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Isotopic and Elemental Variations of Carbon and Nitrogen in a Mangrove Estuary

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Variations in elemental and isotopic ratios of suspended particulate matter (SPM) were investigated in the Guayas River Estuary Ecosystem (GREE) that empties into the Gulf of Guayaquil, Ecuador. Detritus in the system was identified on the basis of extremely high carbon:chlorophyll *a* ratios (>1000). This material had mean $\delta^{13}C$ of $-26{\cdot}4 \pm 0{\cdot}3$, $\delta^{15}N$ of $+4{\cdot}8 \pm 0{\cdot}2$, and $(C{:}N)_{atomic}$ of $14{\cdot}1 \pm 0{\cdot}9$. The isotopic data were comparable to measurements reported for fresh and degrading mangrove leaves, whereas the elemental ratio was comparatively enriched in nitrogen. Isotope measurements of SPM throughout the GREE were more similar to values for riverine material and detritus compared with that for the coastal end-member. Values indicative of *in situ* produced algae, sewage and shrimp pond effluent were only found at selected sites. Bacterial bioassays, which were used to document potential sources of dissolved organic matter in the GREE, were isotopically similar to SPM. This correspondence coupled with the relatively low $(C{:}N)_a$ of SPM could be explained by bacterial immobolization of nitrogen onto detritus. Finally, tidal variations of $(C{:}N)_a$ and $\delta^{13}C$ at a brackish mangrove site were similar in magnitude to spatial variations encountered throughout the GREE. Based on these results, the authors caution that care must be taken when samples are taken for food-web studies in these systems.

Introduction

The ' outwelling ' hypothesis that mangroves contribute significant quantities of carbon to coastal waters continues to be controversial in tropical coastal ecology (Odum & Heald, 1972; Odum, 1980; Robertson *et al.*, 1988; Twilley, 1988; Alongi, 1990). Results of studies testing this hypothesis are contradictory because different approaches and techniques have been used on only a few types of coastal systems in the tropics. In addition, these studies have focused on the mangrove contribution to carbon flow in coastal ecosystems, which

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may not necessarily reflect the contribution of mangroves to energy flow of coastal food-webs (Twilley, 1988). The former is based on mass balance of fluxes and accumulation of carbon, while the latter is tested using information on direct utilization of mangrove detritus by various trophic levels. Both approaches are limited by the inability to trace the transformations and fates of detritus from mangroves to complex energy and material cycles of coastal waters. Stable isotope ratios (e.g. D:H, ^{13}C:^{12}C, ^{15}N:^{14}N, ^{34}S:^{32}S) are uniquely suited to analyse the utilization of detritus from several sources in estuarine food webs (for general reviews see Fry & Sherr, 1984; Peterson & Fry, 1987). These techniques have been used to estimate the contribution of mangrove productivity to carbon accumulation in coastal sediments (Torgeson & Chivas, 1985) and to energy flow in coastal food webs (Rodelli *et al.*, 1984; Zieman *et al.*, 1984; Stoner & Zimmerman, 1988; Rezende *et al.*, 1990). Conflicting confusions about the extent of mangrove contribution, however, may have resulted from spatial and temporal variations and/or from a poor understanding of how isotope ratios change during organic matter degradation.

Recent work in temperate estuaries has documented significant spatial and temporal variations of isotopic ratios in suspended particulate organic matter, which is often dominated by algae (Mariotti *et al.*, 1984; Cifuentes *et al.*, 1988; Horrigan *et al.*, 1990; Fogel *et al.*, 1992). In some cases, these variations are influenced more by isotopic fractionation during algal fixation and degradation than by changes in the relative contributions of terrestrial and estuarine-derived organic matter (Fogel & Cifuentes, 1993). Isotope data reported for tropical estuaries also indicate spatial (Rodelli *et al.*, 1984) and temporal (Rezende *et al.*, 1990) variations, but measurements are limited and do not describe the mechanisms for reported variations. In addition, the issue of how microbial activity changes the isotopic ratio of organic matter during estuarine transport has not been addressed adequately. Thus, in order to constrain the field of potential substrates in a mangrove food-web study isotopically, and to arrive at a more accurate picture of the importance of mangrove detritus, one must first understand which factors influence their isotopic variations.

The temporal and spatial patterns in the availability of mangrove-derived organic matter should depend on a combination of ecological, geophysical and geomorphological characteristics of the estuary (Twilley, 1988). These factors control the levels of primary productivity and types of habitat within coastal waters. External factors such as river flow and tidal amplitude influence inputs of organic matter from uplands and intertidal wetlands. Daily and seasonal patterns in river flow and tides cause temporal variations that are superimposed on spatial variations of indigenous sources of organic matter. Thus, the relative contributions of *in situ* and allochthonous sources of detritus are specific to the environmental conditions of an estuary (Mann, 1975; Welsh *et al.*, 1982; Zieman *et al.*, 1984; Twilley, 1988).

This study was conducted in the Guayas River Estuary Ecosystem (GREE) that includes three tropical sub-estuaries: the Guayas River and Churute River estuaries that are influenced by discharge from the Guayas River; and the Salado Estuary, which is uncoupled from river flow, but is influenced heavily by urban and shrimp pond effluents. Spatial variations in elemental and isotopic ratios of suspended particulate matter (SPM) were investigated in all three sub-estuaries, and these parameters were sampled during a tidal cycle on the Churute River Estuary. In the following discussion, the spatial distributions and tidal variations of different organic sources in the region are considered. It is then demonstrated that the latter should be examined when sampling detrital organic matter for food-web studies.

