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Spatial Variation in Elemental and Isotopic Compositions of Carbon and Nitrogen in a Coastal Lagoon Reveals Natural and Anthropogenic Influences

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ABSTRACT

Increased delivery of particulate organic matter (POM) to coastal lagoons has caused disturbance in nutrient cycling, water quality and aquatic biodiversity. Therefore, the major concern in coastal management is assessment of sources and pathways of POM in order to maintain ecosystem health. Elemental and isotopic of POM compositions are being increasingly used as useful markers for sources of POM in coastal ecosystems to obtain understanding of the nutrient sources and anthropogenic influences. In this study, variation in elemental and stable isotope compositions of carbon (C) and nitrogen (N) were measured in Cau Hai lagoon, Central Vietnam to characterize the possible sources of C and N. Variations in values of C/N ratios (8.17 ± 0.14) and δ^{13}C (-22.00‰ ± 0.13) indicated that POM was mainly of autochthonous origin due to high microalgae productivity. The values of δ^{15}N (3.83‰ ± 0.08) were likely inferred from potential input of POM from aquaculture practices. These results suggest that main sources of POM in the lagoon were derived from autochthonous microalgae and slightly anthropogenic impacts through aquaculture activities. Control POM load should be taken into account in maintaining water quality and biodiversity in the lagoon to inhibit microalgae biomass and shading due to nutrient enrichment.

Keywords: *Carbon, Nitrogen, C/N, δ^{13}C, δ^{15}N.*

Introduction

Coastal lagoons not only provide habitats for a wide range of aquatic species, but also contribute to micro–climate regulation, cultural recreational and commercial values (Gledhill and James 2012; Waajen *et al.*, 2014). Because most of these ecosystems are quite shallow, the influence of natural and anthropogenic disturbances is ranked among the greatest threats to ecosystem health and integrity worldwide (Davis and Koop, 2006; Waajen *et al.*, 2014). Increased delivery of POM to coastal lagoons has caused detrimental changes in habitat, food web structure and nutrient cycling (Bouillon *et al.*, 2012). Coastal lagoons receive POM from multiple sources, including terrestrial plants, soil, freshwater, estuarine and marine phytoplankton as well as domestic and industrial sewage. Because of their physiology and nutrient sources, different organisms exhibit different elemental and/or isotopic signatures. As a result, these signatures represent as tracers to quantify their contribution to the composition of POM.

Commonly used tools for distinguishing marine versus terrestrial POM are elemental ratios (Briggs *et al.*, 2013). The C/N ratios are considered as indicators of organic matter because terrestrial and marine plants have distinctive C/N ratios. The C/N has been widely used to distinguish between autochthonous and allochthonous origins of sedimentary organic matter and to infer the source of POM in aquatic environments. Stable isotope analysis is also an excellent tool to obtain important insights into sources of POM particularly carbon (C) and nitrogen (N) (Zeng *et al.*, 2010). Stable isotopes are measured as the ratio of the two most abundant isotopes for a given element, such as $^{13}C/^{12}C$ for carbon and $^{15}N/^{14}N$ for nitrogen. The $\delta^{13}C$ values in the main pools of global C cycle (atmosphere, terrestrial ecosystems and ocean) are significantly different. Thus, the $\delta^{13}C$ signature of POM is determined by the $\delta^{13}C$ values of carbon sources to the POM pool and fractionation that occurs during processes of the aquatic carbon cycle. Values of $\delta^{15}N$ in aquatic ecosystems vary according to sources of N and assimilation mechanisms (Fourqurean *et al.*, 1997; Lepoint *et al.*, 2004; Chappuis *et al.*, 2017). In general, the $\delta^{15}N$ signature ranges from approximately -5‰ to 20‰. Values of $\delta^{15}N$ around 0‰ in POM can indicate that the N was derived from artificial fertilizers or from (symbiotic) N_2–fixation (Yamamuro *et al.*, 2003; Derse *et al.*, 2007). Positive $\delta^{15}N$ values point to human and animal waste inputs (McClelland *et al.*, 1997; Savage and Elmgren, 2004). Negative $\delta^{15}N$ values can indicate that N is derived from nitrification (Yu *et al.*, 2015) or mycorrhizal associations (Hobbie *et al.*, 2005). Hence, studying spatial patterns of $\delta^{13}C$ and $\delta^{15}N$ values in POM can provide insight into the degree of C and N enrichment from natural or anthropogenic activities (Vizzini and Mazzola, 2008; Bannon and Roman, 2008).

