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Isotopic ratios and elemental contents as indicators of seagrass C processing and sewage influence in a tropical macrotidal ecosystem (Madagascar, Mozambique Channel)

GILLES LEPOINT [1], BRUNO FRÉDÉRICH [2], SYLVIE GOBERT [1] and ERIC PARMENTIER [2]

[1] MARE centre, Laboratoire d'Océanologie, Institut de Chimie, Bât B6c, Université de Liège, 4000, Liège, Belgique.
E-mail: G.Lepoint@ulg.ac.be
[2] MARE centre, Laboratoire de Morphologie Fonctionnelle et Evolutive.

SUMMARY: Isotopic ratios and elemental concentrations of carbon and nitrogen were measured in seven seagrass species colonising different tidal flats near Toliara (SW Madagascar) in order to determine the potential use of these parameters for assessing C processing and sewage use by tropical seagrasses. Nitrogen concentrations measured in upper intertidal seagrasses near Toliara were almost twice those measured on the tidal flat near a healthy mangrove situated 20 km away from Toliara town. At Toliara Beach, δ^{15}N values were correlated with the N concentrations of *Halodule* sp., one of the dominant species on the tidal flat. This correlation did not exist for *Halophila ovalis*, the other dominant species. An increase in N concentrations and δ^{15}N values demonstrates the influence of sewage coming directly onto Toliara Beach on the N cycles of intertidal seagrasses. Nevertheless, this influence seems restricted to the upper littoral zone and was not the main cause of seagrass die-off. On the other hand, at the mangrove site, δ^{15}N values were not correlated with the N concentrations of *Halodule* sp. or *Thalassia hemprichii*, showing that natural δ^{15}N variability is driven by other factors than the δ^{15}N of N sources. Moreover, inter-individual variability of δ^{15}N values was greater than inter-specific or inter-site variability, making the δ^{15}N difficult to interpret in the context of human-disturbance influence on the N cycle of tropical seagrasses. δ^{13}C values were close to -9‰, indicating the use of HCO_3^- as an inorganic carbon source by the seven investigated species. Contrary to our hypothesis, variation between sites and location on the tidal flat was limited, suggesting limited impact on δ^{13}C values of sewage, emersion duration and mechanisms for HCO_3^- incorporation.

Keywords: sewage impact, coastal ecosystem, seagrass, stable isotopes, tidal habitat, SW Indian Ocean.

RESUMEN: Relaciones isotópicas y composición elemental como indicadores del procesamiento de C en fanerógamas marinas, e influencia de los efluentes urbanos en un ecosistema intermareal tropical (Madagascar, canal de Mozambique). – Se midieron las relaciones isotópicas y las concentraciones elementales de carbono y nitrógeno en 7 especies de fanerógamas marinas colonizadoras de diferentes zonas intermareales cerca de Toliara (SO Madagascar) con el fin de determinar el uso potencial de estos parámetros para evaluar el procesamiento de C y el uso de los efluentes urbanos por parte de estas fanerógamas marinas. Las concentraciones de nitrógeno medidas en las fanerógamas marinas de cerca de Toliara fueron al menos dos veces las de aquellas de la zona intermareal próxima a un manglar sano situado a 20 km de la ciudad de Toliara. En la playa de Toliara, los valores de δ^{15}N estuvieron correlacionados con las concentraciones de N de *Halodule* sp., la especie dominante en el intermareal. El incremento en las concentraciones de N y de los valores de δ^{15}N demostraron la influencia que los efluentes urbanos directos a la playa de Toliara ejercen sobre los ciclos de N en las fanerógamas marinas intermareales. Sin embargo, esta influencia parece restringida a la parte alta del litoral y no es la principal causa de la muerte de las fanerógamas marinas. Por otra parte, los valores de N isotópico mostraron diferencias significativas entre los lugares investigados y, en el manglar los valores de δ^{15}N no estuvieron correlacionados con las concentraciones de N de *Halodule* sp. La variabilidad interindividual de δ^{15}N fue mayor que la interespecífica o inter lugar de muestreo, haciendo de éste un parámetro difícil de interpretar en el contexto de la influencia debida a perturbaciones humanas sobre el ciclo del N de fanerógamas marinas tropicales. Los valores de δ^{13}C fueron próximos a -9‰, indicando el uso de HCO_3^- como una fuente de C inorgánico por parte de las 7 especies estudiadas. La variación entre sitios y localización en el intermareal fue limitada, sugiriendo un impacto limitado de los efluentes urbanos, tiempo de emergencia y mecanismos de incorporación de HCO_3^- en los valores de δ^{13}C.

Palabras clave: impacto de efluentes urbanos, ecosistema costero, fanerógamas marinas, isotopos estables, habitat mareal, Océano Índico.