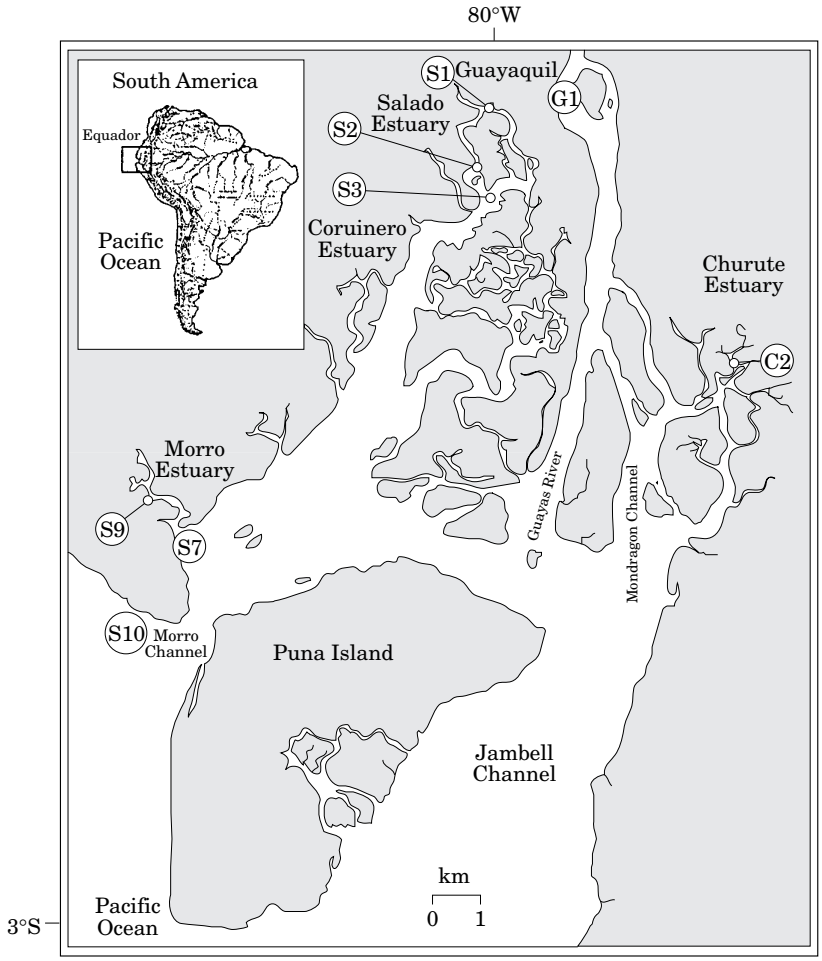

Figure 1. Map of the Guayas River Estuary Ecosystem (GREE), Ecuador. Station locations are indicated by their respective designations.

Study site

The Guayas River Estuary Ecosystem (GREE) is located in the southern coastal province of Ecuador (Figure 1), and, together with the Gulf of Guayaquil, is the largest estuarine ecosystem on the Pacific coast of South America (Cucalon, 1986). The Guayas River receives runoff from a watershed of 51 230 km^2 and has an average discharge of 1144 $m^3 s^{-1}$, which is the highest among the 30 rivers in the coastal zone of Ecuador. Mean precipitation in the Guayas River drainage basin north of Guayaquil is 885 mm $year^{-1}$, which may range from less than 400 to more than 1800 mm during any 1 year. Discharge is strongly seasonal ranging from 200 $m^3 s^{-1}$ in the dry season to 1600 $m^3 s^{-1}$ in the wet season during a year of average precipitation. Tides are semi-diurnal and are of equal amplitude (1·8 m) in the Gulf of Guayaquil, but are amplified to 3–5 m in the Guayas River Estuary near Guayaquil.

The GREE is a coastal margin linked to the Gulf of Guayaquil by three sub-estuaries: the Churute River, Guayas River, and Salado Estuaries. The intertidal zone of these

estuaries is inhabited by eight species of mangrove distributed along a relatively narrow zone, due to the high rates of evapotranspiration relative to precipitation that occur in the southern regions of Ecuador. Thus, the existence of the 121 464 ha of mangrove in the Guayas Province has been attributed to extensive river flow and high tidal amplitude (Schaeffer--Novelli, 1983). These mangrove resources have been heavily exploited recently for the development of shrimp mariculture that provided an export revenue of $383 million U.S. dollars for Ecuador in 1987 (Twilley *et al.*, 1993). Mangrove loss in the Guayas Province has been recorded at 1500 ha year^{-1} from 1984 to 1987 due to construction of shrimp ponds in the intertidal zone (CLIRSEN, 1991; Twilley *et al.*, 1996). Therefore, it is important to establish the role of these wetland ecosystems as a source of organic matter in these coastal waters.

The present study included stations at the river (G1, adjacent to Guayaquil) and gulf (S10) extremes of the Guayas River Estuary. The Salado Estuary was sampled in two tributaries, the Estero Morro (S7 and S9) and Estero Salado (S1, S2, S3). Intensive sampling was performed during a tidal cycle at one station in the Churute River Estuary (C2). Freshwater end-members were collected from stations in Babahoyo (RB) and Daule (RD) Rivers that join to form the Guayas River, about 5 km north of Guayaquil.

It was hypothesized that sources of organic matter would differ in each of the three sub-estuaries because of differing influences of wetland, river and urban inputs to each system. The Churute Estuary is relatively unaffected by urban wastes and is forested heavily by mangroves, due primarily to the existence of the Churute Ecological Preserve. Here, organic matter was characterized through a tidal cycle at one station (C2; see Figure 1), beginning with the ebb tide and ending during flooding tide. Samples were taken from 08.00 to 18.00 h at 30-min intervals.

In contrast, the watershed of the Salado Estuary has a higher level of human activity. In the Morro tributary, there is considerable deforestation of mangroves for the development of shrimp ponds that exchange effluent with estuarine waters. Shrimp ponds pump for nearly 12 h daily during high tide from the estuary, and this water, fertilized to enhance shrimp productivity, is returned to the estuary by canals (Twilley *et al.*, 1993*a*). To characterize and trace organic inputs from a shrimp pond, samples were taken in a commercial shrimp pond adjacent to Station S9 in Morro Tributary. The Salado Tributary, in the upper region of the Salado Estuary, is in an area that receives effluent from about 20% of the population of the city of Guayaquil (population >2 million) (Solorzano, 1989). These stations, along with the river and gulf stations, represent the diversity of inputs to the GREE.

Methods

Sample collection

Sampling took place between 24 and 27 May 1991.

Suspended particulate matter (SPM) for stable carbon and nitrogen isotope analyses was collected by filtering 5–10 l of water through a 4·7-cm GF/C filter (nominal pore size of about 1 μm). These were pumped onboard with a peristaltic pump and passed directly through the filter. Samples were taken below the surface to avoid including surface films. Filters were stored at −20 °C prior to analysis. Samples for elemental analysis were collected by filtering through 2·5-cm GF/C filters. Ten millilitres of the filtrate were sealed in glass ampules and frozen at −20 °C for dissolved organic carbon

measurements. The ampules had been pre-baked at 500 °C for 2 h. All glass-fibre filters were pre-baked at 480 °C for more than 2 h.

