nutrient sources altered isotopic signatures. We first compared isotopic signatures in leaves of *R. mangle* and *P. rhizophorae* collected soon after the strong upwelling event of dry season of 2009. There were no significant differences in isotopic signatures measured during sampling periods affected by upwelling, compared to during non-upwelling sampling periods (t -test for independent samples, $P > 0.05$). It may have been that upwelled water did not penetrate into sediments, or that isotopic signatures in mangroves did not respond to short-term events such as the March 2009 upwelling.

Second, to ascertain if isotopic signatures in mangrove vegetation might be altered by longer-term exposure to upwelling, we made use of the geographic contrast in upwelling frequency in our area of study. We compared samples of *R. mangle* and *P. rhizophorae* taken from the more northern estuaries—where no upwelling was reported by D'Croz and O'Dea (2007)—and southern estuaries (where upwelling was more frequent). There was, however, no evidence that this geographic contrasts in upwelling frequency altered isotopic signatures (Fig. S1).

All these external variables—seasonal differences, deforestation of watersheds, and upwelling—seemed to have little effect on stable isotopic signatures of mangrove plants in the Pacific coast of Panama. Yet, there was substantial variation in isotopic signatures, so we now turn to other possible sources of that variation, including down-estuary effects and species-specific plant-sediment interactions.

3.2.4. Effects of down-estuary differences in water column

We anticipated, from earlier work on nutrient concentrations, that watershed influences in the studied Panama estuaries, are most clearly within fresher ranges, and become less apparent as estuarine water became saltier (Valiela et al., 2014). To test whether we could define these effects on mangrove trees, we plotted stable isotopic values of the mangrove species versus salinity in the stations where the plants were sampled (Fig. 4).

$\delta^{15}\text{N}$ values in leaves of *R. mangle* and *P. rhizophorae*, the two

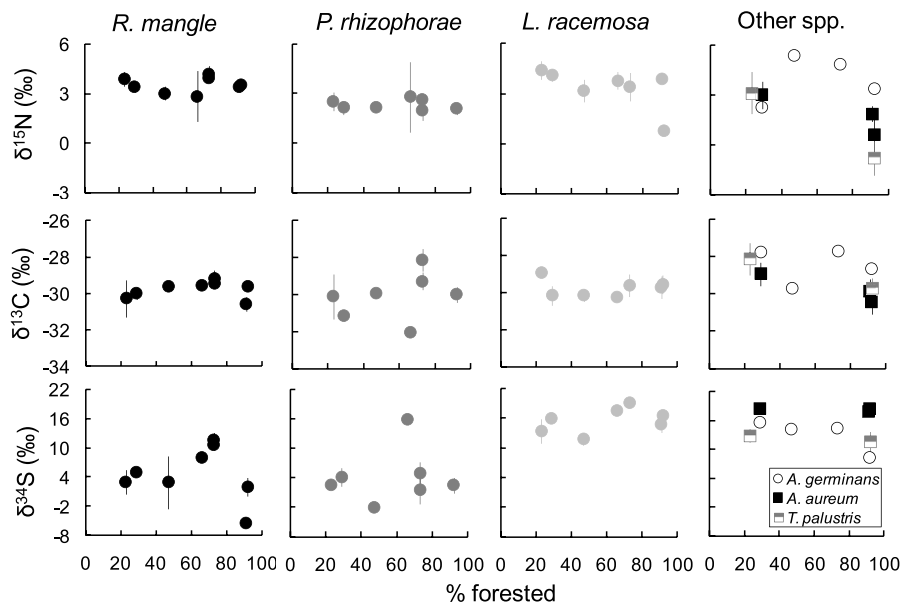


Fig. 3. N, C and S isotopic mean \pm se values (‰) in leaves of mangrove species (*R. mangle*, *P. rhizophorae* and *L. racemosa*) and the other species sampled (*A. germinans*, *A. aureum* and *T. palustris*), plotted versus % forest cover on contributing watersheds (Table 1). Regressions fitted to the points were not significant and are not shown.