INTRODUCTION

Tropical East Africa offers a highly specific diversity of seagrasses (from 5 to 11 species, depending on the location), which is characteristic of tropical areas (e.g. Aleem, 1984; Coppejans *et al.*, 1992). Local inhabitants use them for fishing seagrass-associated fauna, which sometimes constitute the primary protein source for the people (de la Torre and Rönnbäck, 2004). However, seagrass ecosystems are undergoing a global decrease linked to human activity. For example, along the East African coast, shellfish collection, which involves digging the sediment and trampling at low tide, are a major cause of intertidal seagrass loss (Bandeira and Gell, 2003).

Seagrasses occupy the intertidal and subtidal zones, experiencing varying degrees of emersion time. This ecological factor has an impact on the species distribution along the tidal gradient because not all species have the same tolerance to the high irradiance experienced during emersion (Björk *et al.*, 1999). This tidal gradient also affects carbon and nutrient acquisition and processing. Indeed, seagrasses have the possibility of performing photosynthesis during emersion time, sometimes at a greater rate than during immersion (Silva *et al.*, 2005). Other factors such as the nature of the sediment, the water turbidity and the organic content of the sediment also determine the species occurrence and distribution. East African seagrasses have a wide range of life strategies, morphologies, biomasses, nutrients and carbon processing modes. Pioneer species such as *Halophila ovalis* Brown and *Halodule uninervis* (Forsskål) Ascherson are encountered at the upper limit of seagrass beds, where they experience the longest emersion time. These species are also observed in the mid- or lower intertidal zones in association with climax species such as *Thalassia hemprichii* (Ehrenberg) Ascherson or *Cymodocea rotundata* Ehrenberg and Hemprich ex Ascherson. *Syringodium isoetifolium* (Ascherson) Dandy, *Thalassodendron ciliatum* (Forsskål) den Hartog and *Cymodocea serrulata* (Brown) Ascherson and Magnus colonise the subtidal zone, sometimes in association with other species.

However, seagrass research in this area is still scarce and has been identified as an important gap in the understanding of seagrass ecology (Gullström *et al.*, 2002). In this paper, the variability of the nitrogen and carbon processing pattern of the seagrass species that colonise a reef, a mangrove bay and a human-disturbed beach was assessed along a tidal gradient using elemental contents and stable isotope ratios of carbon and nitrogen.

Stable isotopes are classically used in seagrass ecology to assess food web structure. They are also useful in the study of carbon and nitrogen cycles because stable isotope ratios are related to nutrient sources and processing. Nitrogen stable isotope ratio variations in seagrasses are not well understood but are related to inorganic N sources incorporated by seagrasses and to sediment and column water geochemistry (e.g. Kamermans *et al.*, 2002; Carruthers *et al.*, 2005; Fourqurean *et al.*, 2005). Seagrasses have complex and variable strategies for meeting their N requirement, involving both leaf and root uptake and internal resorption (Touchette and Burkholder 2000). Nitrate and ammonium are two possible sources of inorganic N. Moreover, associations with N_2-fixing organisms are thought to be widespread among tropical seagrass species. Along the Kenyan coast, N stable isotope measurement has been used to assess the influence of groundwater on local seagrass meadows (Kamermans *et al.*, 2002).

Carbon isotopic ratios are primarily determined during photosynthesis by the nature of incorporated inorganic carbon (i.e. bicarbonate or dissolved CO_2) (Raven *et al.*, 2002) and by the photosynthesis rate and irradiance level (e.g. Hemminga and Mateo, 1996). The photosynthesis rate and irradiance level vary both temporally and spatially, so the isotopic ratio of C in seagrasses is often depth-related and shows variations according to season, location and community structure.

SITE DESCRIPTION

This study was carried out near the town of Toliara (SW Madagascar, Mozambique Channel, Indian Ocean) (23.22°S, 43.40°E) at the Laboratoire Aqua-Lab of the Institut Halieutique et des Sciences Marines (IH.SM) (Toliara University, Madagascar) (Fig. 1). The region, situated near the Capricorn Tropic, is a semi-desert and the terrestrial vegetation is a xerophilous bush. Toliara is a city of about 140000 inhabitants (mainly fishermen and farmers) and is surrounded by only a few industries (Billé and Mermet, 2002). Deforestation and erosion bring terrigeneous sediment to Toliara Bay via the River Fihérénana. This is a macrotidal bay with a semi-diurnal tide. Toliara Bay receives urban effluence