Acid-precipitated matter (APM) was recovered by precipitating about 5 l of the GF/C filtrate. After acidifying the filtrate to pH 2, the precipitate was recovered on a GF/C filter. The usual designation for this portion of the organic pool is humic acid (e.g. Fox, 1983). Instead, the present authors have chosen to refer to this pool as APM to indicate that other types of dissolved organic matter (DOM), such as proteins and colloids, may also precipitate out of solution at low pH (Thurman, 1985).

Bacterial assays were conducted at selected stations (see Coffin *et al.*, 1989; Coffin & Cifuentes, 1993). First, up to 18 l of sample were filtered through a 0·2-μm cartridge filter and placed in a cubitaner. The sample was then inoculated with a 1-μm filtrate of the same water and incubated in the dark for 48 h. The sample was filtered through a 4·7 GF/F filter to concentrate bacteria for isotopic analysis. This filter was frozen immediately and stored at − 20 °C.

Isotopic analyses

Filters containing SPM, APM and bacteria were dried at 60 °C in an oven flushed continually with high-purity N_2 gas. Filters with SPM were placed in glass petri dishes in a glass desiccator with concentrated HCl fumes to remove carbonates. These filters were subsequently placed in the 60 °C oven again to remove HCl without loss of labile nitrogen. When completely dry, the filters were ground carefully with a mortar and pestle.

The ground filters and plant samples were analysed isotopically by a modified Dumas combustion that converts organic carbon and organic nitrogen to CO_2 and N_2 for mass spectral analysis (Macko, 1981). Samples were placed in quartz tubes with Cu and CuO, evacuated and sealed. The quartz tubes were then heated to 850 °C at a rate of 450 °C h^{-1} kept at 850 °C for 2 h, and cooled to room temperature at a rate of 60 °C h^{-1}. The slow cooling cycle ensured that any oxides of nitrogen were decomposed to N_2. The CO_2 gas was separated from N_2 gas by cryogenic distillation. The N_2 gas was then analysed on a Nuclide 3-60-RMS isotope ratio mass spectrometer. In turn, CO_2 gas was analysed on a Finnigan MAT 251 isotope ratio mass spectrometer. The results are presented in standard notation:

$$\delta^h X = \left[\frac{\left(\frac{^hX}{^lX} \right)_{\mathrm{SAM}}}{\left(\frac{^hX}{^lX} \right)_{\mathrm{STD}}} - 1 \right] \times 1000$$

where X is either carbon or nitrogen, h is the heavier isotope, l is the lighter isotope, SAM is the sample, and STD is the standard. The standard for nitrogen was atmospheric N_2 and the standard for carbon was Pee Dee Belemnite. For $\delta^{15}N$, the precision was ± 0·3‰ for particulate samples, ± 0·5‰ for NH_4^+, and ± 0·8‰ for NO_3^-. The precision of the $\delta^{13}C$ measurement was ± 0·2‰.

Other analyses

Organic carbon and nitrogen concentrations of SPM, APM and bacterial concentrates were measured on a Carlo-Erba CNS analyser. Filters containing the particulate

TABLE 1. Suspended particle matter (SPM) concentration, particulate carbon (PC), organic carbon content (OC), particulate nitrogen (PN), organic nitrogen content (ON), chlorophyll *a* (chl *a*), bacterial abundance (AODC), atomic carbon:nitrogen ratio [$(C:N)_a$], and $\delta^{13}C$ and $\delta^{15}N$ of organic matter for riverine, estuarine and coastal end-members (values for organic matter collected from a commercial shrimp pond are also included)

Station	Sample date	SPM ($mg\,l^{-1}$)	PC–OC ($mg\,l^{-1}$)–(%)	PN–ON ($mg\,l^{-1}$)–(%)	chl *a* ($\mu g\,l^{-1}$)	AODC ($10^9\,l^{-1}$)	$(C:N)_a$	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
River Babahoyo	5/24	–	0·69	0·07	–	3·4	12·0	−27·4	4·8
River Daule	5/24	–	1·94	0·24	–	5·6	9·6	−26·3	6·0
River Guayas[a]	5/27	696	4·29–0·6	0·50–<0·1	–	4·5	9·9	−24·3	3·0
Salado Estuary[b]	5/27	101	2·43–2·4	0·17–0·2	1·35	2·5	14·9	−25·7	5·5
Morro Estuary[c]	5/26	57·1	1·83–4·5	0·20–0·5	15·0	4·8	11·0	−21·5	6·2
Gulf[d]	5/26	60·5	0·58–1·0	0·07–0·1	5·53	3·1	10·3	−21·6	6·1
Shrimp pond[e]	5/26	42·6	5·58–13·1	0·68–1·6	14·1	30·0	9·6	−22·7	0·9

[a]Station G1; [b]Stations S1, S2 and S3; [c]Stations S7 and S9; [d]Station S10; [e]Adjacent to Station S9.
See Figure 1 for locations of stations.
The data for Salado and Morro Estuaries are averages of two and three stations, respectively.

material were thawed and dried in a vacuum oven at 60 °C. Dissolved organic carbon was measured by high-temperature combustion at 680 °C in the presence of Pt catalyst with a Shimadzu TOC-5000 analyser. To remove dissolved inorganic carbon, 25 µl of Ultrex HCl was added to the samples followed by purging for 5 min with high-purity N_2 gas. Salinity, suspended particulate matter (SPM), dissolved oxygen and chlorophyll (fluorometric method) were measured by standard methods (Parsons *et al.*, 1985). Bacterioplankton were counted by epifluorescence microscopy with the acridine orange direct count technique (Hobbie *et al.*, 1977). Finally, particulate organic carbon was divided by the SPM to give an estimate of organic carbon content. A similar calculation was made for organic nitrogen content. This calculation does include a small error because the particulates for elemental analyses were isolated with GF/C filters (nominal pore size of 1·2 µm), and the seston analyses were performed with material retained on 0·45-µm Nuclepore filters.