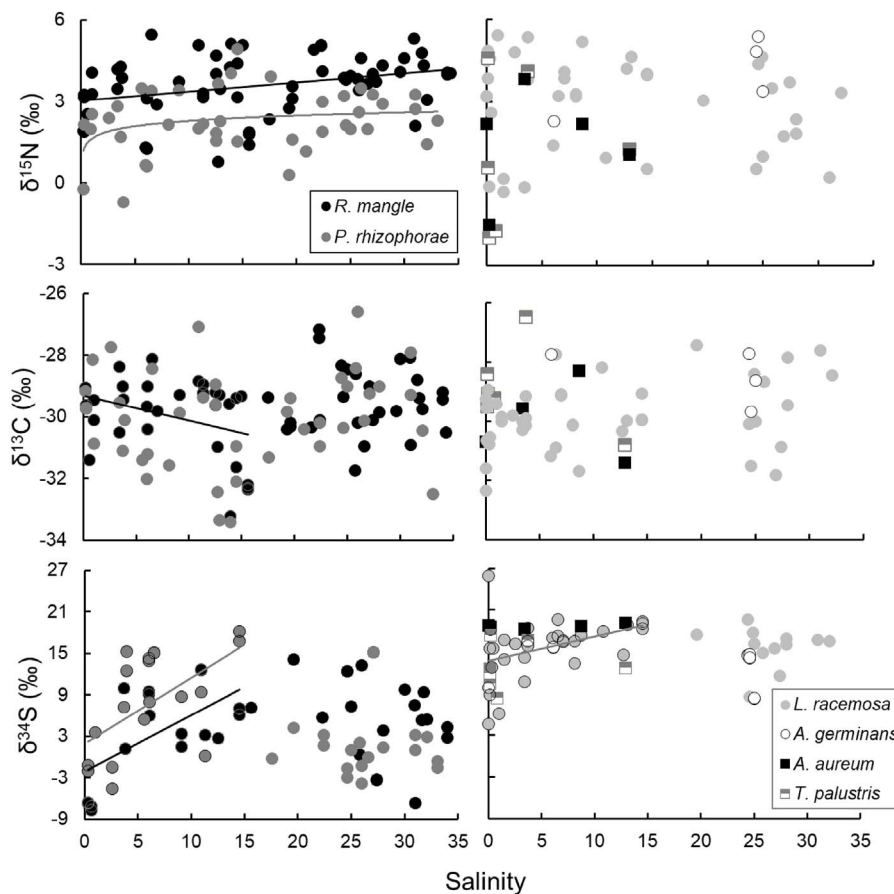


Fig. 4. N, C and S isotopic values (‰) in plant leaves, plotted versus salinity down-estuary. Data were pooled across all estuaries and sampling years. Left panels: data for the two dominant species, *R. mangle* (black points and lines) and *P. rhizophorae* (grey points and lines). Right panels: data for *L. racemosa* (grey points and lines), *A. germinans*, *T. palustris*, and *A. aureum*. Regression lines for *A. germinans*, *T. palustris*, and *A. aureum* were not calculated because of low number of points. Regression lines are shown only when significant, see Table S1 for statistical results.

Table 3

Stable isotope values and nutrient content of N, C and S (mean \pm se) in surface sediments collected from mangrove forests within the different estuaries of this study. Samples are means from all samples collected from estuaries of Table 1. Data were pooled into bins of salinities < 5, 5–25, and > 25. The number of samples for C and N is shown below the salinities; the number of samples for S is shown in parenthesis.

	Salinity		
	< 5	5–25	> 25
	<i>n</i> = 19 (19)	<i>n</i> = 17 (14)	<i>n</i> = 35 (13)
$\delta^{15}\text{N}$ (‰)	2.7 \pm 0.2	2.8 \pm 0.2	3.4 \pm 0.3
$\delta^{13}\text{C}$ (‰)	–27.6 \pm 0.3	–27.3 \pm 0.4	–24.8 \pm 2.2
$\delta^{34}\text{S}$ (‰)	8.3 \pm 2.3	–3.8 \pm 2.3	4.3 \pm 3.2
%N	0.1 \pm 0.02	0.2 \pm 0.04	0.2 \pm 0.08
%C	1.5 \pm 0.3	3.1 \pm 0.8	5.8 \pm 3.2
C/N	12.9 \pm 0.6	14.7 \pm 1.1	18.7 \pm 4.3
%S	0.1 \pm 0.02	0.3 \pm 0.08	0.2 \pm 0.07

overwhelmingly dominant mangrove species, were variable (Fig. 4 top left), but differed consistently down the entire estuarine salinity gradient (Table S1). $\delta^{15}\text{N}$ of *R. mangle* were consistently heavier than those of *P. rhizophorae* by about 1.5‰ (Fig. 4 top left). These differences could be due to inter-specific contrasts in habitats, or presence of mycorrhizal symbionts.

The isotopic differences between the mangrove species could reflect habitat differences: *P. rhizophorae* grows on relatively stable, coarse-grained mineral sediments, while *R. mangle* is most often found on more unconsolidated, finer, organic sediments. These contrasting substrates could provide N with different isotopic signatures, owing to differences in rates of denitrification and regeneration (Valiela et al., 2014) that could lead to heavier $\delta^{15}\text{N}$ of the available N in sediments preferred by *R. mangle*.

