Botanica Pacifica. A journal of plant science and conservation. 2020. 9(2): 61–72
DOI: 10.17581/bp.2020.09207

Interspecific variation in foliar nutrients and isotopes of submerged macrophytes in the Cau Hai Lagoon, the typical brackish lagoon in Vietnam

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Manuscript received: 04.05.2020
Review completed: 15.07.2020
Accepted for publication: 22.07.2020
Published online: 25.07.2020

ABSTRACT

Submersed macrophytes are key elements of shallow coastal ecosystems but globally declined due to anthropogenic disturbances and climate change. Foliar elemental and isotopic compositions of macrophytes are being increasingly used to identify nutritional status and environmental conditions including potential stress factors, thereto insight is required in its interspecific variation. In this paper, we present interspecific variations in foliar nutrients (C, N, P) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of four macrophyte species (*Najas indica*, *Halophila beccarii*, *Halodule uninervis*, *Halophila ovalis*) in Cau Hai lagoon (Vietnam) in relation to key environmental variables. The foliar C, N, $\delta^{13}C$ and $\delta^{15}N$ values varied among species and related to salinity, water depth and/or DIN concentrations. Foliar N and C:N values were correlated to DIN concentration in the water (*N. indica* and *H. beccarii*, respectively), whereas foliar C:P was correlated to P concentrations in sediment (*N. indica*) or water depth (*H. uninervis*). Foliar $\delta^{13}C$ of *H. beccarii* and *H. uninervis* increased with salinity and $\delta^{15}N$ of *N. indica* with both salinity and DIN concentration in the water. These aquatic plants likely varied either in resource acquisition strategies (e.g. uptake of CO_2 or HCO_3^-, N and P from different sources) or in their usage (e.g. C investment in structural tissue) and in their response to changes in salinity, water depth and DIN concentration in the water. The studied macrophytes likely experienced relatively high N and P availability and another factor than P or N probably inhibited C-fixation and/or carbon storage of these plants.

Keywords: *Najas*, *Halophila*, *Halodule*, salinity, depth, DIN, phosphorus

РЕЗЮМЕ

Данг Ти Нху И, Нгуен Тиен Хоанг, Фам Хак Лию, Харада Х., Коба К., Брион Н., Ван Хью Д., Ван Хоп Н., Сиренз Т., Вентеринк Г.О. Межвидовая изменчивость листовых питательных веществ и изотопов погруженных видов макрофитов в типичной солоноватой лагуне. Погруженные макрофиты являются ключевыми компонентами мелководных прибрежных экосистем. В глобальном масштабе их сообщества существенно сократились из-за антропогенных нарушений и изменения климата. Элементные и изотопные составы листьев макрофитов все чаще используются для определения их пищевого статуса и условий окружающей среды, включая потенциальные стрессовые факторы, для чего необходимо понимание их межвидовой изменчивости. В настоящей работе мы представляем результаты исследования межвидовых вариаций внекорневых питательных веществ (C, N, P) и стабильных изотопов ($\delta^{13}C$ и $\delta^{15}N$) четырех видов макрофитов: *Najas indica*, *Halophila beccarii*, *Halodule uninervis*, *Halophila ovalis* в лагуне Кау-Хай (Вьетнам) в зависимости от ключевых переменных окружающей среды. Содержания C, N, значения $\delta^{13}C$ и $\delta^{15}N$ варьировали в зависимости от вида и были связаны с соленостью, глубиной воды и/или концентрацией DIN. Содержание N и значения C:N коррелировали с концентрацией DIN в воде у *N. indica* и *H. beccarii*, тогда как значения C:P коррелировали с концентрацией P в детрите (*N. indica*) или в толще воды (*H. uninervis*). $\delta^{13}C$ у *H. beccarii* и *H. uninervis* увеличивалась с повышением солености, а $\delta^{15}N$ у *N. indica* – как с повышением солености, так и с концентрацией DIN в воде. Макрофиты различались в стратегиях потребления ресурсов (например, поглощение CO_2 или HCO_3^-, N и P из различных источников), в их использовании (например, инвестиции C в структурные ткани), а также по их реакции на изменения солености, глубины воды и концентрации DIN в воде. Изученные макрофиты находились в условиях доступности N и P, а фиксация C ингибировалась другими факторами.

Ключевые слова: *Najas*, *Halophila*, *Halodule*, соленость, глубина, DIN, фосфор

(*переведено редколлегией*)

Submersed macrophytes constitute key elements of shallow coastal ecosystems, but they are currently declining at an alarming rate (globally 7 % per year according to Waycott et al. 2009). This decline is partly due to nutrient enrichment in aquatic ecosystems worldwide (Paerl 2009, Van Katwijk et al. 2011). Elemental

and isotopic compositions of macrophyte leaves provide insight into the environmental conditions and availability of essential resources (N, P and light) for growth of aquatic plants and/or to detect stressors (shading, high salinity and high temperature) that predict an upcoming macrophyte decline (Fourqurean et al. 2007, Walton et al. 2016). Therefore, it is important to have insight in the variation in foliar concentrations of the key elements such as carbon (C), nitrogen (N), phosphorus (P) and their ratios – among species, as well as in relation to important environmental factors.

Nutrient (N and P) concentrations in water and sediment as well as water depth, salinity, temperature and light conditions are considered to be key factors in controlling submerged macrophyte distribution, growth and foliar C, N and P concentrations (Hoyer et al. 2004, Ferdie & Fourqurean 2004, Campbell & Fourqurean 2009). Foliar concentrations of C, N, P and their ratios depend on the relative availability of N and P in water and sediment and on the macrophyte growth, which also depend on C assimilation during photosynthesis and hence on light and other growth limiting resources (Fourqurean et al. 2007). The benthic macrophyte Redfield Ratio C:N:P of 550:30:1 (molar base) has been used as an indicator for light, N or P limitation of macrophyte growth (Atkinson & Smith 1983, Johnson et al. 2006), although some authors have debated the use of this ratio (Ferdie & Fourqurean 2004, Walton et al. 2016). Plants growing in nutrient poor waters display significantly higher C:N and/or C:P ratios than those from nutrient rich conditions, provided they receive sufficient light (Atkinson & Smith 1983).