Results

Rivers and sub-estuaries of GREE

Salinity was measurable in the Guayas River (0·3) but waters were fresh in the Daule and Babahoyo Rivers. In contrast, salinities in the Salado Estuary were much higher (23·4–23·9) owing to high tidal energy and restricted river flow into this region. Salinities in the Morro Estuary were also high, ranging between 26·0 and 28·8. Although Station S10 in the Gulf of Guayaquil was the most coastal station, salinity at this site was only 26·3. The highest salinity (32·7) was measured in the shrimp pond, adjacent to Station S9 in the Morro Estuary, suggesting that significant evaporation occurred at this site (Twilley *et al.*, 1996).

Biomass, suspended particle load and organic content (Table 1): No pigment data (chl *a*) were taken at the river stations. The highest chl *a* values were measured in the

Morro estuary (15·0 $\mu g\,l^{-1}$) and at the shrimp pond station (14·1 $\mu g\,l^{-1}$). These were considerably higher than those from Station S10 and the Salado Estuary, 5·53 and 1·35 $\mu g\,l^{-1}$, respectively. Bacterial abundance at the river and sub-estuaries was generally high, ranging from 2·5 and $5{\cdot}6 \times 10^9$ cells l^{-1}. The bacterial abundance at the shrimp pond, 30×10 cells l^{-1}, coupled with its high chl *a* content confirmed that this was a highly organic-rich system.

The suspended particulate load (SPM) in the Guayas River, 696 mg l^{-1}, was the highest measured in this study. With the exception of the shrimp pond, both particulate carbon (PC) and particulate nitrogen (PN) concentrations were also higher in Guayas River compared to the other sites. Owing to the high SPM, however, carbon (%OC) and nitrogen (%ON) content was the lowest, comparatively. The Salado Estuary had twice the SPM compared with the Morro Estuary, the coastal station (S10) and the shrimp pond. The latter two sites had similar SPM. In turn, the Salado Estuary had lower chl *a*, bacterial abundance and PC concentrations. Finally, PC and PN concentrations were much higher at the shrimp pond than at the Morro Estuary, which, in turn, was higher than at Station S10.

$\delta^{13}C$ and $\delta^{15}N$ (Table 1): The $\delta^{13}C$ of SPM in Guayas River, $-24{\cdot}3‰$, was more positive than at the Babahoyo and Daule Rivers, $-27{\cdot}4‰$ and $-26{\cdot}3‰$, respectively. In contrast, the $\delta^{15}N$ of SPM in the Guayas River, $+3{\cdot}0‰$, was lighter compared with the Babahoyo ($+4{\cdot}8‰$) and Daule Rivers ($+6{\cdot}0‰$). Also, the $\delta^{13}C$ of SPM in the Salado Estuary, $-25{\cdot}7‰$, resembled values measured in the rivers, but was more negative than values observed at the other sites, which ranged from $-21{\cdot}5$ to $-22{\cdot}7‰$. The $\delta^{15}N$ of SPM were similar in the sub-estuaries and at Station S10, whereas the lowest value, 0·9‰, was measured in the shrimp pond.

The Churute Estuary: tidal cycle study

Salinity during the tidal cycle ranged from 5·7 at the beginning of the study (ebb tide) to 4·6 by low slack tide, and remained constant for a period of 4 h [Figure 2(a)]. Prior to flood tide, salinity dropped sharply to a minimum of 4·0 at 15.00 h (all times are local time). Thereafter, salinity increased steadily to a value of 5·3.

Chlorophyll *a* (chl *a*) variations were generally the mirror image of the salinity changes [Figure 2(a)]. The chl *a* concentration was relatively uniform (2·53–3·16 $\mu g\,l^{-1}$) during ebb tide, but then increased to values in the range of 4·46–5·38 $\mu g\,l^{-1}$ in the period of low slack tide. There were large fluctuations in chl *a* during the period when salinity decreased prior to flood tide. At that time, the maximum chl *a* value of 11·07 $\mu g\,l^{-1}$ was measured. Finally, chl *a* decreased steadily during flood tide and reached values similar to those measured at the beginning of the study.

Suspended particulate matter (SPM) fluctuations exhibited distinct minima and maxima that appeared to be related to changing currents [Figure 2(b)]. The minimum value, 46·85 mg l^{-1}, was observed at the end of low slack tide. The highest SPM, 153·5 mg l^{-1}, was measured during flood tide.

Dissolved O_2 (DO) variations were opposite to those of SPM [Figure 2(b)]. Through the tidal cycle, DO varied from 2·27 to 3·26 ml l^{-1} with the lowest value observed at the beginning of low slack tide. During low slack tide, DO increased steadily reaching a maximum at 14.00 h, which corresponded to a maximum in chl *a*. During flood tide, DO dropped sharply reaching consistent values between 2·45 and 2·51 ml l^{-1} at the end of the study.