Alternatively, the isotopic contrasts between the mangrove species could be associated with differences in presence of fungal symbionts. Mycorrhizae release N with lighter isotopic signatures, N that is taken up by host plants and lowers foliar signatures (Nadelhoffer et al., 1996; Hobbie and Colpaert, 2003). Mycorrhizae might be responsible for the consistently lighter $\delta^{15}\text{N}$ of *P. rhizophorae*, the species that prefers the less organic, coarse-grain sediments that also are likely to be less reduced. In contrast, the finer, organic sediments where *R. mangle* tends to dominate are likely to be more reduced, and hence inhibit fungal activity. This conjecture raises the interesting notion that perhaps Pan-tropical shifts toward anoxic sediments might have favored expansion of *R. mangle* at the expense of *P. rhizophorae* across space and time.

The mechanisms and processes affecting differences in $\delta^{15}\text{N}$ among mangrove plant species need further study. These studies would clarify larger ecosystem-level questions such as the controls of land-sea coupling and sedimentary conditions, as well as more specific questions, such as nitrogen-processing contrasts in symbiosis and biogeochemistry that might be responsible for the continuing geographic replacement of *P. rhizophorae* by *R. mangle* across geological history of the neotropics.

$\delta^{15}\text{N}$ of leaves in the less-common mangrove taxa, as *A. germinans*, *L. racemosa*, and *T. palustris*, and ferns, varied between –1.5 and 5.5‰ (Fig. 4 top right). The $\delta^{15}\text{N}$ values spanned a similar range as those of the two dominant species, but lacked the down-estuary gradient in $\delta^{15}\text{N}$.

There was substantial variation in isotopic values in all the species, and the relation to down-estuary salinity was only associated with part of the variability measured. The large un-accounted residuals from regressions in plots such as Fig. 4 (top left) may depend on quite small-scale local effects of still-undefined variables. It may also be that mangroves are particularly variable, since variation we report here seems larger than found in other ecosystems, such as temperate salt marshes and coastal bays (McClelland et al., 1997; Cole et al., 2004;

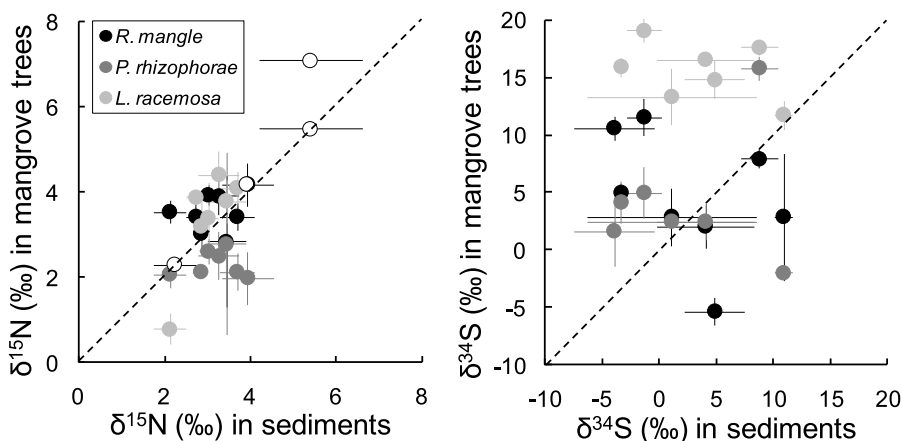


Fig. 5. $\delta^{15}\text{N}$ (left panel) and $\delta^{34}\text{S}$ (right panel) in leaves (mean \pm se) of the three main mangrove species (*R. mangle*, *P. rhizophorae*, and *L. racemosa*), plotted versus $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ in sediments (mean \pm se). Open circles are data from Japanese mangroves in Kuramoto and Minagawa (2001) and Prasad and Ramanathan (2009). Dashed lines represent line 1:1.