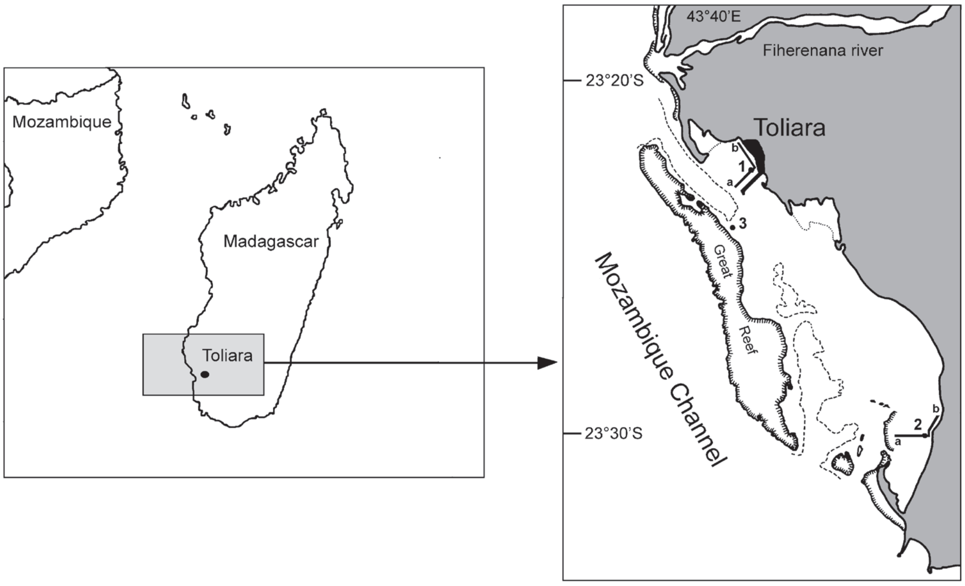

FIG. 1. – Sampling locations near Toliara (SW Madagascar, Mozambique Channel). Numbers refer, respectively, to Toliara Beach (1), Beloza Mangrove (2), Toliara Great Reef (3). Letters refer, respectively, to the sampling transect across the tidal flat (a) and the sampling transect along the upper intertidal area (b).

directly without any treatment or sewage plan. The significance of this effluence is unknown but, in the absence of industry and productive agriculture, it is probably almost completely constituted by human and animal waste, and is quantitatively limited and diffuse. Furthermore, the intertidal flat is used as a toilet by both humans and animals at low tide. The mangrove near the town has disappeared. A barrier reef extends out from Toliara, forming the Grand Récif of Toliara and delimiting Toliara Bay (Fig. 1). This reef near the town has been devastated, suffering from low water quality (i.e. high sediment load from the local river), unselective fishing and trampling at low tide. The quality of water and the preservation of the coral reef and mangrove increase toward the south, which is only occupied by a few small villages of pirogue fishermen. An integrated coastal management plan was launched in this area in 1997 (Billé and Mermet, 2002).

MATERIALS AND METHODS

Sampling was carried out in November 2005 across the intertidal flat near Toliara town and 20 km away at the relatively preserved mangrove area of Beloza (Fig. 1). The sampling area went from the upper intertidal area to the subtidal area, which represents a distance of about 600 m. A third sampling was carried out on Toliara Great Reef from the lower intertidal zone to a maximum of a 3 m depth. The sampling was done to encompass the diverse seagrass associations, according to four zones with different emersion durations: subtidal, lower intertidal, mid-intertidal and upper intertidal. To evaluate small-scale variability, in each zone we selected two seagrass patches (three for the mid-intertidal zone), separated by about 20 m. Three replicates of each encountered species were collected at each station. A replicate is composed of one fragment of rhizomes bearing one or more leaf bundles. For small species such as *H. ovalis* and *Halodule* sp., leaves from two to three leaf bundles were gathered in order to obtain a sufficient amount of matter to measure elemental contents and stable isotope ratios.

An additional horizontal transect was made along the highest seagrass settlement (corresponding to upper intertidal) on Toliara Beach (about 800 m) and at the mangrove site (about 400 m). Along these transects, we sampled each time we encountered a new seagrass patch. Therefore, sampling sites were randomly distributed along the upper intertidal area. Three replicates were collected at each sampling site as for transects across the tidal flat.

Leaves were scraped with a razor blade to remove epiphytes, oven dried for 48 h at 50°C and then ground to a homogenous powder. After grinding, samples were placed for 24 h under a glass bell with fuming HCl (37%) (Merck, for analysis quality) in order to eliminate remaining calcareous epiphytes.

Measurements were performed with a mass spectrometer (Optima, GV Instrument, UK) coupled to a C-N-S elemental analyser (Carlo Erba, Italy). Isotopic ratios are presented as δ values (‰), expressed relative to the vPDB (Vienna Peedee Belemnite) standard and to atmospheric N_2 for carbon and nitrogen, respectively. Reference materials were IAEA-N1 (δ^{15}N=+0.4 ± 0.2‰) and IAEA CH-6 (sucrose) δ^{13}C= -10.4 ± 0.2‰). Experimental precision (based on the standard deviation of replicates of an internal standard) was 0.3 and 0.4‰ for carbon and nitrogen, respectively. Elemental results are expressed as a percentage of the considered element relative to the total dry weight (% DW).