Stable isotope signatures of C and N in macrophyte tissues have been used to elucidate the sources and relative availability of C and N (Anderson & Fourqurean 2003, Lepoint et al. 2004, Campbell & Fourqurean 2009) as well as light condition in their habitats (Gorman et al. 2017). Generally, the ^{13}C values in seagrasses range from -23 ‰ to -3 ‰ (Anderson & Fourqurean 2003). This large variation can be ascribed to interspecific differences in discrimination against the heavier ^{13}C isotope during photosynthetic fixation of CO_2 (Lepoint et al. 2004, West et al. 2006) or variations in $\delta^{13}C$ of the available carbon sources (CO_2 and HCO_3^-) (Falkowski et al. 1989). Hence, the variation in foliar $\delta^{13}C$ also depends on light conditions and ambient temperature which influence the photosynthetic rate (Campbell & Fourqurean 2013, Fourqurean et al. 2015), or factors which affect HCO_3^- availability, such as salinity (Fry 2002). Plants that assimilate HCO_3^- for photosynthesis are more enriched in $\delta^{13}C$ compared to those that assimilate CO_2, because HCO_3^- has a less negative $\delta^{13}C$ than CO_2 (0 ‰ vs. -9 ‰) (Raven et al. 2002, Chappuis et al. 2017). Thus, submerged plants have enriched values of $\delta^{13}C$ (-16.7 ‰ to -10.5 ‰) in comparison to aquatic plants with leaves in contact with the atmosphere (-34.8 ‰ to -14.6 ‰) (Chappuis et al. 2017). The $\delta^{15}N$ values in macrophytes range from -5.2 ‰ to 20.1 ‰ (Chappuis et al. 2017), with tropical macrophyte genera including *Halodule* spp., *Halophila* spp. and *Syringodium* spp. being among the most depleted (Fourqurean et al. 2015, Walton et al. 2016). Foliar $\delta^{15}N$ is affected by N sources and plant acquisition mechanisms (Sun et al. 2011, Peipoch et al. 2014, Walton et al. 2016). The use of $\delta^{15}N$ in aquatic plants to distinguish between natural and sewage derived N is well documented (Risk et al. 2009). A high foliar $\delta^{15}N$ (above 5 ‰) in macrophytes indicates that the acquired N associated to human and animal waste inputs (Yamamuro et al. 2003, Connolly et al. 2013, Fourqurean et al. 2015). Instead, low foliar $\delta^{15}N$ values (~0 ‰) in macrophytes documents that N was derived from nitrate (fertilizer), from re-mineralised N from locally produced organic matter, or from N derived from atmospheric N_2 fixation (Peipoch et al. 2013, Fourqurean et al. 2015, Walton et al. 2016). Negative $\delta^{15}N$ values in plants can indicate that N was derived from nitrification (Yu et al. 2015) or to mycorrhizal associations (Hobbie et al. 2005). Hence, proportion of C and N isotopes in macrophytes can reflect the availability of essential resources and provide insight in their growth conditions (Campbell & Fourqurean 2009). However, knowledge about interspecific differences and the effects of environmental factors on isotope ratios is still limited for many seagrasses and macrophytes.

Although there are many studies that have evaluated variation in foliar nutrients of submerged macrophyte species such as *Halodule uninervis*, *Halophila ovalis* (Yamamuro et al. 2003, Campbell & Fourqurean 2009, Walton et al. 2016), there is still limited information about elemental and isotopic compositions of *Najas indica* and *Halophila beccarii* so far. Additionally, the effects of environmental factors in tropical lagoons on such differences are still limited. In this study, we examined interspecific variation in foliar nutrients (C, N, and P) and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of four macrophyte species in Cau Hai lagoon (Vietnam) in relation to key environmental variables (such as salinity, water depth or nutrient concentrations in water and sediment). This is the first attempt to characterize the sources of variation in elemental and isotope ratios of these submerged macrophyte species and discuss role of ecological and/or physiological characteristics in a tropical monsoon lagoon. We focused on the four macrophyte species *Najas indica* (Willd.) Cham., *Halophila beccarii* Aschers., *Halodule uninervis* (Forssk.) Boiss. and *Halophila ovalis* (R. Br.) Hook. f. which are common in Cau Hai lagoon, but internationally considered as threatened species (Short et al. 2011, Odelu et al. 2014). Although *N. indica* is generally classified as a freshwater macrophyte, it is quite abundant in the Cau Hai lagoon and usually forms mixed beds with *H. beccarii*.

MATERIAL AND METHODS

Study site

The Cau Hai lagoon (16°19'22"N 107°50'59"E) is the southern part of the large Tam Giang-Cau Hai lagoon system, located on the central coast of Vietnam (Fig. 1). It has an area of approximately 11 200 ha. Besides precipitation, there are three main sources of water flows joining into the lagoon: freshwater from Dai Giang and Truoi Rivers in the west of the lagoon, brackish water from the connected Thuy Tu lagoon in the north and saline water from the sea through the Tu Hien inlet in the east. The shallowest areas close to the lagoon edge are used for aquaculture practices (Disperati & Virdis 2015). The

area close to the river mouths and the adjacent lagoon are almost completely covered by shrimp farms, whereas the area close to the inlet contains some fish cages (small scale). In general, the strongest aquaculture activity is in the north-western region of the lagoon. Therefore, this region is considered to be a main source of nutrient enrichment for the Cau Hai lagoon (Nguyen & Yabe 2014, Disperati & Virdis 2015).

The benthic macrophyte communities in the Cau Hai lagoon are largely dominated by both freshwater macrophytes (*Najas indica*, *Vallisneria spiralis* L. and *Myriophyllum spicatum* L.) and seagrass species (*Zostera japonica* Asch. & Graebn., *Halophila beccarii*, *Halophila ovalis*, *Halodule pinifolia* (Miki) Hartog, *Halodule uninervis* and *Ruppia brevipedunculata* ShuoYu & Hartog) (Phan et al. 2018). *N. indica* and *H. beccarii* are the most dominant species (Phan et al. 2018). During the past decades, the area covered with macrophytes has declined by more than 50 % compared to that in 1990–2000 (Cao et al. 2012). Changes in environmental variables such as salinity, water depth and nutrient availability related to natural and anthropogenic disturbances, may have caused negative effects on macrophyte growth within the lagoon (Thanh et al. 2004, Le 2012, Phan et al. 2018).

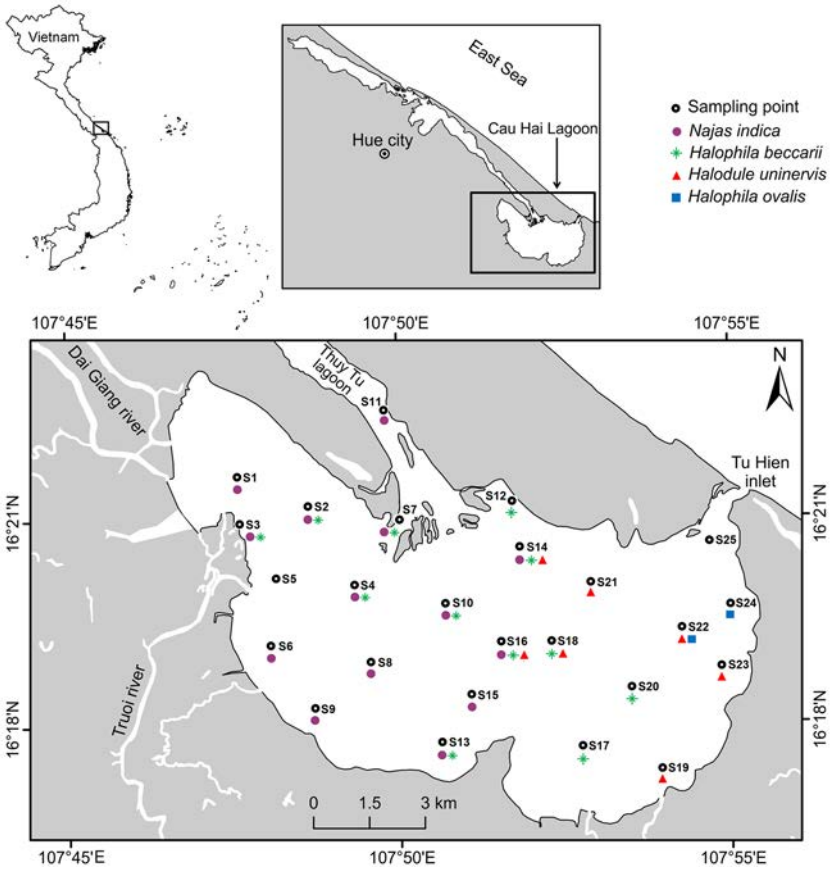

Figure 1 Location of sampling points for water, sediment and macrophytes in 2015 in the Cau Hai lagoon, central Vietnam. The black dots indicate coordinators of the 25 permanent sampling sites; the colour dots indicate the occurrence of the four macrophyte species in sampling events

Sampling and measurements

Samples of water, sediment and plants were collected simultaneously at 25 sites (located with Garmin GPS MAP® 78) in Cau Hai lagoon in March, May, July and September 2015 (Fig. 1). Water and sediment were sampled during all four events (n=100 for water and sediment samples; n=50 for chlorophyll a (Chl-a) which was only measured in the July and September), and macrophytes of the four focus species were collected when present (n=23 for *N. indica*; n=28 for *H. beccarii*; n=18 for *H. univervis*; n=8 for *H. ovalis*). Samples of water, sediment and macrophytes were taken together in four events and formed a gradient of environmental and foliar variables to interpreter correlation among foliar nutrients and environmental variables. All glassware and containers were cleaned with a 5 % H_2SO_4 solution and distilled water prior to use. All samples were placed in ice boxes after collection and transported to the laboratory. The details of sampling and measurements are described as follows.