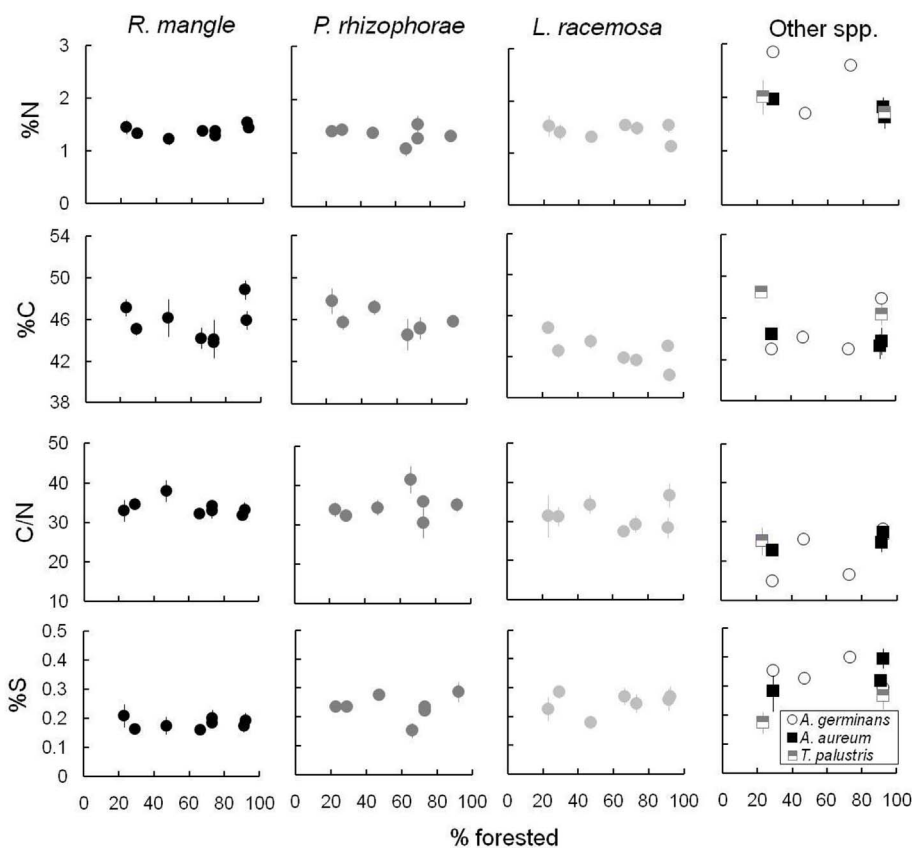


Fig. 6. Mean (\pm se) foliar %N, %C, C/N, and %S in the three main mangrove species (*R. mangle*, *P. rhizophorae*, and *L. racemosa*) and other species sampled (*A. germinans*, *A. aureum*, and *T. palustris*) plotted versus % forest cover on contributing watersheds (Table 1). Regressions fitted to the points were not significant and are not shown.

Olsen et al., 2011). In any case, even after we systematically sought to quantify effects of drivers that create variation in mangrove isotopes, much of the variation remained un-accounted-for.

$\delta^{13}\text{C}$ signatures of the different plant species were also variable for all taxa sampled (Fig. 4 middle panels). There was a significant decrease in $\delta^{13}\text{C}$ from fresh reaches up to salinity of about 15, with an upward jump to higher values in saltier reaches (Fig. 4 middle panels). Increases in salinity decrease stomatal conductance, a mechanism that enriches $\delta^{13}\text{C}$ in mangrove leaves (Lin and Sternberg, 1992; Medina and Francisco, 1997; Kao et al., 2001). Such an effect, however, does not seem consistent with heavier $\delta^{13}\text{C}$ at higher salinities in Fig. 4 (middle panels), so here again we have un-explained trends and variation.

$\delta^{34}\text{S}$ values of mangrove plants varied greatly (-8 to 26‰) across the different estuaries (Fig. 4 lower panels). The range in variation paralleled ranges reported elsewhere (Okada and Sasaki, 1995, 1998; Fry and Smith, 2002). $\delta^{34}\text{S}$ increased significantly from the upper limit of mangrove in freshwater reaches, towards down-estuary to about a salinity of 15 (Fig. 4 bottom panels). Down-estuary increases demonstrated the influence of marine sulfate, characteristically $\sim 20\text{‰}$ (Fry, 2006). Sulfur signatures of *P. rhizophorae* growing at salinities of 0–15 tended to be heavier than those of *R. mangle* (Fig. 4 lower left). The other species showed similar trends, with a larger uptake of marine sulfate likely (Fig. 4 bottom right). At salinities > 15 , isotopic values in leaves of all species remained somewhat lower than values