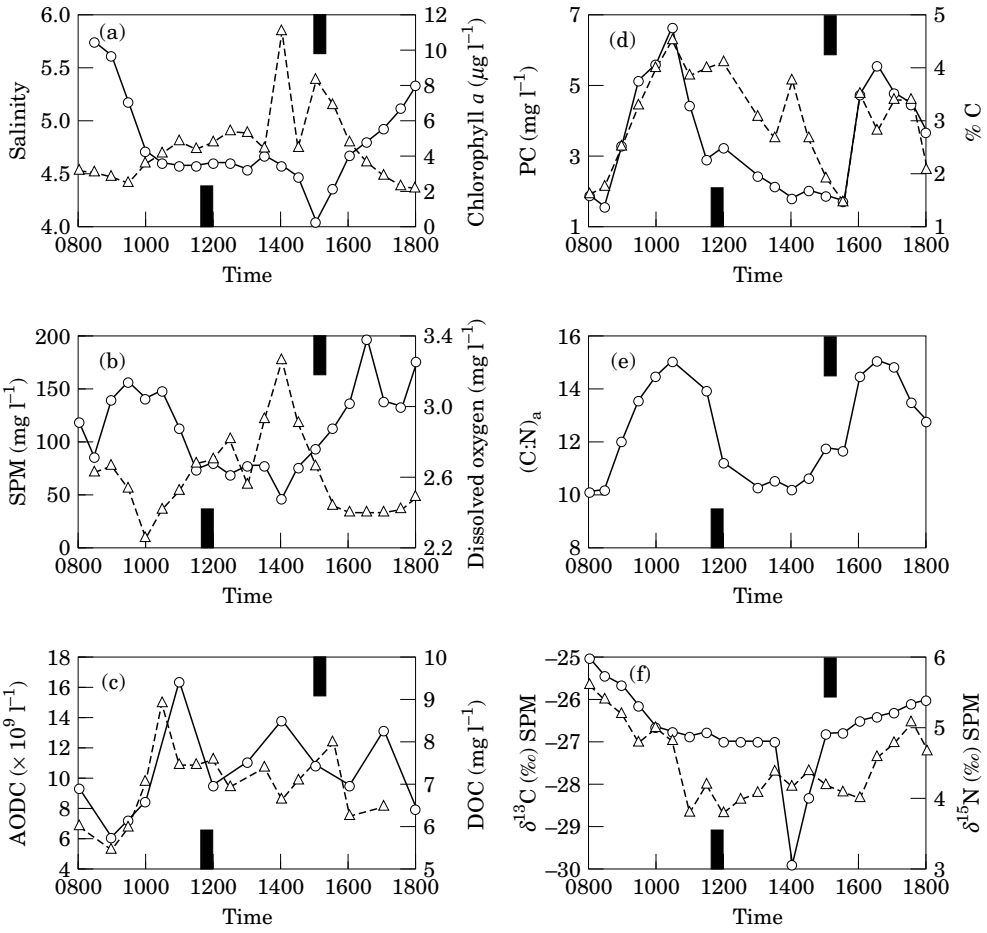

Figure 2. (a) Salinity and chlorophyll *a* (chl *a*), (b) Suspended particulate matter (SPM) and dissolved O_2, (c) Bacterial abundance (AODC) and dissolved organic carbon (DOC), (d) Particulate organic carbon (PC) and organic carbon content (%C), (e) Atomic carbon:nitrogen ratio [$(C:N)_a$], and (f) Stable carbon isotope ratio ($\delta^{13}C$) and stable nitrogen isotope ratio ($\delta^{15}N$) *vs.* time during the tidal cycle at Station C2 (refer to Figure 1). ○, left-hand parameter; △, right-hand parameter. The lower bar indicates slack tide and the upper bar designates the beginning of flood tide.

Bacterial abundance (AODC) remained elevated during periods of high tidal energy (ebb and flood waters) and the slack ebb tide [Figure 2(c)]. Values for AODC ranged from 6·0 to 14 × 10^9 cells l^{-1}.

Dissolved organic carbon (DOC) was 5·98 mg l^{-1} at the start of the study and increased to a maximum of 8·91 mg l^{-1} through the flood tide [Figure 2(c)]. Values decreased slightly by slack tide and increased again during flood tide. Throughout the later part of the tidal cycle, DOC values were variable and trends were not evident.

Particulate carbon (PC) had maxima at the end of ebb tide and during the flood tide [Figure 2(d)]. Values for PC ranged from 1·50 to 6·62 mg l^{-1}. The lower concentrations corresponded with low slack tide.

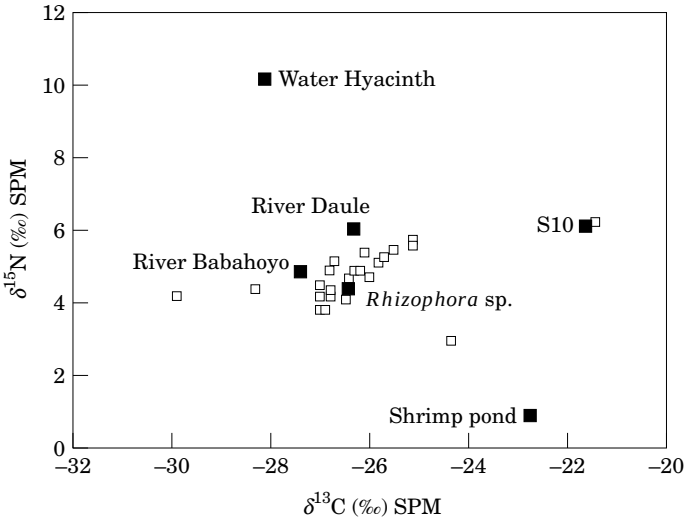

Figure 3. Stable nitrogen isotope ratio (δ^{15}N) *vs*. stable carbon isotope ratio (δ^{13}C) of suspended particulate matter (SPM) (□) in the Guayas River Estuary system. Included (■) are values for *Rhizophora* sp. and water hyacinth leaves, and SPM from the Daule and Babahoyo Rivers, Station S10 in the Gulf of Gauyaquil, and a shrimp pond.

Carbon content (%OC) maxima coincided with those in PC [Figure 2(d)]. The %OC, however, did not decrease as rapidly as PC during low slack tide.

Atomic carbon:nitrogen ratios $(C:N)_a$ peaks corresponded with peaks in PC [Figure 2(e)]. Values of $(C:N)_a$ ranged from 10 to 15.

Stable carbon isotope ratios (δ^{13}C) varied by about 2‰ with the exception of a sharp decrease [Figure 2(f)], which corresponded with a minimum in SPM [Figure 2(b)], and distinct maxima in dissolved O_2, chl *a* and %OC [Figure 2(a–d)].

Stable nitrogen isotope ratios (δ^{15}N) of SPM behaved similarly to δ^{13}C of SPM with the exception of the sharp drop in δ^{13}C observed at 2 pm [Figure 2(f)]. The highest δ^{15}N, +5·6, occurred at the start of the tidal cycle. Values dropped to a low of +4·0‰ at low slack tide, followed by relatively uniform values until flood tide, when δ^{15}N values increased.

Discussion

Isotopic source characterization

The Guayas River Estuary Ecosystem (GREE) is visually dominated by fringing mangrove stands of *Rhizophora mangle* and *R. harisonii*. Massive rafts of water hyacinths float downstream occasionally from the Guayas River. The extent to which intertidal and upland sources of organic matter mix with *in situ* produced material can be demonstrated by plotting δ^{15}N against δ^{13}C SPM data (Figure 3). Here, freshwater (Stations RD and RB) and coastal end-members (Station S10) are emphasized along with data for the dominant mangrove species, *Rhizophora*, water hyacinth and shrimp pond effluent. Most SPM data were within the mixing domain that included organic matter from coastal waters (Station S10), rivers, and *Rhizophora*. With the exception of samples from the Morro Estuary, the data clustered within the riverine SPM and intertidal mixing

members, suggesting that allochthonous sources dominated organic matter in the GREE system. Based on these isotope results, neither water hyacinths nor effluent from shrimp ponds contributed significantly to the particulate material of GREE. Only Station G1 on the Guayas River had isotopic ratios that implicated contributions from shrimp pond organic matter. This station, however, was located near the city of Guayaquil, far from the influence of any shrimp ponds. Guayaquil has a population of 2 million people with little sewage treatment facilities, and, thus, the shift in both carbon and nitrogen isotope ratios could be explained instead by sewage inputs (see Rau *et al.*, 1981; Requejo *et al.*, 1986). Possible sources of the extremely negative $\delta^{13}C$ that were dissimilar from any end-members used in this study are discussed below.