Water. During each sampling event, *in-situ* measurements were done about 30 cm below the water surface for water temperature, pH, dissolved oxygen, turbidity and salinity, using a water quality monitoring unit with specific sensors (HORIBA U–5000, Horiba, Japan). Water depth was measured with a depth sounder (Hondex PS–7, Honda Electronics, Japan). Surface water samples were collected with a Van Dorn sampler at 50 cm below the surface. The water samples were transferred into glass bottles and transported to the lab in an icebox. Samples for Chl-a analysis were obtained by filtration on glass fibre filters (Whatman GF/F) and were stored in the dark at -20°C until analyses. Unfiltered water samples were stored in glass bottles and frozen until they were submitted to a digestion procedure for total N (TN) and total P (TP) analyses. Filtered samples were stored in glass bottles and frozen until they were analyzed for nitrate + nitrite (NO_X) and ammonium (NH_4^+) spectrophotometrically. All nutrient measurements followed standard protocols (APHA 1998). The dissolved inorganic nitrogen (DIN) was calculated by summation of NO_X–N and NH_4–N. Trophic state indices (TSI) of the water were calculated using equations described by Carlson (1977), Kratzer & Brezonik (1981) for Chl-a, TN and TP, respectively.

Sediment. At each site three replicate sediment cores were taken to a depth of 10 cm, using a sediment core sampler (4.5 cm diameter). The fresh sediments were stored in aluminium foil and transported refrigerated to the laboratory. Extractable N (NO_X^- and NH_4^+) and dissolved inorganic phosphorus (DIP) were determined by extracting fresh sediment with 2M KCl or 0.5M $NaHCO_3$, respectively (Estefan et al. 2014). Concentrations of NO_X^-, NH_4^+ and

DIP were measured spectrophotometrically. Fresh sediment samples were dried at 50°C for 48 h, ground and analysed for TN using an elemental analyser coupled to an isotope ratio mass spectrometer (EA–IRMS) as described below. TP was analysed using a modification of the combustion (500°C for 2 hours) and hot 1M HCl extraction procedure of Andersen (1976) according to Johengen (1997). Subsequently, phosphate was measured spectrophotometrically using a molybdate/ascorbic acid procedure (Murphy & Riley 1962) with an automatic segmented flow nutrient analyser (QuAAtro, Seal Analytical).

Macrophytes. Four focus species (*Najas indica*, *Halophila beccarii*, *Halodule uninervis* and *Halophila ovalis*) were collected at each site when present. The four macrophytes showed a distinctive spatial distribution in the Cau Hai lagoon (Fig. 1). Green leaves were scraped to remove epiphytes and then rinsed again using distilled water. Once dried (50°C for 48 h in the oven), leaf samples were ground and analysed for C, N, $\delta^{13}C$ and $\delta^{15}N$ values using an elemental analyser (Flash1120 series EA, Thermo) coupled to an isotope ratio mass spectrometer (DeltaV, Thermo). Analyses were calibrated against reference materials: IAEA–CH6 ($C_{12}H_{22}O_{11}$) and IAEA–N2 $(NH_4)_2SO_4$) for C and N respectively. Samples' isotopic ratios (R) are reported in the standard delta notation (δ) of the heavy to the light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$ in either sample or standard) as follows: δ (‰) = $[(R_{Sample}/R_{Standard})-1] \times 1000$ (West et al. 2006). TP was determined as described above for sediment samples. C:N:P elemental ratios of studied macrophytes were compared with the benthic macrophyte Redfield Ratio (SRR) of 550:30:1 (Atkinson & Smith 1983) to evaluate potential growth limiting factors.

Data analysis

One-way ANOVA and Tukey's post hoc test were used to examine which species were different from each other. Linear regressions were used – per species – to determine the (correlative) influence of water and sediment parameters on foliar nutrients concentrations or isotopic ratios. The effects of environmental variables (explanatory variables) on foliar nutrients of macrophytes (response variables) were visualised with redundancy analysis (RDA) using the vegan package in R (Oksanen et al. 2013). Multicollinear variables were discarded using the Variance Inflation Factor (VIF<4), if not already excluded by step-wise selection (Oksanen et al. 2013). For three of the studied plant species, datasets were large enough to calculate a RDA with foliar nutrients and isotopes as well as environmental variables; however, for *H. ovalis* the dataset was too limited. This had to be done per species because of interspecific differences in foliar nutrient and isotopes. To meet test assumptions of parametric statistics, data were log-transformed if needed. All analyses were carried out with R version 3.4.0 on R-Studio (Logan 2010, R Core Team 2017).

The geostatistical kriging technique was used to interpolate the spatial distribution of key water variables in the lagoon that have significant spatial variation (saliity, depth and DIN in the water). Kriging assumes that the distance or direction between sample points reflects a spatial correlation that can be used to predict unknown values for any geographic point data (Goovaerts 1997, Daya & Bejari 2015). The method with smaller prediction errors will be selected for the spatial interpolation. The model type, nugget, sill and range were modified to fit each of the variogram models by using ISATIS Software (Geovariances 2016).

RESULTS

Distribution of key environmental factors and macrophytes

Water and sediment variables showed strong variation in space or time (Table 1). There was a clear west-east gradient in salinity (Fig. 2A; Fig. 3A), as well as a north-south gradient in water depth (Fig. 2B). There were also spatial gradients in DIN concentrations in the water with higher values in the western part of the lagoon (Fig. 2C; Fig. 3C), whereas, the P concentrations did not show spatial variation (Table 1; Fig. 3D). Additionally, salinity, concentrations of N and P in the water and sediment varied in time (Table 1).

The four macrophytes showed a distinctive spatial distribution in the Cau Hai lagoon (Fig. 1, 3). *N. indica* and *H. beccarii* occurred in the western and central parts of the lagoon with lower salinity and higher DIN concentrations (Fig. 3A, C). Whereas, *H. uninervis* occurred in the central and eastern part and *H. ovalis* occurred only at two sites in the eastern part with higher salinity and lower DIN concentrations) (Fig. 3A, C). Furthermore, *H. uninervis* and *H. ovalis* distributed in the narrow range of salinity, water depth, DIN and DIP concentrations (Fig. 3).

Interspecific variation in macrophyte foliar C, N, P, $\delta^{13}C$ and $\delta^{15}N$

There were significant differences in foliar C, N and C:N among the four studied species (averaged over time and space), but not in foliar P, C:P or N:P (Fig. 4). *H. beccarii* had a relatively high foliar N (3.1±0.1 %) and a low foliar C (32.0±0.8 %) concentration and therefore a lower foliar C:N (12.7±0.5) than the three other species (Fig. 4D), whereas *H. ovalis* had the lowest foliar N concentration (2.3±0.1 %) (Fig. 4B).