RESULTS

As at other sites in East Africa, seagrass diversity was found to be relatively high, and diverse seagrass associations representing seven species were found across the tidal range. The most frequent species collected in our study was *Halodule* sp. (probably *H. uninervis*, in both narrow and large leaf form, *sensu* Brouns and Heijs, 1991). Communities on the mangrove beach were more diversified than on the Toliara Beach. The upper littoral zones of the three sampled sites were colonised by monospecific stands of *Halodule* sp. or of *H. ovalis*. On the mangrove tidal flat, the mid-intertidal zone was progressively colonised by mixed associations of *H. uninervis* and *T. hemprichii*. *C. rotundata* was sometimes present in small patches or was associated with these

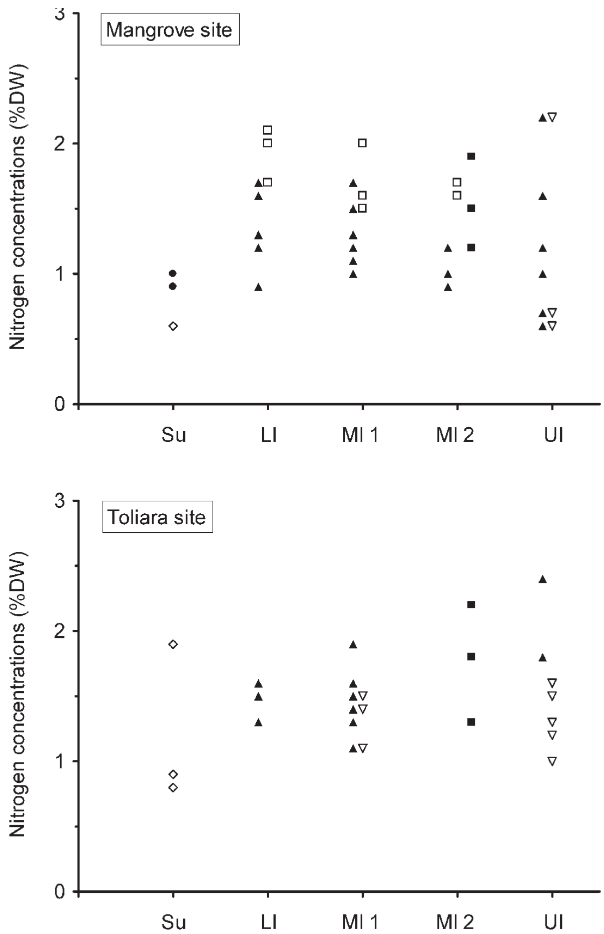

FIG. 2. – Nitrogen concentrations (% DW) measured in seagrass species collected on Toliara Beach and Beloza Mangrove (Madagascar) (Sub: subtidal; LI: Low Intertidal; MI1: Mid-intertidal community 1, MI2: Mid-intertidal community 2; UI: Upper intertidal). ■: *Cymodocea rotundata*, □: *Thalassia hemprichii*, ●: *Thalassodendron ciliatum*, ▲: *Halodule* sp., ▽: *Halophila ovalis,* ◊: *Syringodium isoetifolium*.

TABLE 1. – Mean values (n=3) of δ^{13}C (‰), δ^{15}N (‰), nitrogen concentration ($\%_{dw}$) and C/N ratios (w:w) of seagrass collected along two transects made in the upper littoral zone of Toliara Beach and a mangrove site. Stations were randomly distributed along the upper intertidal limits, according to the seagrass patch occurrence.

Place	Station	Species	n	δ^{13}C	δ^{15}N	%N	C/N
Mangrove	1	*Halodule* sp.	3	-13.2±0.4	5.3±0.1	1.3±0.2	18.7±0.5
	2	*Halodule* sp.	3	-11.8±0.2	4.9±1.4	1.1±0.1	24.9±3
	3	*Halodule* sp.	3	-10.2±0.1	3.4±0.1	1.1±0.0	24.1±0.9
	4	*Halodule* sp.	3	-10.5±0.4	5.2±0.4	0.9±0.1	25.1±1.4
	5	*Halodule* sp.	3	-11.3±0.8	4.6±1.3	1.3±0.2	21.7±3.9
	average	*Halodule* sp.	15	-11.4±1.2	4.6±1.0	1.1±0.2	22.7±3.2
Toliara Beach	1	*Halophila ovalis*	3	-9.0±0.1	4.8±0.9	2.0±0.1	10.9±0.8
	2	*Halodule* sp.	3	-10.4±0.9	2.7±3.2	2.2±0.3	11.4±0.7
		Halophila ovalis	3	-10.5±0.4	4.1	1.5±0.2	11.4±0.9
	3	*Halodule* sp.	3	-9.5±0.7	3.4±1.4	2.2±0.1	12.4±0.5
		Halophila ovalis	3	-9.4±0.3	7.1±1.3	2.3±0.7	10.0±2.0
	4	*Halodule* sp.	3	-9.5±0.9	3.6±0.9	2.2±0.4	14.1±2.7
	5	*Halophila ovalis*	3	-8.6±0.6	2.4±1.0	1.7±0.6	12.1±1.5
	6	*Halophila ovalis*	3	-9.5±0.2	3.2±0.8	1.3±0.3	15.8±4.8
	average	*Halodule* sp.	9	-9.7±0.9	3.2±1.8	2.2±0.3	13.4±3
		Halophila ovalis	15	-9.3±0.7	4.4±2.0	1.8±0.5	12.1±3
		both species	24	-9.5±0.8	3.8±1.9	1.9±0.5	12.6±3

TABLE 2. – Mean values (n=3) of δ^{13}C (‰), δ^{15}N (‰), nitrogen concentration ($\%_{dw}$) and C/N ratios (w:w) of seagrass collected on the Great Reef of Toliara.