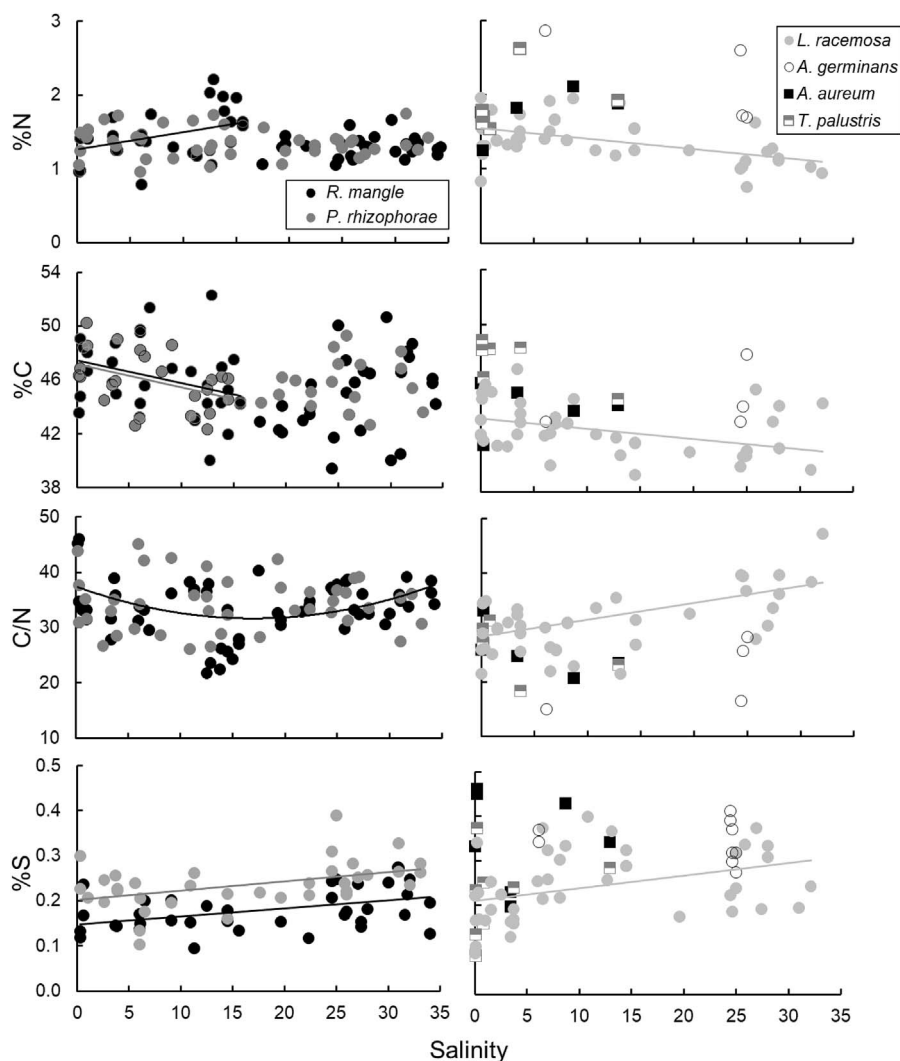


Fig. 7. Foliar %N, %C, C/N, and %S plotted versus salinity down-estuary. Data pooled across estuaries and dates. Left panels: data for *R. mangle* (black points and lines) and *P. rhizophorae* (grey points and lines). Right panels: *L. racemosa* (grey points and lines), *A. germinans*, *T. palustris*, and *A. aureum*. Regression lines for *A. germinans*, *T. palustris*, and *A. aureum* were not calculated because of low number of points. Regression lines shown only when significant, see Table S1 for statistical results.

characteristic of marine sulfate, perhaps evidencing uptake of sulfide from sedimentary sources (note the difference between *R. mangle* and *P. rhizophorae*, which may be related to the different substrate preferences between these two species). The shifts and high variability in $\delta^{34}\text{S}$ along the different reaches of the estuaries need further study.

3.2.5. Effects of plant-sediment interactions

The $\delta^{15}\text{N}$ of rooted mangrove plants could have been influenced by the nitrogen status of the sediments on which they grew. To find a link between tree and sediment nutrients, we plotted the mean isotopic values of N and S of the three main mangrove species versus those of sediment for each estuary (Table 3), the putative N and S source to the trees (Fig. 5). The N isotopic values extended across similar ranges (Table 3, Fig. 5) and there were no significant differences between foliar and sediment N isotopic signatures in the mangroves (runs test, $P > 0.05$; Fig. 5 filled symbols). The distribution of points was indistinguishable from the 1:1 line, suggesting some degree of coupling between sediment and foliar signatures in mangrove forests. Similar data from mangroves from other regions fell into the overall pattern (Fig. 5 open symbols). Nevertheless, some inter-specific differences in plant-sediment links merit notice in Fig. 5 (left). Virtually all values (15 out of 16) for foliar $\delta^{15}\text{N}$ for *R. mangle* and *L. racemosa* in Panama, and from mangroves elsewhere, were heavier (by up to 2‰) than

sedimentary $\delta^{15}\text{N}$ (Fig. 5 left). The heavier signatures suggest that mycorrhizae might not be involved (Nadelhoffer and Fry, 1994), and that there was a loss of light nitrogen from mangroves of these species, perhaps through leaching of dissolved nitrogen compounds by the intense rainfall in the region. In contrast, all points for *P. rhizophorae* were lower than those in underlying sediments, a result consistent with the notion that mycorrhizae may play some role in the nitrogen economy of this poorly-studied species.