The macrophyte species clearly differed in foliar $\delta^{13}C$, with most negative values for *N. indica* (-18.4±0.3 ‰) and *H. beccarii* (-17.3±0.5 ‰) and least negative for *H. ovalis* (-10.7±0.5 ‰) (Fig. 4G). Foliar $\delta^{15}N$ of *H. uninervis* (1.3±0.5 ‰) was significantly lower than that of the three other species (Fig. 4H).

Correlations among macrophyte foliar nutrients and environmental variables

The foliar N of *N. indica* and foliar C:N of *H. beccarii* were significant correlated to DIN concentrations in the water (Fig. 5A, B; 6A, C). Correlations of foliar P and C:P and DIP concentrations in the sediment were observed in *N. indica* (Fig. 5A, 6B). For *H. uninervis*, foliar P and C:P were correlated to water depth (Fig. 5C, 6D).

Foliar $\delta^{13}C$ values of *H. beccarii* and *H. uninervis* were positively correlated to salinity (Fig. 5B, C; 6E). Noteworthy, the difference in foliar $\delta^{13}C$ among studied species did not depend on salinity; i.e., the interaction between species and salinity was not significant (F = 1.0 and p = 0.39). A significant

Table 1. Summary statistics for water and sediment parameters (means ± S.E) and one-way ANOVA results for effects of sampling time and sampling site. The number of observations for each quantity=100; for Chl-a: n=50. The significant statistics are shown in bold. Levels of significance are as follows: *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Parameter	Min	Max	Mean ± S.E	ANOVA (F ratio)	
				Time	Site
Water quality parameters					
Temperature (°C)	26.2	35.2	31.38 ± 0.20	**170.99*** **	0.25
pH	5.4	11.6	8.52 ± 0.10	**9.35*** **	0.96
DO (mg/L)	3.8	11.2	7.22 ± 0.15	**18.46*** **	0.46
Salinity (‰)	0.2	29.9	13.65 ± 0.61	**15.96*** **	**4.64*** **
Turbidity (NTU)	0.0	50.8	4.93 ± 0.68	41.38	0.89
Water depth (m)	0.5	2.4	1.51 ± 0.42	0.50	**26.46*** **
NO_x–N (µg/L)	7.1	54.9	18.97 ± 0.81	**2.82***	1.18
NH_4–N (µg/L)	17.4	241.2	58.48 ± 3.81	**3.06***	0.82
DIN (µg/L)	31.4	269.7	77.45 ± 4.20	1.52	**3.83***
TN (µg/L)	114.2	770.6	403.77 ± 12.91	0.36	**9.67** **
TP (µg/L	7.0	132.8	29.07 ± 2.03	**7.89** **	0.95
TN:TP (Molar base)	8.6	79.5	39.51 ± 1.79	2.65	1.18
Chl-a (µg/L)	0.4	105.9	6.3 ± 2.1	1.90	**3.39***
TSI	31.3	79.0	44.1 ± 0.6	1.04	**4.96** **
Sediment quality parameters					
NO_x–N (mg/Kg)	0.3	19.2	1.96 ± 0.22	**14.57*** **	1.22
NH_4–N (mg/Kg)	4.3	30.7	15.46 ± 0.53	1.51	**3.85***
DIN (mg/Kg)	4.7	32.5	17.03 ± 0.53	0.56	**3.39***
PO_4–P (mg/Kg)	1.1	17.0	4.07 ± 0.26	**2.40***	0.87
DIN:DIP (Molar base)	1.2	33.2	12.69 ± 0.75	1.78*	1.74
TN (%dw)	0.5	2.9	1.53 ± 0.05	**1.87***	0.99
TP (g/Kg)	0.05	0.5	0.20 ± 0.01	**6.16***	0.88
TN:TP (Molar base)	7.4	63.1	23.02 ± 1.13	1.21	0.65

correlation between foliar $\delta^{15}N$ and environmental variables was only found for *N. indica*, foliar $\delta^{15}N$ was negatively correlated to salinity and positively correlated to DIN in water (Fig. 5A, 6H).

DISCUSSION

Variation in foliar C, N and P and their ratios

Foliar C and N concentrations differed among species, whereas P did not. Foliar C and C:N were relatively low in *H. beccarii*. This species might have less structural carbon (as indicated by depleted C:N and C:P ratios) than the other three species, as was also found for *Cymodocea nodosa* (Ucria) Asch. near the Balearic Islands (Fourqurean et al. 2007). Foliar N was low in *H. ovalis*, but this difference was likely a consequence of the limited spatial occurrence in the far eastern part of the lagoon where N concentrations in the water and sediment were relatively low, rather than an inherent difference between species. At two other sites in Australia and Fiji where *H. ovalis* co-occurred with one of the other species (*H. uninervis*), *H. ovalis* had a higher N concentration (Yamamuro et al. 2003), which supports that the lower N concentration of *H. ovalis* in the Cau Hai lagoon was likely not due to inherent interspecific differences and/or insignificant fluctuation of DIN concentration in its distributed areas (Fig. 3C). Noteworthy, numbers of replication was too low for statistical comparisons among species that occurred at the same sites.

Variation in foliar N and C:N was associated with variation in DIN concentration in the water, although only observed for *N. indica* and *H. beccarii*. This suggests that DIN concentration in the water column was an important source of N for these macrophyte species, particularly in the north western part of the lagoon. In this region, a relatively high DIN concentration in the water compared to plant demand might have led to excess uptake of DIN or 'luxury consumption' by the macrophytes, as observed in other studies (cf. Gordon et al. 1981, Stapel & Hemminga 1997, Ferdie & Fourqurean 2004, Apostolaki et al. 2011, Vizzini et al. 2004, Walton et al. 2016). Similarly, *Zostera*

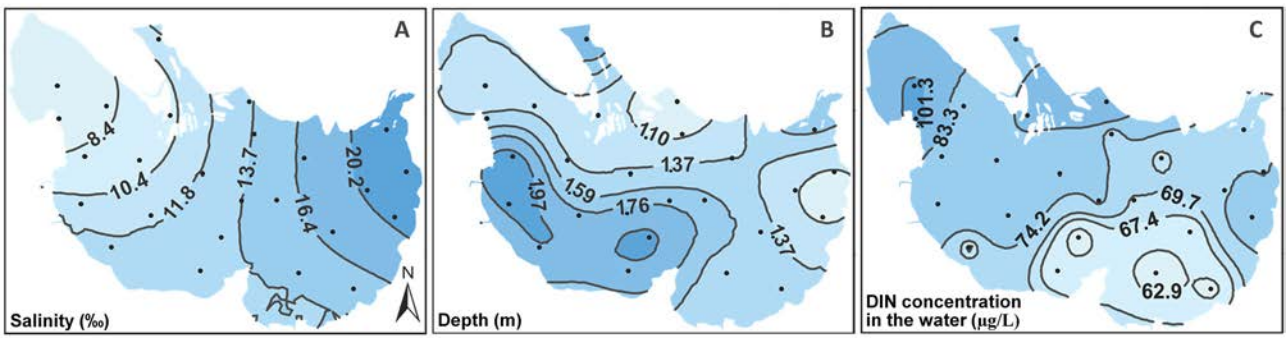

Figure 2 Spatial variation in mean salinity, depth, DIN concentration in the water collected between March and September 2015 in the Cau Hai lagoon. Black dots indicate locations of the 25 permanent sampling sites used for spatial interpolations. The darkness of the shading increases with the value of variables