The Cau Hai lagoon, located in Thua Thien Hue province, is a typical brackish water body in central Vietnam with a total surface area of 11,200 ha. It provides diverse habitats for a variety of flora and fauna. In recent decades, human activities such as aquaculture practices combined with a limited water exchange have caused a decline in the water and sediment quality of the lagoon, which likely lead to a loss of biodiversity (Le, 2012; Nguyen and Yabe, 2014; Disperati and Virdis, 2015). Development of low tide aquaculture ponds is considered a significant source of nutrient enrichment for the surrounding water and which might be an important threat to biodiversity in the Cau Hai lagoon. However, characteristics of POM (C, N) in the lagoon have not been investigated so far. Therefore, the goal of this study was to characterize elemental (C, N and C/N ratio) and stable isotope ($\delta^{13}C$, $\delta^{15}N$) compositions of POM in order to clarify sources of POM in the lagoon.

Materials and Methods

Study site

This study was conducted in Cau Hai lagoon (16.23°N and 107.97°E), a southern part of the Tam Giang–Cau Hai lagoon system which belongs to the Central coast of Vietnam. It is a semi–circle water body with an area of approximately 11,200 ha, with the water depth ranges within a few meters (0.5 to 2.4m, Nhu Y, 2019) and salinity ranges from 5.4 to 11.6‰. The Cau Hai lagoon is a typical brackish coastal lagoon, influenced by freshwater flow from inland rivers and the adjacent lagoon in the North and seawater flow from the East. Aquaculture practices concentrate on the shallowest areas close to the lagoon edge (Disperati and Virdis, 2015). Shrimp farms completely covered the area close to the river mouths and the adjacent lagoon, whereas fish cages occupy the area close to the inlet (small scale). In general, the strongest aquaculture activity is in the North–Western region of the lagoon. Moreover, the north western part of the lagoon is classified as eutrophic, the central and eastern parts mesotrophic and the water near the Tu Hiet inlet is oligotrophic (Nhu Y, 2019). This suggested that there is an increase of nutrient load in POM into the lagoon affecting nutrient status and water quality of the lagoon.

Sampling and measurements

The POM in the sediment samples were collected from 25 sites in the Cau Hai lagoon in March, May, July and September 2016 (Figure 1). Samples were taken to a depth of 10cm using a sediment core sampler (4.5cm diameter). After drying, sediment was homogenized and packed into silver cups. All samples were treated with HCl to remove inorganic carbon. The carbon and nitrogen stable isotope analysis was performed using the Continuous flow Stable Isotope Mass Spectrometer coupled to an Elemental Analyzer. The carbon results are corrected to three standards: IAEA–CH_6 (sugar) and the nitrogen results are corrected to IAEA–N_1 and IAEA–N_2. 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] × 1000 (West *et al.*, 2016). Water salinity was measured by the Water Quality Monitor (HORIBA U–5000).

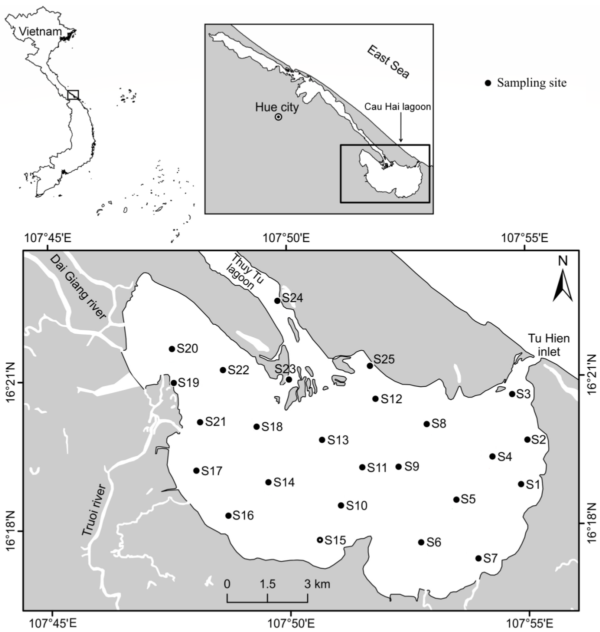

Fig. 1. Location of sampling sites in the Cau Hai lagoon. The black dots indicate coordinators of the 25 permanent sampling sites.

Statistical analysis

One–way ANOVAs were used to determine the effect of sampling sites on variation of C, N, C/N, δ^{13}C and δ^{15}N values. Correlations among variables were made using parametric tests (Pearson's) by the Performance Analytics package. All analyses were performed on R–studio (Crawley, 2007; Logan, 2010; R Core Team, 2018). The geostatistical kriging technique was used to interpolate the spatial distribution of studied variables by using ISATIS Software (Geovariances, 2016). 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 and Bejari, 2015).

Results and Discussion

Spatial distribution of C, N and C/N values

The values of C and N in POM samples collected from surface sediments displayed large variations as well as spatial pattern (Figure 2). The C values fluctuated between 0.26 and 2.24% while N values varied between 0.05 and 0.29%. On average, the contents of C and N indicated a similar distribution pattern with the highest C and N values observed on the West part of the lagoon (Figure 2). Similar to the data of C, the carbon to nitrogen ratio C/N also showed a wide range of variations from 3.59 to 11.06 and reflected spatial variation (Table 1).