There was no apparent link between plant and sedimentary $\delta^{34}\text{S}$ (Fig. 5 right), although the ranges of values for sulfur isotopes were similar. This contrasts with increased isotopic sulfur values in mangroves down-estuary, related to increased soil sulfide, found in Florida mangroves (Fry and Smith, 2002). Overall, $\delta^{34}\text{S}$ data from the Panama mangroves were dominated by variability, large enough that it is hard to discern much, but there may be some species-specific contrasts. For example, $\delta^{34}\text{S}$ for *L. racemosa* tended to lie above the 1:1 line (Fig. 5 right), at levels near those of marine sulfate, and its foliar signature did not change with changes in sediment signatures. The implication is not clear, because *L. racemosa* in the region did not clearly grow nearer the sea. $\delta^{34}\text{S}$ values for *R. mangle* and *P. rhizophorae* showed high variation, with no relation to sediment values (Fig. 5 right). There must be key, small-scale, local contrasts that substantially alter sulfur isotopic signatures in these mangrove species, and remain unidentified.

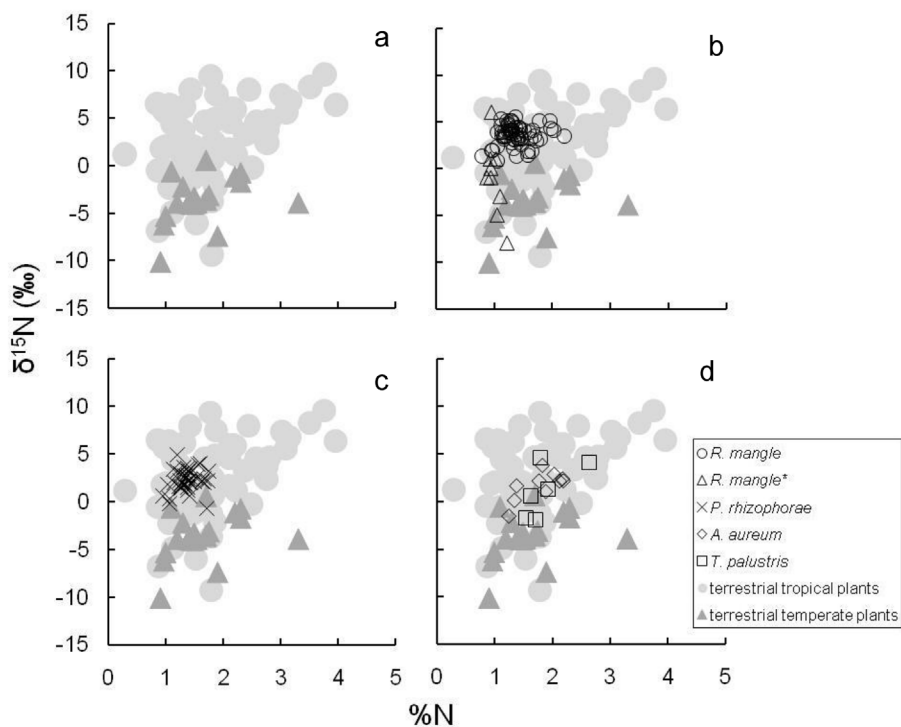


Fig. 8. Foliar $\delta^{15}\text{N}$ (‰) versus foliar %N in tropical and temperate forest plants and mangroves. a) data for tropical and temperate terrestrial forest vegetation from Martinelli et al. (1999) (these data are included as background comparisons in the other panels); b) data for *R. mangle*; c) data for *A. aureum*; and d) data for *T. palustris*. Black symbols are from this study, except for the triangles in b (*R. mangle**), which show data from Belize experiments by McKee et al. (2002), with %N data kindly provided by Karen McKee, pers. comm.