Localisation	Assemblages	Species	δ^{13}C	δ^{15}N	%N	C/N ratios
subtidal	*Thalassodendron ciliatum*	*T. ciliatum*	-9.1±0.3	3.8±0.6	1.0±0.1	30.8±3.6
subtidal	*Halophila ovalis/ Cymodocea serrulata*	*H. ovalis*	-8.0±0.3	1.6±0.6	1.7±0.5	12.1±1.2
		C. serrulata	-8.4±0.7	1.7±0.9	1.1±0.1	28.4±1.5
subtidal	*Cymodocea serrulata*	*C. serrulata*	-9.3±0.1	2.7±1.6	1.0±0.1	23.2±1.4
subtidal	*Syringodium isoetifolium*	*S. isoetifolium*	-3.3±0.5	1.2±0.1	1.0±0.1	23.9±2.2
intertidal	*Halophila ovalis/ Cymodocea rotundata*	*H. ovalis*	-7.6±0.5	0.7±1.5	1.3±0.3	14.8±0.6
		C. rotundata	-9.9±1.0	2.0±0.7	1.2±0.2	23.9±1.3
higher intertidal	*Halodule* sp./ *Cymodocea serrulata*	*C. rotundata*	-7.8±0.2	-0.2±2.1	1.6±0.2	16.4±2.1
		Halodule sp.	-8.5±0.8	2.7±0.4	1.3±0.2	19.9±2.6

two species. On Toliara Beach, seagrass communities were reduced to small patches of *Halodule* sp. and *H. ovalis*. Small water pools sheltered mixed associations of *C. rotundata* and *Halodule* sp. *C. rotundata* was also present on the reef site, where it formed monospecific patches or mixed associations with *Halodule* sp.

The subtidal upper limit was colonised by *S. isoetifolium* in monospecific stands at the three sites.

C. serrulata and *T. ciliatum* occurred in the deeper subtidal zone at the mangrove and barrier reef sites, but were not collected at Toliara Beach. *H. ovalis* was sometimes found in-mixing with *C. serrulata* in this subtidal area.

Nitrogen concentrations relative to dry weight (% DW) in seagrass leaves at Toliara Beach and the mangrove beach varied between 0.6 and 2.6% DW (Fig. 2). Along the horizontal transects in the upper intertidal zone, N concentrations measured in *Halodule* sp. from Toliara Beach were almost twice those measured at the mangrove beach (Table 1) (Mann-Whitney U test, $p< 0.001$). Here, C:N ratios (w:w) showed the same significant differences as the N concentrations. On the Great Reef, nitrogen concentration varied between 1 and 1.6% DW (Table 2).

Delta ^{15}N values ranged from -0.5 to +6.0‰ (Fig. 3). There was no clear pattern along transects across the tidal flats but there was a relatively high variability in these δ^{15}N values. For *Halodule* sp. from the upper intertidal, δ^{15}N values tended to be significantly greater at the mangrove location than on Toliara Beach (Mann-Whitney U test, $p< 0.05$).

Delta ^{13}C values varied between -11‰ and -2.9‰ (Fig. 4). However, the δ^{13}C values of the seagrass species did not show any specific differences, except for *S. isoetifolium*, which had, on average, less negative δ^{13}C values than those of other species (Fig. 4). There was no apparent pattern of δ^{13}C values in relation to emersion zone on tidal flats at the Toliara beach (Fig. 4) and reef sites (Table 2). Indeed, for these two sites, values were relatively constant along the transect across the tidal flat and were comprised of between -8 and -10.5‰, with the exception of *S. isoetifolium*, which showed less negative values than other species at all locations.