In aquatic ecosystems, sources of POM are determined by the distinctive elemental ratios and isotopic composition of marine versus terrestrial matter (Yu *et al.*, 2010; Ramaswamy *et al*., 2008; Briggs *et al*., 2013). Generally, POM dominated by terrestrial sources contains less N relative to C and lighter δ^{13}C values than organic matter dominated by marine sources. Due to their greater protein content (~34%), microalgae have C/N values in the range 6–8, which is 2–20 times lower than terrestrial organic matter (Wetzel, 1983; Meyers, 1994). Seagrasses and seaweed (C/N ~15 to 50) often have elevated C/N ratios relative to microalgae because they contain higher levels of structural carbohydrates (lignin and cellulose; 8–10%) and slightly lower amounts of protein (10–15%) (Khan *et al.*, 2015). In the Cau Hai lagoon, mean C/N ratio in surface sediment (8.17 ± 0.14) are in the range of microalgae produced POM. Relatively higher C and N concentrations were found in the West part of lagoon indicating that organic matter may be predominantly of in–situ origin and/or terrestrial origin. The dominance of POM in

Table 1. Data statistics on measured variables of POM collected in surface sediment in Cau Hai lagoon and ANOVA results for effects of sampling time and sampling site. The number of observations for each quantity =100. Significant values are in bold and levels of significance are as follows: *$p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$.*

Variable	Mean ± SE	Min	Max	ANOVA *F ratio*
C (%)	1.09 ± 0.04	0.26	2.24	**9.41*****
N (%)	0.15 ± 0.004	0.05	0.29	**4.32***
C/N (Molar)	8.17 ± 0.14	3.59	11.06	**6.40*****
δ^{13}C (‰)	-22.00 ± 0.13	-25.32	-18.97	**23.89*****
δ^{15}N (‰)	3.83 ± 0.08	1.80	5.72	**6.88***
Salinity (‰)	8.52±0.10	5.41	11.64	**4.64*****

the West part of the lagoon is attributed to the existence of favourable conditions, such as high nutrients discharged from rivers, agriculture and aquaculture farms surrounding this area for production of estuarine phytoplankton (Nhu, 2019).

Previous study on elemental and stable isotopes of macrophytes in Cau Hai lagoon also found that the mean of C/N in macrophytes was 14.6 ± 0.36 suggesting that macrophytes contribute insignificantly to the source of organic matter in the lagoon (Nhu, 2019). These results also strongly imply that estuarine, marine microalgae and their detritus are the major source of POM in the lagoon. Furthermore, the negative correlation between salinity and C/N ($r = -0.59$, $p<0.001$) also reveals high in–situ production and increase of organic in the West part of lagoon. Hence, the POM of the sediment in the Cau Hai lagoon was likely derived primarily from algae rather than other aquatic plants or allochthonous sources.

Spatial distribution of $\delta^{13}C$ and $\delta^{15}N$ values

The $\delta^{13}C$ values of POM samples in the Cau Hai lagoon varied between -25.3‰ and -19.0‰ with the relatively high values measured at sites near sea inlet of the lagoon and low mostly at sites near the estuaries (Fig.2). The $\delta^{13}C$ values are commonly used to elucidate carbon source in coastal lagoon, such as freshwater phytoplankton (~ -31.5‰; Middelburg and Herman 2007), estuarine phytoplankton (~ -25.3‰; Sarma *et al.*, 2010) and marine phytoplankton (~ -23.2‰, Dehairs *et al.*, 2000). In this study, the $\delta^{13}C$ values were found in the range of -25.32‰ to -18.97‰ strongly suggested sources of POM in Cau Hai lagoon including the freshwater, estuarine and marine microalgae. In addition, aquaculture wastes are generally characterized by depletion of $\delta^{13}C$ values (Vizzini and Mazzola, 2004), hence the enrichment of $\delta^{13}C$ with salinity indicates the decreasing influence of POM from the West of the lagoon. Indeed, the more enriched $\delta^{13}C$ in the East of the lagoon may also indicate some degree of carbon limitation or a shift toward a carbon concentration mechanism whereby bicarbonate (HCO_3^-) is used as a carbon source by autochthonous microalgae because of salinity influences the relative uptake of dissolved CO_2 and HCO_3^- as was previously observed in other coastal waters (Unger *et al.*, 2013; Yu *et al.*, 2010). This result is inline with the data on $\delta^{13}C$ of macrophytes in the lagoon with the increased values towards the sea inlet (Nhu, 2019).