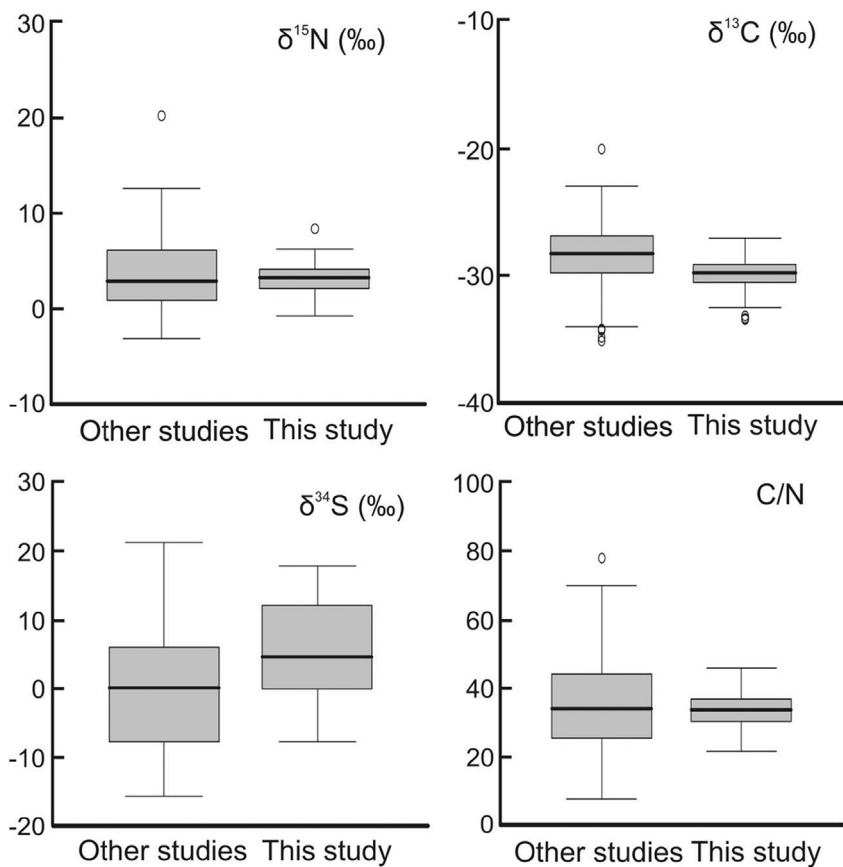


Fig. 9. Box plot comparisons of foliar $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, $\delta^{34}\text{S}$, and C/N values for Panama mangrove vegetation from this study and published values (sources listed in text).

3.3. Factors affecting foliar %N, C, and S

The percentages of N and C, C/N, and % S in leaves of mangroves and other species were narrowly constrained (Fig. 6), and relatively low compared to other reports (Golley et al., 1975; Rao et al., 1994; Feller et al., 2003), and rainforest trees (Alongi, 2009). Most stands of well-developed mangroves are nitrogen limited, which tied to the low foliar and sedimentary N content, and implying high N use efficiency (Alongi, 2009).

There were no discernible effects of degree of deforestation on the % N, %C, C/N, or %S in leaves of any species of plants (Fig. 6). The lack of linkage of nitrogen and carbon to land covers corroborates earlier findings (Valiela et al., 2013b), based on nutrient concentrations and ratios, and stable isotopic results (Fig. 3), that in this region, mangrove trees seem uncoupled from the contributing terrestrial watersheds.

We found no differences in N, C, or S content between dry and wet seasons or rainfall (*t*-test for independent samples, $P > 0.05$) or effects of upwelling (Fig. S1). So in Fig. 7 we pooled all data of the different watersheds together to check differences down estuary. Nevertheless, the large variation in elemental composition made it difficult to discern trends down estuary, but there were some statistically distinct aspects (Fig. 7, Table S1), with uncertain explanation. For the two dominant mangrove species, %N increased, while %C decreased as salinity increased to about 15 (Fig. 7 left panels). Sulfur content of leaves increased significantly down estuary, with higher values at the sites most influenced by oceanic waters (Fig. 7 bottom), even though there was no increase in %S in sediments (Table 3). The trends for the less abundant species were less distinct.

C/N in the dominant species of mangroves decreased between fresh water and mid-estuary (Fig. 7), perhaps a result of the ammonium peak we reported in the water column at mid-estuary reaches (Valiela et al., 2013b). The trough of C/N implies highest nutritive status in mangrove trees in mid-estuary, and some decrease in saltier reaches. C/N in the less-abundant species of plants (Fig. 7 right panels) either showed no down-estuary trend, or, for *L. racemosa*, a lower nutritive status nearer the sea.