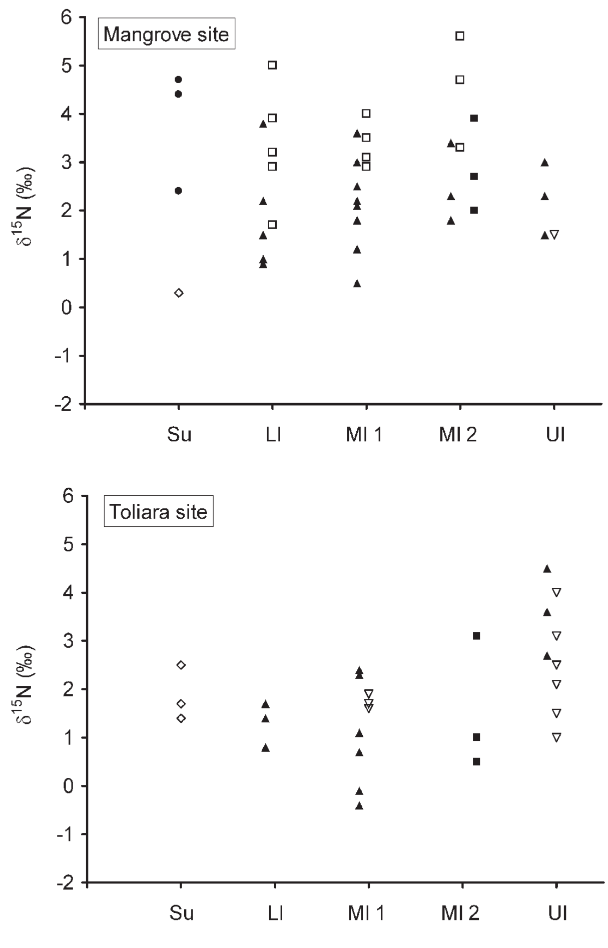

FIG. 3. – Nitrogen isotope ratios measured in seagrass species collected on Toliara Beach and Beloza Mangrove (Madagascar). (Sub: subtidal; LI: Low intertidal; MI1: Mid-intertidal community 1, MI2: Mid-intertidal community 2; UI: Upper intertidal). ■: *Cymodocea rotundata*, □: *Thalassia hemprichii*, ●: *Thalassodendron ciliatum*, ▲: *Halodule* sp., ▽: *Halophila ovalis,* ◊: *Syringodium isoetifolium.*

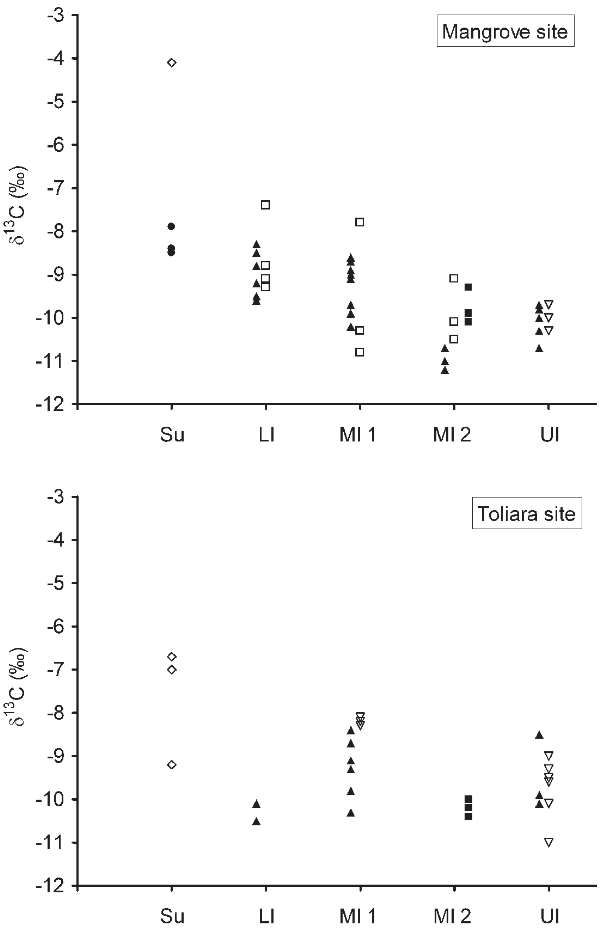

FIG. 4. – Carbon isotope ratios measured in seagrass species collected on Toliara Beach and Beloza Mangrove (Madagascar). (Sub: subtidal; LI: Low intertidal; MI1: Mid-intertidal community 1, MI2: Mid-intertidal community 2; UI: Upper intertidal). ■: *Cymodocea rotundata*, □: *Thalassia hemprichii*, ●: *Thalassodendron ciliatum*, ▲: *Halodule* sp., ▽: *Halophila ovalis,* ◊: *Syringodium isoetifolium*.