Elemental source characterization

The $(C:N)_a$ is often indicative of the predominant source of organic matter in a system. Phytoplankton $(C:N)_a$ range from 7·7 to 10·1 (Holligan *et al.*, 1984). Bacterioplankton are nitrogen-rich and have $(C:N)_a$ from 2·6 to 4·3 (Lee & Fuhrman, 1987). In contrast, vascular plant material can have significantly higher $(C:N)_a$ (>50; e.g. Hedges & Mann, 1979). The $(C:N)_a$ of SPM at GREE (Table 1) were at the upper end of the range reported for phytoplankton, but much lower than those reported for vascular plant material. The carbon isotope data discussed above, however, implied that terrestrial and/or intertidal sources of organic matter predominated in much of the GREE, suggesting other nitrogen sources were immobilized.

When mangrove leaves decompose, a significant amount of material is lost initially as dissolved organic matter that is utilized by bacteria, part of which colonize the decaying leaves (Benner *et al.*, 1986, 1988; Twilley *et al.*, 1986). For samples with $\delta^{13}C$ in the range of terrestrial plant material, nitrogen immobilization by bacteria could have led to the lower $(C:N)_a$. Nitrogen immobilization associated with decaying mangrove leaves has been observed in a variety of tropical intertidal environments (Heald, 1969; Gotto & Taylor, 1976; Zuberer & Silver, 1978; Rice & Tenore, 1981; Twilley *et al.*, 1986; Benner *et al.*, 1990). In most of these studies, $(C:N)_a$ shift from >75 to <40 while decomposing on the forest floor. Thus, the even lower $(C:N)_a$ in the estuarine SPM may have resulted from adding bacterial biomass to decaying leaf material or from the incorporation of organic nitrogen produced by bacteria onto the leaf detritus through humification processes (Rice, 1982; Rice & Hanson, 1984). Both these processes occurring in the water column would result in reduction of the $(C:N)_a$ of the original leaf material. An alternative explanation for low $(C:N)_a$ and ^{12}C-enrichment in estuarine SPM is algae that fixed isotopically light CO_2. This will be discussed below.

A regression of %OC against %ON for selected stations in the GREE was linear with a slope of 8·2 ($r^2=0·99$; Figure 4, closed circles). This slope translates to a $(C:N)_a$ of 9·6, which is slightly depleted in nitrogen compared with typical algae and bacteria. Part of the data from Station C2 and the Salado Estuary had an unusually narrow $\delta^{13}C$ range of $-26·9$ to $-25·1$ (mean of $-26·4 \pm 0·5$) and were relatively nitrogen-poor (Figure 4, open circles). In contrast, the other data set had a $\delta^{13}C$ range of $-29·9$ to $-16·3$. The most positive values came from the shrimp pond, Stations S7 and S9 in the Morro Estuary, and the coastal station. At these sites, salinity was >25 and both elemental and isotopic data indicated significant contributions by algae. The data from Station G1 in the Guayas River, the Salado Estuary and tidal Station C2 in the Churute Estuary, however, had more negative $\delta^{13}C$ ranging from $-29·9$ to $-24·3$. These values were in the range usually quoted for terrestrially-derived material (see Fry & Sherr, 1984).

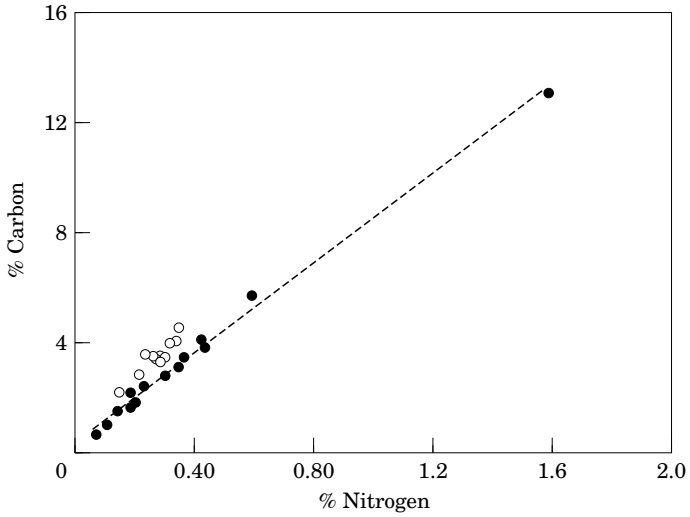

Figure 4. Organic carbon content (%C) *vs.* organic nitrogen content (%N). ○, includes Stations S2 and S3, and selected samples from Station C2.

The combination of low $(C:N)_a$ and $\delta^{13}C$ values for terrestrially-derived material in SPM could be explained by a significant contribution of algae growing on isotopically light CO_2 (see Fogel *et al.*, 1992). At station C2, there was sufficient chl *a* data to make a first-order estimate of algal-derived carbon. Two assumptions were used to make this calculation. First, it was assumed that the extracted chl *a* was derived primarily from algae, and not from intact remains of allochthonous plant matter. Second, the range of PC:chl *a* for algae was taken to be 20–200 (see below). At Station C2, PC concentrations were higher than values observed typically in temperate and arctic estuaries [Figure 2(d); compare with Loder & Hood, 1972; Spiker & Schemel, 1979; Cifuentes, 1991]. Pigment concentrations, however, were not unusually high [Figure 2(a)]. Based on an average chl *a* concentration over the tidal cycle, it was estimated that only 0·1 to $0{\cdot}9 \pm 0{\cdot}4$ mg l^{-1} or 3–26% of PC was algal in origin. This suggests that, on average, the living algal component at Station C2 was not as large as the detrital component. Thus, in view of the low algal biomass, algae growing on ^{12}C-depleted CO_2 can not be the principal explanation for the coexistence of low $(C:N)_a$ and negative $\delta^{13}C$ in SPM.