3.4. Relation of isotopic signatures and nutrient content of plants

Net effects of differences in magnitude and pathways of nitrogen gain, storage, and loss result in larger ranges in $\delta^{15}\text{N}$ and %N in vegetation of tropical forests compared to temperate forests (Fig. 8a). For example, foliar $\delta^{15}\text{N}$ values of temperate forests tend to be below 0‰, while tropical values extend to 10‰. $\delta^{15}\text{N}$ of tropical vegetation show some increase where foliar %N is higher, a trend not seen in temperate plants. $\delta^{15}\text{N}$ and %N of mangrove species from Panama fell within narrower bounds than described for other tropical forests (Fig. 8 b, c, d), with no evident slopes. The relative narrow ranges documented for mangrove vegetation might, in part, be a result of limited taxonomic diversity, but on the whole, conditions within mangrove estuaries created fairly rigid controls. The ranges of $\delta^{15}\text{N}$ and %N in the two dominant species, *R. mangle* and *P. rhizophorae*, also overlap tightly (Fig. 8b and c), perhaps as an evidence of similar niche spaces of these two competing species. For comparison, we added values for experimental mangrove plots in Belize (Fig. 8b, triangles). These values extend to $\delta^{15}\text{N}$ ranges seen in temperate sites, an effect that might result from uptake of added fertilizer that was fixed from atmospheric N_2 . The Belize data are also of interest because the %N fell at the low extreme of Panama mangroves. The height of the Belize stands was at most 6 m, compared to the much taller stands we have measured in Panama (up to 30-plus m in height). Further study of the controls of these contrasts in height (and biomass) in relation to internal and external nitrogen supply will be of interest.

Variation of stable isotopic ratios of mangrove vegetation in Panama differed, with ranges for $\delta^{13}\text{C} < \delta^{15}\text{N} < \delta^{34}\text{S}$ (Fig. 9). These ranges were quite similar, albeit somewhat narrower, to those measured for mangrove taxa across a wide variety of other tropical coasts (Fig. 9, compiled from Hemminga et al., 1994, Rao et al., 1994, Woitichik et al., 1997, Kuramoto and Minagawa, 2001, Bouillon et al., 2008 and references therein, Prasad and Ramanathan, 2009, Tue et al., 2011). Again, sulfur isotopes seem to be the most variable, a feature that asks for more study.

Foliar C/N values in this study were similar to those from other sites elsewhere (Fig. 9 bottom right). C/N in mangrove vegetation ranged 21–46 (Fig. 7), well within the 17–78 span of mangrove species reported from elsewhere (Fig. 9, bottom right).

On the whole, judging by isotopic signatures and elemental contents, the mangrove ecosystems along the Pacific coast of Panama included in this study seemed not affected by seasonal rainfall, biogeographic position of the estuary, % forest cover of the watershed, or even La Niña exceptional events. These potentially important external variables failed to significantly alter stable isotope values or nutrient content in mangrove leaves. In contrast, within-ecosystem, down-estuary changes in both stable isotopes and nutrient content were evident, as were certain inter-specific differences in regard to plant-sediment features. Within-mangrove transformations seemed powerful enough to significantly de-couple mangrove ecosystems—or at least mangrove plants and sediments—in the Pacific coast of Panama from the influence of both watershed and marine inputs, but inter-specific differences remained.

Deforestation of tropical watersheds is widespread and the consequent effect of exports to receiving waters is therefore important for agencies dealing with environmental management. In the watersheds we studied within mangrove-estuary transformation erased effects of different land cover, even up to about 70% deforestation. These results speak for the importance of conservation of mangrove estuaries, since our results suggest that mangrove estuaries provide valuable ecological services, as filters that uncouple land use practices on contributing watersheds from water quality of receiving coastal waters.

Acknowledgments

This work was funded by NSF grant DEB-0842413. We would not have been able to carry out the fieldwork without the availability of the excellent resources of the Liquid Jungle Laboratory built by Jean Pigozzi, and we are much indebted to the LJL staff for providing excellent support and facilities for our work. We thank Laurence Madin and the Ocean Life Institute at the Woods Hole Oceanographic Institution for initial support and throughout the work. The support of the Woods Hole Consortium was instrumental to facilitating work by our inter-institutional research team. Sarah Wilkins, Rita Monteiro, Sandy Baldwin, Jim Brennan, Katuska Hernández, Richard McHorney, Sam Kelsey, Ned Mueller, and Jason Bissonette helped carry out the demanding fieldwork involved in the project. Inés Viana was supported with a postdoctoral fellowship from Xunta de Galicia (Consellería de Cultura, Educación e Ordenación Universitaria grant ED481B 2016/189-0). We thank Gonzalo Nieto Feliner, Director, Real Jardín Botánico, Madrid, for identification of mangrove taxa. We thank Karen McKee for sharing data from Belize used in Fig. 8 and Steve Bouillon for sharing some useful references used in Fig. 9.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.marenvres.2018.03.003>.

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