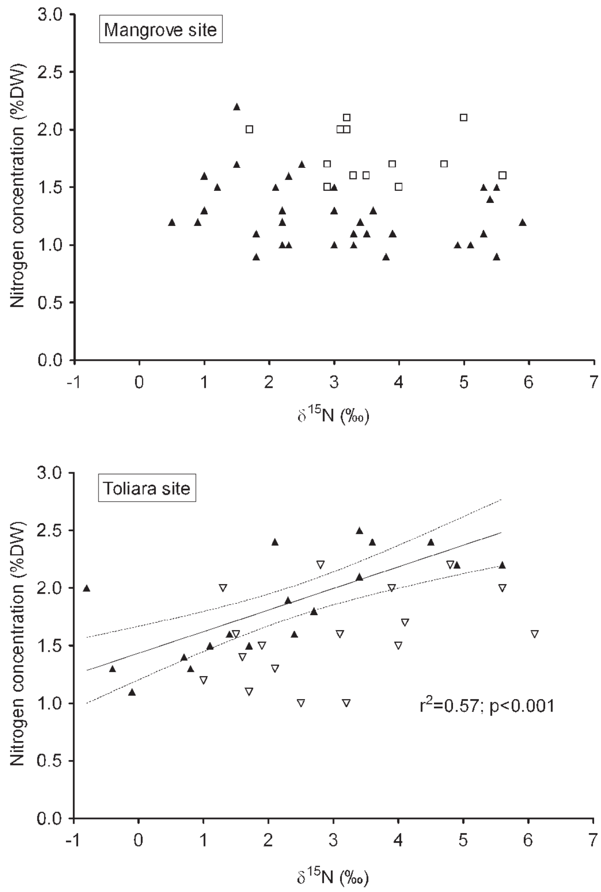

FIG. 5. – Relation between $\delta^{15}N$ and nitrogen concentrations in leaves of *Halodule* sp. (▲) *Halophila ovalis* (▽) and *Thalassia hemprichii* (□) collected on Toliara Beach and Beloza Mangrove (Madagascar).

The range of $\delta^{13}C$ values observed across the tidal flat at the mangrove location was larger (from -7 to -11‰) than those measured at the Toliara beach and reef locations. Moreover, $\delta^{13}C$ values of *Halodule* sp. coming from the upper littoral were significantly depleted in ^{13}C along the mangrove horizontal transect in comparison with those from the Toliara Beach horizontal transect (Table 1) (Mann-Whitney U test, $p< 0.001$).

At Toliara Beach, there was a significant correlation between $\delta^{15}N$ values and nitrogen concentrations ($r^2 = 0.57$, $p < 0.001$) (Fig. 5). This correlation did not occur at the mangrove site, which showed a greater variability of $\delta^{15}N$ values, or for the other dominant species, *T. hemprichii* and *H. ovalis*. The variability between sampling sites separated by 20 m was high and greater than the variability between locations separated by 20 km.

DISCUSSION

Seagrass communities are deeply affected by human disturbance and, among other factors, by the increase in the nutrient load to coastal areas. Measurements of nitrogen concentrations in seagrasses have demonstrated this influence in diverse tropical ecosystems (e.g. Yamamuro *et al*., 2003). In our study, the N concentrations measured in the intertidal seagrass living on the upper part of the tidal flat near Toliara were found to be almost twice the concentrations measured in seagrass living on a relatively preserved tidal flat situated 20 km away from the city. The influence of anthropogenic nitrogen inputs on seagrass nitrogen concentration seems, however, to be limited to the upper littoral zone, as the transect across the tidal zone did not show any significant difference between the two investigated areas. This is possibly due to the influence of the tidal cycle on the dilution of human-produced effluent. The zone that is exposed for the longest time was shown to be significantly affected by the waste water arriving from the town. Waste water discharge onto Toliara Beach is relatively diffuse and unequally distributed along the beach, as there is no waste treatment plan in this town. This distribution explains the variability of N concentrations along the horizontal transect at Toliara Beach.

The N inputs contribute to the increase in N concentrations in seagrasses on Toliara Beach, at least in its upper part. Nevertheless, the primary causes of seagrass disappearance on this tidal flat are the increase in organic matter load, which makes the sediment completely anoxic (pers. ob.) and toxic for seagrass settlement, and above all trampling by people at low tide and the digging involved in the shellfishing process (Bandeira and Gell, 2003).

Generally, the anthropogenic impact on $\delta^{15}N$ in macrophytes is an increase in $\delta^{15}N$ values relative to $\delta^{15}N$ "natural" values (e.g. MacClelland *et al.*, 1997; Costanzo *et al.*, 2001). The $\delta^{15}N$ values measured at Toliara Beach were found to be, on average, lower than those measured at the mangrove site, which is not the habitual trend observed for anthropogenic influence on the $\delta^{15}N$ values of macrophytes (but see Rogers, 2003). However, there was a correlation between $\delta^{15}N$ and N concentrations of *Halodule* sp. collected at Toliara Beach. This fits with the positive correlation between $\delta^{15}N$ and N concentrations in seagrasses observed by Yamamuro *et al.* (2003) in a human impacted reef.

Intra-specific variability observed at the mangrove site encompasses a range of 7‰. This variability is not correlated to N concentrations. Fourqurean *et al.* (2005) have shown that $\delta^{15}N$ signals of seagrasses may vary seasonally in tropical areas. These natural seasonal variations may be of the same order as differences between anthropogenic and natural $\delta^{15}N$ signals. Therefore, there are no $\delta^{15}N$ values that may be unambiguously interpreted as proof of human impact. In agreement with Fourqurean *et al.* (2005), our results call for experimental studies on $\delta^{15}N$ variability in seagrasses before this parameter is used as an effective tracer of the human impact on the tropical seagrass nitrogen cycle.

$\delta^{13}C$ values of seagrasses in the literature range from -3 to -24‰, but median values are between -10 and -12‰ (Hemminga and Mateo, 1996). On the Toliara Reef and the Toliara tidal flat, average $\delta^{13}C$ values were shown to be around -9‰, except in *S. isoetifolium* (-4‰). On the mangrove tidal flat, which extends over several hundred metres, $\delta^{13}C$ values were found to be minimal (-11‰) in the upper intertidal zone near the mangrove and reached values of close to -9‰ in the mid-part of the tidal area.