Detrital organic matter

The SPM recovered from aquatic systems contains both living and detrital organic matter. Cifuentes *et al.* (1988) used the ratio of particulate carbon concentration to chl *a* content (PC:chl *a*) to distinguish SPM samples that contained mostly detritus from those that included a large component of healthy algae. The PC:chl *a* in healthy algae range from 20 to 200 (Parsons *et al.*, 1977). Living plant matter of terrestrial origin can have higher values compared with that of algae. As expected, the lowest PC:chl *a*, 104, was measured at the coastal station, which was furthest from the mangrove forests. Furthermore, the PC:chl *a* value at Station S10 was in the range observed for algae. In contrast, the PC:chl *a* at Station G1 in the Guayas River was over 50 times greater. The average value for all samples, 327, was above the range expected for healthy algae.

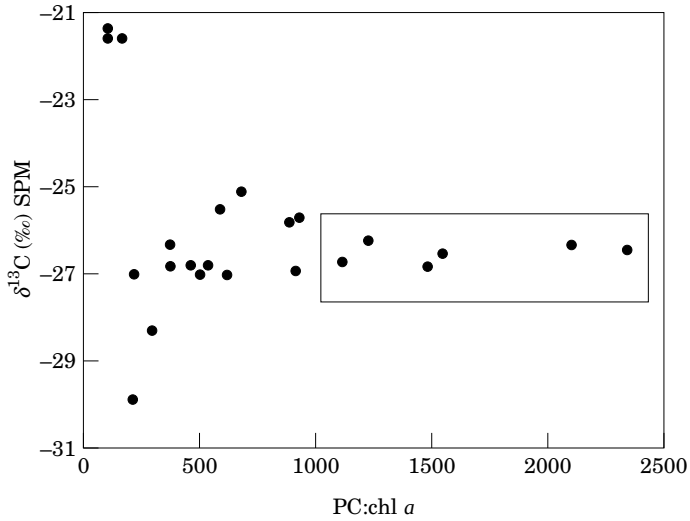

Figure 5. Stable carbon isotope ratio (δ^{13}C) of suspended particulate matter (SPM) *vs.* particulate organic carbon:chlorophyll *a* ratio (PC:chl *a*; wt/wt). Enclosed data points represent detrital samples as defined by PC:chl *a* >1000.

Significant degradation, allochthonous inputs or both of these factors could explain this high PC:chl *a* average.

Cifuentes *et al.* (1988) observed that δ^{13}C and δ^{15}N of SPM from the Delaware Estuary had greater variations at lower PC:chl *a*, but reached more uniform values at higher PC:chl *a*. The present authors found the same trend in the isotopic data from the GREE (Figure 5, only δ^{13}C shown). As in Cifuentes *et al.* (1988), samples containing detritus primarily were distinguished by their extremely high PC:chl *a*. To estimate the elemental and isotopic character of detritus, samples from Station C2 with PC:chl *a* >1000 (n=6; see Figure 5, boxed data) were used, and their elemental and isotopic ratios were averaged. This calculation resulted in a δ^{13}C of $-26{\cdot}4 \pm 0{\cdot}3$, a δ^{15}N of $+4{\cdot}8 \pm 0{\cdot}2$, and $(C:N)_a$ of $14{\cdot}1 \pm 0{\cdot}9$.

Both the carbon and nitrogen isotope ratios calculated for detritus in this tropical mangrove estuary were more negative than those reported for similar material from the Delaware Estuary ($\delta^{13}C = -22{\cdot}6$ and $\delta^{15}N = +6{\cdot}9$; see Cifuentes *et al.*, 1988), an algal-dominated, temperate estuary. This comparison suggests that the GREE had a comparatively larger allochthonous contribution of detritus. Since mangrove primary productivity is a likely source of terrestrial organic matter (Torgeson & Chivas, 1985; Twilley, 1988), it is noteworthy that the carbon isotope estimate for detritus was within the range of reported values for living and dead mangrove leaves (Rodelli *et al.*, 1984; Stoner & Zimmerman, 1988; Rezende *et al.*, 1900; this study). Moreover, the calculated δ^{15}N was also similar to that for mangrove leaves (4·3‰, this study). In contrast, the $(C:N)_a$ of the detritus was much lower than that of senescent and decomposing mangrove leaves (Twilley *et al.*, 1986; see Figure 4 in Benner *et al.*, 1990). Thus, if mangrove leaves were the principle source of detritus, the nitrogen content of the litter had to increase significantly during decomposition, as discussed earlier. While some of this nitrogen enrichment of mangrove litter occurs on the forest floor (e.g. Twilley *et al.*, 1986), additional nitrogen immobilization must occur in the estuary.

TABLE 2. Bacterial cell growth, bacterial carbon production and stable carbon and nitrogen isotope ratios of bacterial bioassays (BIO) and acid precipitated matter (APM)

Station	Growth $\times 10^9$ (cells l^{-1} day^{-1})	C production ($\mu gC\, l^{-1}\, day^{-1}$)	$\delta^{13}C$ BIO	$\delta^{13}C$ APM	$\delta^{15}N$ BIO	$\delta^{15}N$ APM
River Babahoyo	5·96	120	−26·7	−25·8	4·4	4·2
River Daule	1·43	29	−25·8	−25·9	6·3	5·7
M2	0·53	11	−25·3	−26·6	6·1	4·6
S7	1·30	26	−24·6	−23·2	8·2	6·8
S10	1·79	3	−24·0	−22·1	9·3	7·4
Shrimp pond	3·43	69	−23·1	–	4·9	–

See Figure 1 for locations of stations.

Dissolved organic matter and bacteria

Although bacterial production is ultimately a sink for organic carbon, the microbial turnover of carbon can influence isotopic and elemental variations in the system. Bacterial growth in bio-assay incubation experiments can be used to indicate the ' quality ' (Leff & Meyer, 1991) and potential sources (Coffin *et al.*, 1989) of organic matter used by bacteria. A series of bio-assays were conducted at selected stations in the GREE, and the bacterial growth rate varied significantly among the stations (Table 2). Increases in cell abundance over time in these incubation experiments ranged from about 0·5 to $6{\cdot}0 \times 10^9$ cells l^{-1} day^{-1}. Assuming a carbon to cell conversion factor of 20×10^{-15}, the range of bacterial carbon production was estimated at 11–120 $\mu gC\, l^{-1}\, day^{-1}$. Given growth yields of 0·2 to 0·5, it was calculated that between 20 and 600 $\mu gC\, l^{-1}\, day^{-1}$ were turned over by the bacteria. The largest increases in microbial biomass were produced in the Babahoyo River and the shrimp pond, suggesting that, of all the stations examined, these had the largest concentrations of carbon available to bacteria.