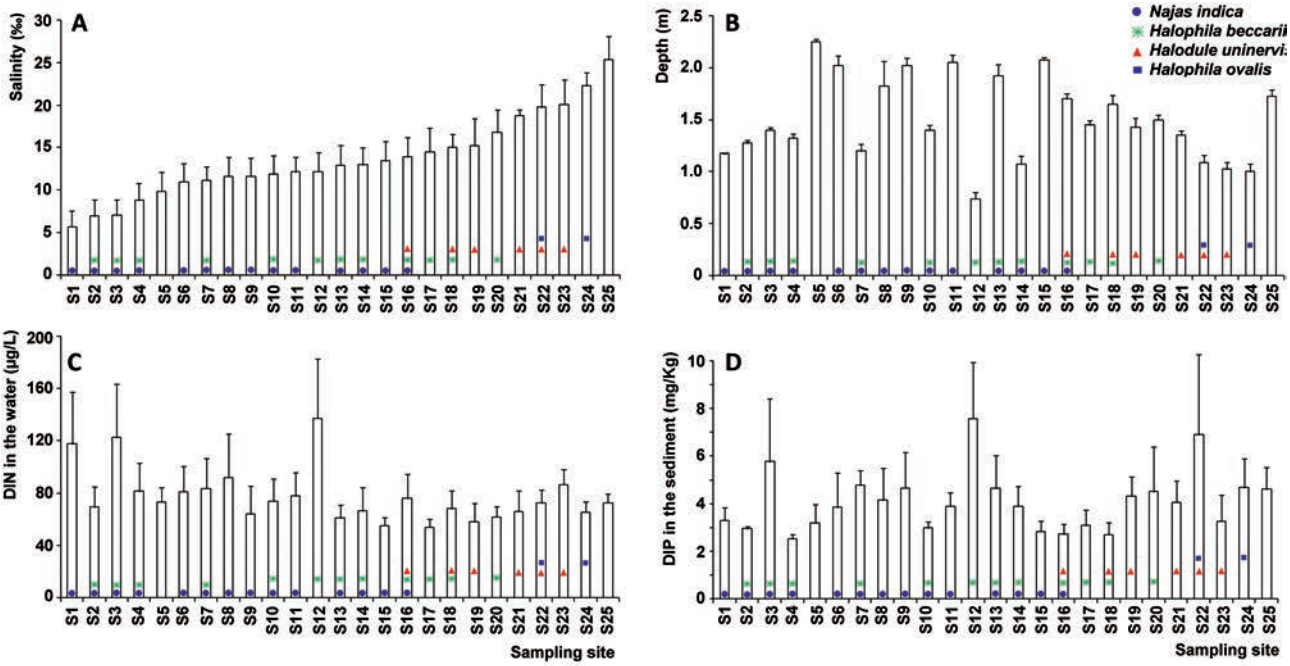

Figure 3 Distribution of key environmental variables and four macrophyte species at 25 sampling sites in Cau Hai lagoon. Bars charts indicate environmental variables: salinity (A), depth (B), DIN concentration in the water (C) and DIP concentration in the sediment (D). Error bars are the standard error of the means of environmental variables. Dots inside the bar charts indicate the presence of macrophyte species: *Najas indica* (Willd.) Cham. (n=23); *Halophila beccarii* Aschers. (n=28), *Halodule uninervis* (Forssk.) Boiss. (n=18) and *Halophila ovalis* (R. Br.) Hook. f. (n=8)

marina L. in Atlantic Canada showed an increasing foliar N concentration with N enrichment although its biomass declined due to shading (Schmidt et al. 2018). Variation in foliar P and C:P could not be ascribed to differences in P in the water, but for *N. indica* foliar P was positively correlated to variation in DIP in the sediment. This indicates that for this species P-acquisition by the roots from the sediment may be an important factor as observed for *Najas marina* in Myall Lake, Australia (Shilla et al. 2006). Foliar P of *H. uninervis* was not correlated to P in water or sediment, but it increased with increasing water depth. There is no good explanation for these correlations, at this point.

The average concentrations of C, N and P and their ratios in the macrophytes of the Cau Hai lagoon indicate that these plants likely experienced relatively high N and P availability. The average C:N (15) and C:P ratios (464) of all species were clearly below the Redfield Ratio for benthic macrophytes (C:N≈18, C:P≈550) (Atkinson & Smith 1983, Duarte 1990, Johnson et al. 2006). Foliar N and P concentrations in this study (2.3–3.1 % for N, 0.19–0.21 % for P) were in the range of the data reported in other coastal areas (1.6–3.1 % for N and 0.14–0.44 % for P; Yamamuro et al. 2003, Walton et al. 2016). Plants growing in excess nutrient conditions reflect this in high N and P concentrations compared to C (Atkinson & Smith 1983, Duarte 1990, Johnson et al. 2006). A moderate to high nutrient availability as indicated by the foliar nutrients in the plants agreed with trophic state indices in Cau Hai lagoon. Hence, it appears that another factor than P or N inhibited C-fixation and/or carbon storage of macrophytes (Burkholder et al. 2007).

Variation in foliar $\delta^{13}C$ and $\delta^{15}N$

On average, foliar $\delta^{13}C$ values differed strongly among the four macrophyte species of this study reflecting interspecific variation in bicarbonate acquisition mechanisms (Invers et al. 1999, Schwarz et al. 2000, Uku et al. 2005, Borum et al. 2016). The difference was also partly due to variation in the spatial distribution and salinity of the macrophyte species' habitats, although the difference among studied species did not depend on salinity (the species × salinity interaction was not significant). The observed interspecific trend in $\delta^{13}C$ (*H. ovalis* > *H. uninervis* > *H. beccarii* > *N. indica*) was consistent with the limited available information about $\delta^{13}C$ values of macrophytes from previous studies (Wetzel 1969 in Keeley & Sandquist 1992). Averaged per species, we observed the most negative values for *N. indica* (-18 ‰) which were in the range of previously observed freshwater macrophytes (-11 to -50 ‰; Wetzel 1969 in Keeley & Sandquist 1992, Chappuis et al. 2017). Moreover, Wetzel (1969) observed that the foliar $\delta^{13}C$ value of the closely related species *Najas flexilis* (Willd.) Rostk. & Schmidt was quite low (-22 ‰) because it did not take up HCO_3^- (Wetzel 1969 in Keeley & Sandquist 1992). This may also count for *N. indica* in the Cau Hai lagoon given its low foliar $\delta^{13}C$ value. Also, the relatively low $\delta^{13}C$ value of *H. beccarii* (17 ‰) in comparison to the other *Halophila* species is consistent with previous studies (McMillan et al. 1980 in Hemminga & Mateo 1996). Highest $\delta^{13}C$ values (-10.7 ‰) were observed in *H. ovalis* which occurred at the shallowest depth (Fig. 3B) and highest salinity suggesting that photosynthesis of this species may depend more on HCO_3^- as a carbon source (Borum et al. 2016) than the three other species. Foliar $\delta^{13}C$ of *H. beccarii* and *H. uninervis* in the Cau Hai lagoon increased with salinity, as was previously observed in other coastal waters (Fry 2002, Hitchcock et al. 2017). Generally, $\delta^{13}C$ in seagrasses ranges from -24 ‰ to -3 ‰ (Hemminga & Mateo 1996, Anderson & Fourqurean 2003), where the least negative values in marine primary producers indicate