With the notable exception of *S. isoetifolium*, the range for $\delta^{13}C$ values is relatively narrow considering the large range of species characteristics and environmental conditions experienced by seagrasses in our collection places (i.e. depth, emersion duration and level of human impact). The absence of a clear difference in $\delta^{13}C$ values between Toliara Beach and the other two sites would indicate that carbon originating from sewage is not incorporated by the plants. Nevertheless, in the absence of isotopic measurements on sewage carbon, it is impossible to answer this question definitively.

Essentially, $\delta^{13}C$ values of seagrasses are determined during photosynthesis. Generally, the $\delta^{13}C$ value of a plant is more negative than its inorganic carbon source (C_i), due to isotopic fractionation against ^{13}C (i.e. the preferential use of ^{12}C), which happens during photosynthesis. This fractionation occurs during the entrance of C_i into the plant and during carboxylation catalysed by the Ribulose-1.5.-Biphosphate Carboxylase/Oxygenase (Rubisco). Maximal fractionation by Rubisco is -27‰, leading to very negative $\delta^{13}C$ values in certain red algae (Raven *et al.*, 2002). Nevertheless, when all the C_i trapped in the cell is consumed by photosynthesis (i.e. high photosynthetic rate, efficient C_i utilisation or limitation by C_i supply), fractionation by Rubisco does not occur to its full extent (Hemminga and Mateo, 1996). Therefore, fractionation against the C_i source is minimal and $\delta^{13}C$ values reflect the C_i source signatures, modified by fractionation due to C_i entrance into the cell. Atmospheric and dissolved CO_2 have an isotopic signature of close to -10‰. HCO_3^- displays $\delta^{13}C$ values close to +1‰. The use of HCO_3^- leads to less negative values for $\delta^{13}C$ of seagrasses (Raven *et al.*, 2002). Some seagrass species can efficiently use HCO_3^- as a C_i source (Beer *et al.*, 2002 and reference therein) and all the species investigated in our study do this (Uku *et al.*, 2005). Beer *et al.* (2002) have demonstrated the occurrence of some mechanisms for HCO_3^- entrance (i.e. diffusion, active uptake using or not using an H^+ gradient). According to Uku *et al.* (2005), *C. serrulata* uses external conversion of HCO_3^- without the creation of an H^+ gradient, *H. ovalis* establishes an H^+ gradient associated with intracellular carbonic anhydrase

(CA), and other species associate H^+ gradient and external CA. This different mechanism for HCO_3^- use should lead to a different ^{13}C signature. Nevertheless, our data do not indicate isotopic differences in relation to different C_i acquisition mechanisms. Consequently, observed values suggest that, in the present study, fractionation by Rubisco against C_i sources was probably at a minimum due to a high photosynthetic rate and/or a high C_i use efficiency independently of the species, the site (Toliara Beach, Toliara Reef, the mangrove site) and the location on the tidal flat (i.e. different emersion times).

The only significant site effect on seagrass $\delta^{13}C$ was the proximity of mangrove at the mangrove tidal flat site, which was shown to lead to more negative $\delta^{13}C$ in samples collected near the mangrove border. This effect has already been recorded and can probably be explained by a change in the isotopic signature of the C_i source. Indeed, a significant decrease in the $\delta^{13}C$ of dissolved inorganic carbon has been observed near mangrove (Bouillon *et al*., 2004). This is due to the contribution of inorganic carbon produced by the re-mineralisation of mangrove material, which has low $\delta^{13}C$ values (i.e. -27‰) (Mook and Tan, 1991).

The specific difference between *S. isoetifolium* and the other species is probably linked to morphological differences and not necessarily to C_i source differences. Indeed, *S. isoetifolium* has a cylindrical shape, while the other species display oblong or strap-like morphologies. This morphological difference may reduce the fractionation during C_i entrance into the plant, for example, by affecting the thickness of the boundary layer.

Differences in life strategies (i.e. pioneer *vs*. climax species) or experienced emersion time did not have an effect on $\delta^{13}C$, so atmospheric CO_2 is not an important C_i source in this area. This would be explained by the very high level of irradiance experienced during emersion time, which may stop photosynthesis during emersion in tropical seagrass ecosystems (Beer *et al*., 2006). This contrasts with temperate seagrass intertidal ecosystems, where production during emersion can be significant (Silva *et al*., 2005).

In conclusion, an increase in N concentrations and of $\delta^{15}N$ values demonstrates the influence of sewage coming directly onto Toliara Beach on the N cycles of intertidal seagrasses. Nevertheless, this influence seems restricted to the upper littoral zone and was not the main cause of seagrass die-off. Contrary to our hypothesis, variation between sites and location of the $\delta^{13}C$ values on the tidal flat was limited, suggesting limited impact on $\delta^{13}C$ values of sewage, emersion duration and mechanisms for HCO_3^- incorporation.

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