The $\delta^{15}N$ values in the Cau Hai lagoon fluctuated between 1.80 and 5.72‰ with statistical differences among sampling sites (Table 1, Figure 2). The relatively high values of $\delta^{15}N$ were observed at sites in the North part of the lagoon (Figure 2). The variation in $\delta^{15}N$ values of POM is commonly used to distinguish between natural and sewage derived N (Risk *et al.*, 2009). The $\delta^{15}N$ values related to nitrogen fixation process closing to 0‰, $\delta^{15}N$ related to agriculture and aquaculture ranging from 1 to 3 ‰ and from 4 to 11‰ respectively (Yamamuro *et al.*, 2003; Derse *et al.*, 2007). In this study, the range of $\delta^{15}N$ may be indicated for both agriculture and aquaculture discharge into the lagoon with the highest impact in the aquaculture area in the North-West of the lagoon as observed in macrophytes. Excreted nitrogen, mainly in the form of urea from aquaculture ponds, is hydrolysed to ammonia and converted into nitrate which are subsequently taken up by primary producers and enter the food web (Vizzini and Mazzola, 2004). Particularly in the sites with the highest $\delta^{15}N$ values occupied by many aquaculture ponds, which might indicate that these sites might be an important source of N enrichment in the lagoon. Other potential sources are run off from agricultural fields or sewage (Truong *et al.*, 2018), but in the first case $\delta^{15}N$ would be rather low instead of high, and in both cases the highest $\delta^{15}N$ values would be expected further up North-West of the lagoon.

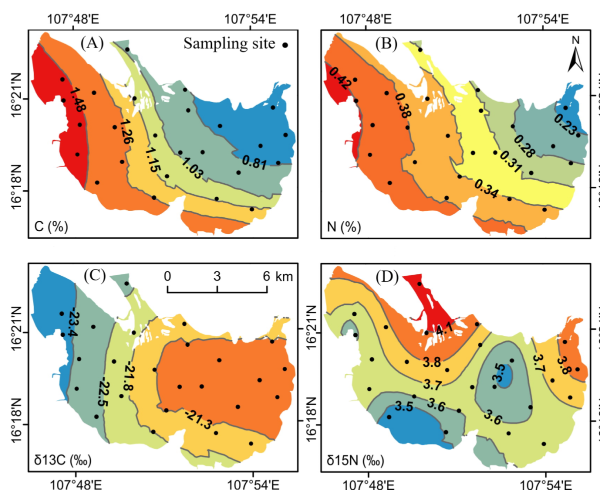

Fig. 2. Spatial variation in mean C, N, $\delta^{13}C$, $\delta^{15}N$ in sediment collected between March and September 2016 in Cau Hai lagoon. Blackdots indicate locations of the 25 permanent sampling sites used for spatial interpolations. The red colour of the shading increases with the value of variables.

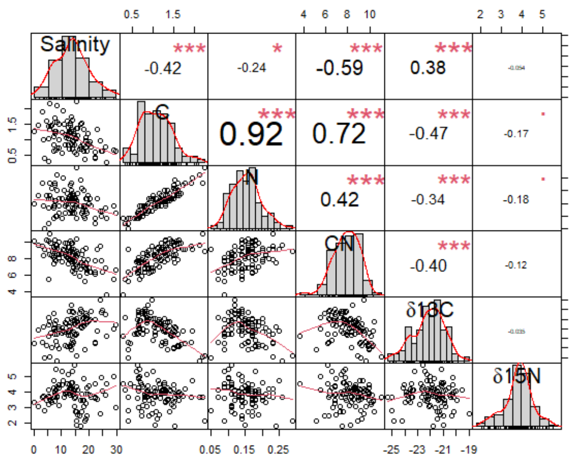

Fig. 3. Pearson correlation coefficients (r) of elemental (C, N, C/N) and isotopic ($\delta^{13}C$, $\delta^{15}N$) variables in the sediment as well as bottom water salinity. Levels of significance are as follows:*$p<0.05$; **$p<0.01$. n=100.

Conclusion

In general, the values of elemental and isotope of sediment in the lagoon showed a spatial pattern with the highest concentrations in the west and North–West part, which likely indicated anthropogenic enrichment from human activities in this part of the lagoon. The spatial pattern of C, N suggests that some organic matter might enter from the most western corner in the north. There was a trend of heavier $\delta^{13}C$ values with increasing salinity, microalgae appear to acquire relatively high amounts of C from bicarbonate and hence increase of $\delta^{13}C$ with salinity. Indeed, the highest $\delta^{15}N$ values were observed at sites near aquaculture ponds, which may indicate that these might be an important source of N enrichment in the lagoon. Lack of wastewater treatment facilities and environmental awareness which leads to discharge waste directly into water bodies are the main causes of water pollution in the Cau Hai lagoon. There is a need for increased investment in treatment plants for aquaculture ponds to avoid further nutrient enrichment and eutrophication in the lagoon as elsewhere.

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Conflict of Interest

The author declares no conflict of interest.

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