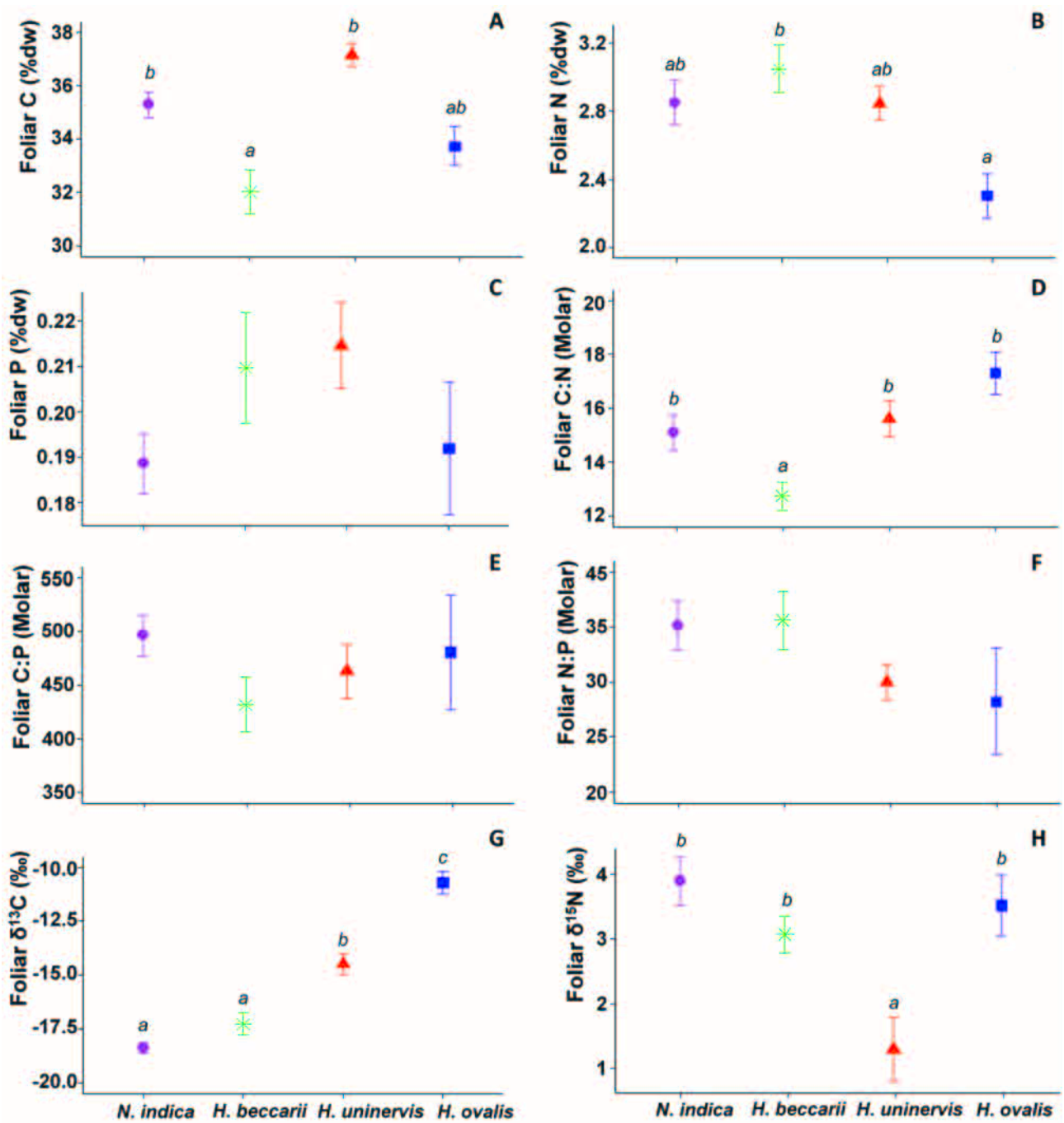

Figure 4 Mean foliar carbon, C (A) , nitrogen, N (B), phosphorus, P (C) concentrations, ratios of C:N (D), C:P (E) and N:P (F) as well as stable isotopes $\delta^{13}C$ (G) and $\delta^{15}N$ (H) of four macrophyte species collected in 2015 in the Cau Hai lagoon. Error bars are the standard error of the means. Significant differences are indicated by different letters at $p<0.05$ (Tukey's test after Anova Type II, taking into account the repetitive measurements). Freshwater species: *Najas indica* (Willd.) Cham. (●, purple, n=23); Seagrass species: *Halophila beccarii* Aschers. (*, green, n=28), *Halodule uninervis* (Forssk.) Boiss. (▲, red, n=18) and *Halophila ovalis* (R. Br.) Hook. f. (■, blue, n=8)

a relatively high assimilation of HCO_3^- instead of CO_2 (Hemminga & Mateo 1996) to adapt to marine life (Beer et al. 2002). These foliar $\delta^{13}C$ values were much lower than those observed in the saline habitats such as Shark Bay, Australia ($\delta^{13}C$ = -13.2 ‰ in *Halodule uninervis*, salinity > 42 ‰, Fraser et al. 2012) and Bermuda ($\delta^{13}C$ = -9.6 ‰ in *Halodule* sp., salinity > 36 ‰, Fourqurean et al. 2015). The positive correlation between foliar $\delta^{13}C$ and salinity may also reflect that the isotopic signature of dissolved inorganic C varies with salinity, from close to 0‰ in ocean waters to -10 ‰ to -15 ‰ in freshwaters (Mook 2000). Indeed, according to Henry's law, dissolution of CO_2 in water is a function of temperature and salinity. Under the high pH conditions in the brackish water of the Cau Hai lagoon (8.5±0.1), DIC may be comprised for more than 80 % of HCO_3^- (Michener & Lajtha 2007, Schmidt et al. 2012). Macrophytes species vary in their ability to use HCO_3^-. In Cau Hai lagoon, *H. beccarii* and *H. uninervis* appeared to acquire relatively high amounts of C from bicarbonate.

H. uninervis had a consistently lower foliar $\delta^{15}N$ value compared to the other three species in the Cau Hai lagoon, which supported previous observations for this species

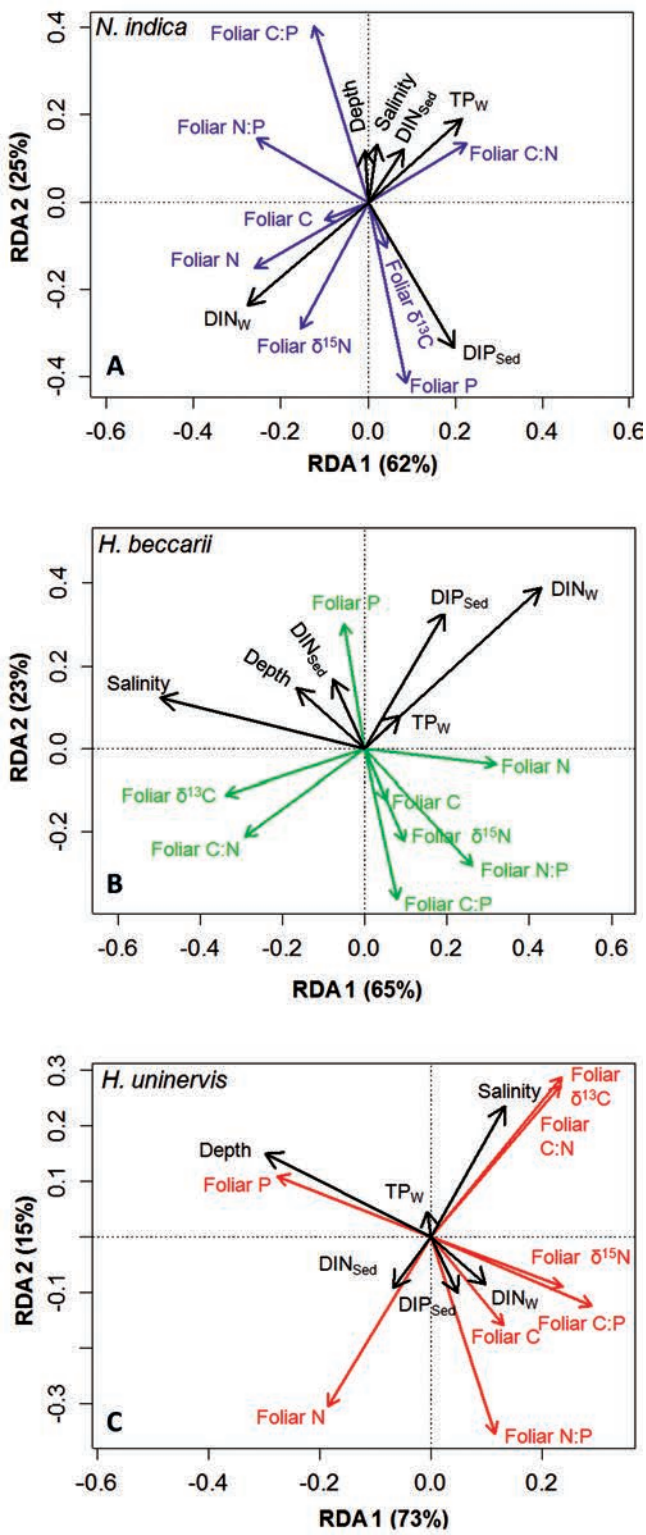

Figure 5 Biplots of the first two axes of the redundancy analysis showing the association of macrophyte occurrence (R^2 = 0.38, p = 0.02), foliar nutrients and environmental variables: *Najas indica* (Willd.) Cham. (R^2 = 0.52, p = 0.04), *H. beccarii* (R^2=0.39, p=0.011), *Halodule uninervis* (Forssk.) Boiss. (R^2 = 0.56, p = 0.012). Abbreviation: DIN_W, DIN_{Sed}: DIN concentrations in the water and sediment, respectively; TP_W: TP concentration in the water; DIP_{Sed}: DIP concentration in the sediment

in other regions (Yamamuro et al. 2003, Frase et al. 2012, Walton et al. 2016). This indicates that this species either differed from the other species in the proportions of N that it acquires from the various N sources (e.g. nitrate and ammonium in water or sediment), or that the relationship between the N demand and N availability was different for this species (Peipoch et al. 2014, Fourqurean et al. 2015, Walton et al. 2016). Although foliar $\delta^{15}N$ of *H. uninervis* was correlated differently to the measured environmental variables than that of *N. indica* (cf. temperature, pH; Table 2), we could not identify a different source of N for *H. uninervis*. With a lower demand for nitrogen, plants discriminate more against the heavier isotope, as was described for *Halodule uninervis* in Al Khor bay (Qatar) (Walton et al. 2016) and Moreton Bay or in an experiment in Green Island (Australia) with an excess supply of nitrogen (Udy & Dennison 1997). Generally, foliar $\delta^{15}N$ values of macrophytes growing in anthropogenically disturbed water are higher than those of macrophytes from undisturbed habitats (Fourqurean et al. 1997, Yamamuro et al. 2003, Lepoint et al. 2008, Herbeck et al. 2014, Hitchcock et al. 2017). In our study, foliar $\delta^{15}N$ values were highest in the north-western part of the lagoon as observed in foliar $\delta^{15}N$ of *N. indica* (3.9±0.8 ‰), where aquaculture activity is most intensive and where also DIN concentrations are at the highest. Foliar $\delta^{15}N$ of macrophytes in the Cau Hai lagoon where high compared to values from undisturbed waters, such as Dravuni (Fiji) and Green Island (Australia) (Yamamuro et al. 2003). This indicates that the Cau Hai lagoon suffers from N enrichment, at least in the north-western part as illustrated by the spatial patterns of DIN concentrations in the water (Fig. 2C). The lagoon on average would be classified as mesotrophic (ranging 40–50) according trophic state indices (TSI) of Carlson (1977), Kratzer and Brezonik (1981), but locally it could vary from oligotrophic (TSI<40) to eutrophic (TSI=50–70) (Table 1).

CONCLUSION

There was a considerable interspecific variation in foliar nutrient concentrations and isotopic composition in the studied macrophytes in the Cau Hai lagoon. This variation was associated to intrinsic interspecific differences (different N source of *H. uninervis*), natural variation in habitat conditions among species (salinity, depth), and anthropogenic influences (N enrichment). Variations in foliar N and P could be explained by the measured variation in environmental conditions only to a limited extent. Variations in foliar $\delta^{13}C$ and $\delta^{15}N$ among species were distinct and were likely related to variation in C and N acquisition and in DIN availability. The high nutrient concentrations in the studied macrophytes in Cau Hai lagoon indicates that possibly another factor was limiting growth of these plants such as excess nutrients affecting their nutrient uptake and growth rate (toxicity or inhibited C-fixation) or shading from algae (phytoplankton, macroalgae and epiphyte) (Whalen et al. 2013).

Competing Interests Statement: The authors declare no competing interests.

Author Contributions Statement: D.T.N.Y. and H.O.V. designed the study. D.T.N.Y. collected field samples, analysed water, sediment and macrophyte samples; N.B.D.V., P.C. and T.S. contributed to the chemical analyses. N.T.H

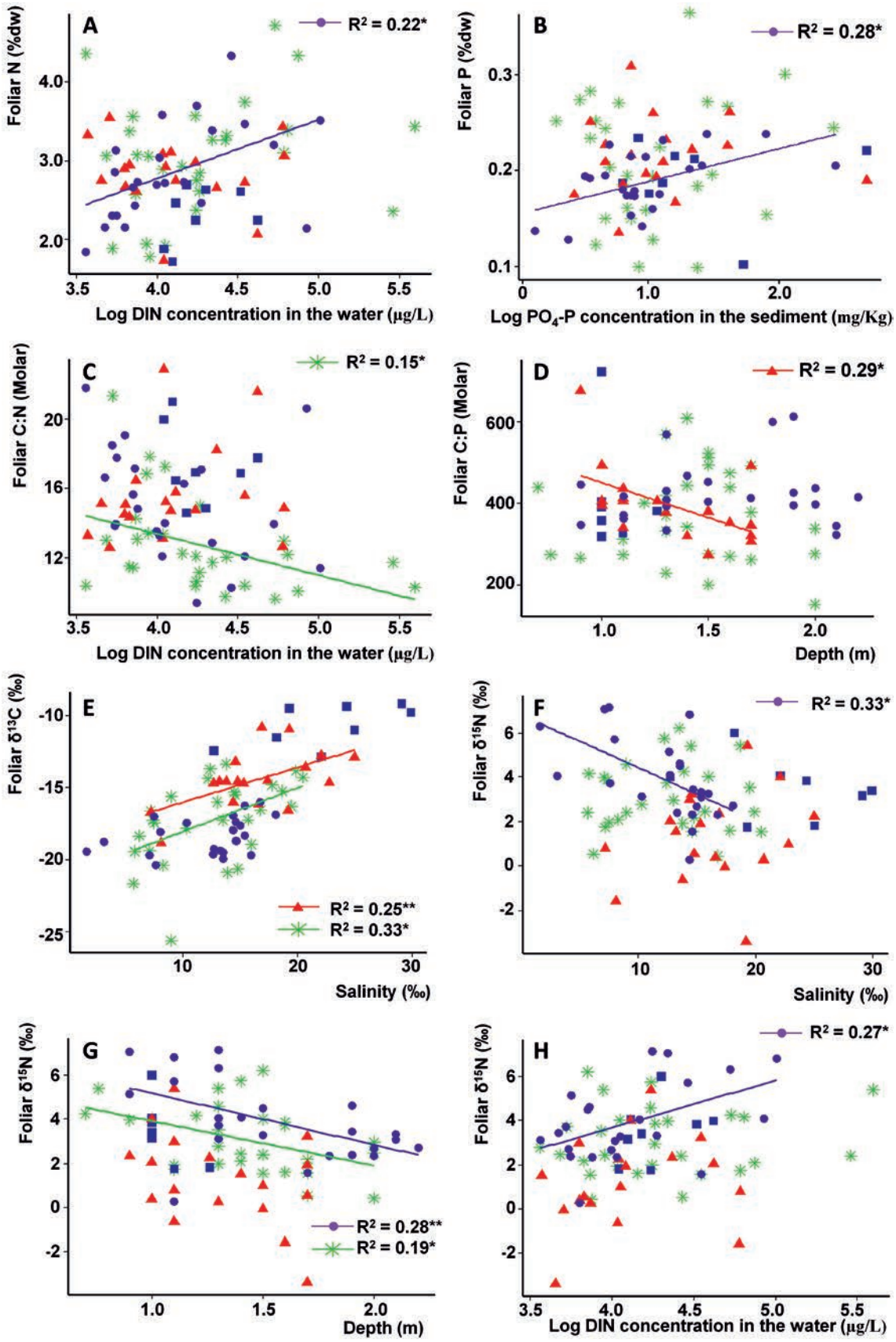

Figure 6 Correlations between foliar nutrients and environmental parameters in Cau Hai lagoon. Only significant regression lines are drawn. Levels of significance are as follows: *p<0.05; **p<0.01. Freshwater species: *Najas indica* (Willd.) Cham. (●, purple, n=23); Seagrass species: *Halophila beccarii* Aschers. (*, green, n=28), *Halodule uninervis* (Forssk.) Boiss. (▲, red, n=18) and *Halophila ovalis* (R. Br.) Hook. f. (■, blue, n=8)

Table 2. Pearson correlation coefficients (r) of plant, water and sediment quality parameters. Levels of significance are as follows: *$p<0.05$; **$p<0.01$. *N. indica:* n=23, *H. beccarii*: n=28, *H. uninervis*: n=18, *H. ovalis*: n=8

Plant parameters		Water parameters								Sediment parameters				
		Temp	pH	Salinity	Depth	DIN	TN	TP	TN:TP	DIN	DIP	TN	TP	TN:TP
Najas indica	**Foliar C**	.02	**.43***	-.38	-.25	.31	-.08	-.08	.07	.13	.12	-.05	.24	-.25
Halophila beccarii		.17	-.09	-.05	-.18	-.18	.17	.32	-.21	-.14	-.13	.03	.26	-.22
Halodule uninervis		-.33	-.20	-.25	-.12	.37	-.29	-.25	.18	-.03	-.07	.11	-.04	.14
Halophila ovalis		-.11	.26	-.21	-.37	.63	-.19	.07	-.13	.17	-.01	**-.81***	-.64	.47
Najas indica	**Foliar N**	.00	.11	-.22	-.12	**.49***	-.33	**-.48***	.40	-.02	.09	.05	-.36	.30
Halophila beccarii		-.09	-.07	-.31	-.34	.24	.15	.21	-.04	-.15	.05	.02	.07	-.03
Halodule uninervis		-.34	-.04	-.47	.35	-.12	-.20	-.24	.17	.11	.20	.40	.15	-.04
Halophila ovalis		-.63	-.02	-.15	-.50	.40	-.38	-.14	-.02	.47	.20	**-.78***	**-.71***	.58
Najas indica	**Foliar P**	.28	.12	-.18	-.21	.23	.19	-.16	.26	-.15	**.53****	-.14	-.10	-.06
Halophila beccarii		.14	-.10	.22	.04	-.06	.24	.13	.06	.03	.09	**-.42***	.04	-.36
Halodule uninervis		.03	.39	-.24	**.61****	-.27	-.05	-.19	.20	-.03	.02	**.47***	.25	-.10
Halophila ovalis		.18	-.08	.33	.07	-.06	.05	.09	-.08	.31	-.04	-.14	.27	-.54
Najas indica	**Foliar C:N**	-.06	.01	.07	-.01	-.38	.25	**.43***	-.38	.06	-.07	-.10	**.43***	-.39
Halophila beccarii		.22	-.04	**.40***	.25	**-.44***	-.06	-.01	-.13	.10	-.16	.00	.13	-.12
Halodule uninervis		.25	-.01	.41	-.38	.24	.12	.16	-.10	-.18	-.23	-.37	-.16	.07
Halophila ovalis		**.77***	.17	.16	.45	-.25	.47	.22	.01	-.50	-.30	.66	.64	-.54
Najas indica	**Foliar C:P**	-.28	.08	.01	.12	-.12	-.25	.15	-.26	.20	**-.49***	.12	.18	-.03
Halophila beccarii		-.13	.06	-.22	-.06	.01	-.19	-.03	-.13	-.13	-.12	.38	.01	.30
Halodule uninervis		-.18	-.46	.17	**-.60****	.32	-.04	.09	-.12	.01	-.06	-.36	-.25	.15
Halophila ovalis		-.32	.10	-.38	-.20	.20	-.14	-.12	.08	-.23	.09	-.11	-.47	.69
Najas indica	**Foliar N:P**	-.13	.04	-.05	.08	.23	-.36	-.25	.14	.08	-.26	.15	-.22	.29
Halophila beccarii		-.25	.07	**-.43***	-.20	.25	-.15	-.03	-.05	-.17	-.03	.36	-.06	.35
Halodule uninervis		-.39	**-.48***	-.16	-.32	.15	-.14	.03	-.05	.15	.13	-.08	-.14	.11
Halophila ovalis		-.58	.01	-.38	-.34	.26	-.31	-.19	.06	.01	.20	-.36	-.65	**.79****
Najas indica	**Foliar $\delta^{13}C$**	.15	-.34	.37	.32	.01	.20	.06	.00	.06	.06	-.04	.05	-.11
Halophila beccarii		-.20	.03	**.50****	.07	-.33	-.01	-.16	.13	-.11	-.27	-.15	-.09	.05
Halodule uninervis		**-.53***	**-.67****	**.57***	-.34	-.06	-.29	-.25	.14	-.38	.29	-.17	-.18	.13
Halophila ovalis		-.03	.08	.62	.05	-.14	.35	-.61	**.91****	-.10	.21	.55	.48	-.49
Najas indica	**Foliar $\delta^{15}N$**	.10	.16	**-.58***	**-.54****	**.48***	**-.42***	**-.50***	.38	-.16	.25	-.09	-.30	.21
Halophila beccarii		-.08	-.11	.02	**-.42***	.08	.03	-.09	.14	.03	.04	-.08	-.25	.22
Halodule uninervis		**-.55***	**-.51***	.23	-.42	.20	-.27	-.14	.03	-.24	.35	-.40	-.08	-.07
Halophila ovalis		-.33	.19	-.31	-.70	.38	-.01	.16	-.13	.28	-.09	-.41	**-.78***	.88

made maps of sampling sites and spatial distribution of key environmental variables. K.K. contributed to the evaluation of the variation in $\delta^{13}C$ and $\delta^{15}N$ of macrophytes. D.T.N.Y. and H.O.V. wrote the manuscript with contributions from all co-authors.

ACKNOWLEDGEMENTS

The study was funded by grants of the Belgian Government (VLIR–UOS) and the Graduate School of Global Environmental Studies (GSGES Seeds, Kyoto University, Japan). I am grateful to various students of the Faculty of Environmental Sciences of Hue University of Sciences (Vietnam) for their assistance with the field and lab activities. Stable isotope facilities were supported by grants from FWO Hercules (HERC46) and VUB SRP2: Tracing and modelling of present and ancient global changes.

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