The $\delta^{13}C$ range for bio-assays, −26·7 to −23·1, encompassed the isotopic range of marine and allochthonous sources, demonstrating that ' potential ' carbon sources varied throughout the GREE. The most negative values were measured in Babahoyo and Daule Rivers, while other values increased in the following order: Station C2, Station S7, Station S10 and the shrimp pond. The $\delta^{15}N$ measured in the bio-assays varied from +4·4 to +9·1 (Table 2), and the most positive value was measured at the coastal station (S10).

The waters in the GREE were highly coloured indicating that dissolved humic substances were present at high concentrations. Although some isotopic measurements have been made of dissolved organic matter, the techniques are relatively new (Peterson *et al.*, 1994). Dissolved organic matter that precipitates at pH 2 is considered, by definition, to be humic acid (Fox, 1983; referred to here as acid precipitated matter, APM). Other DOM, however, such as proteins and molecules that contain acidic groups that are charged at natural pH, can also precipitate out of solution at lower pH (Thurman, 1985). Because APM precipitates out of solution, it can be recovered on a GF/C filter (Fox, 1983) and can easily be measured isotopically.

Humic material has been traditionally considered to be refractory to bacterial degradation. Although the isotopic ratio of this fraction of the DOM pool may not be representative of the whole pool, a close coupling between the isotopic ratio of SPM

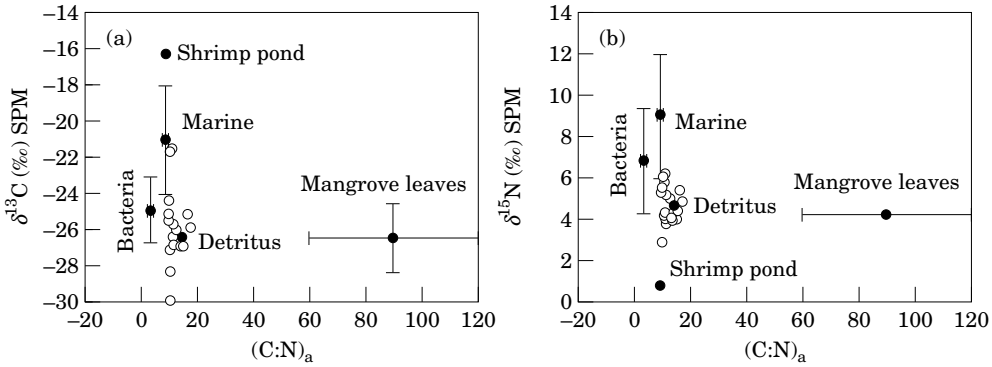

Figure 6. (a)Stable carbon isotope ratio (δ^{13}C of suspended particulate matter (SPM) *vs*. (C:N)$_a$. (b) Stable nitrogen isotope ratio (δ^{15}N) of SPM *vs*. Atomic carbon:nitrogen ratio [(C:N)$_a$]. Individual values or ranges are included for coastal organic matter, bacteria, mangrove leaves/detritus and the shrimp pond.

(Table 1) and APM (Table 2) would suggest similar sources. The average carbon isotope discrimination between SPM and APM was $0{\cdot}0 \pm 1{\cdot}1$‰. The only sample that differed by more than 1 SD from the mean was that from Babahoyo River, where SPM was 1·6‰ more negative than APM. The average nitrogen isotope discrimination between SPM and APM was $-0{\cdot}4 \pm 1{\cdot}2$‰. Only the sample from Guayas River differed by more than 1 SD from the mean. Isotopic similarity was also observed between bacterial bio-assays and APM (see Table 2). The carbon isotope discrimination between bio-assays and APM ranged from $-1{\cdot}9$ to $+0{\cdot}1$‰. In turn, the nitrogen isotope discrimination varied from $+0{\cdot}2$ to $+1{\cdot}9$. The close correspondence between the isotopic ratios of SPM, bio-assay and APM suggested these pools are closely coupled metabolically in time and space within the GREE.

Inconsistencies among the isotopic and (C:N)$_a$ data for various samples, where the former indicated allochthonous organic matter while nitrogen enrichment of the latter indicated *in situ* sources, were discussed above. A first-order estimate based on chl *a* data eliminated isotopically light algae as the explanation. An alternative explanation was that bacterial biomass or incorporation of organic nitrogen produced by bacteria through humification processes (Rice, 1982; Rice & Hanson, 1984) altered the character of allochthonous organic matter of either terrestrial and/or intertidal origin. To test this hypothesis, the δ^{13}C and δ^{15}N of SPM were plotted against their (C:N)$_a$ [Figure 6(a,b)]. Ranges of δ^{13}C, δ^{15}N and (C:N)$_a$ for mangrove leaves/detritus, marine plankton and bacteria are included in the figure. Specifically, the isotopic and (C:N)$_a$ data for marine plankton are from the literature, whereas that for mangrove leaves/detritus included data from this study. Finally, the elemental data for bacteria are from the literature, while the isotopic data for bacteria were taken from the bio-assay experiments discussed earlier (see Table 2).

For both carbon and nitrogen, the (C:N)$_a$, δ^{13}C and δ^{15}N data clustered on a mixing line between mangrove leaves/detritus and bacteria [Figure 6(a,b)]. This observation was based on the assumption that the isotopic measurements made in the bio-assay experiments were similar to what would be added to the allochthonous component by bacteria during detritus formation. If this assumption holds, then it appears that the majority of organic matter in the GREE system was of allochthonous origin, but altered

microbially. The authors suggest that additional modification by bacteria of allochthonous detritus occurred *in situ* resulting in immobilization of nitrogen and, thus, explaining the low $(C:N)_a$. In turn, the similarity among $\delta^{13}C$ and $\delta^{15}N$ ranges in APM and SPM suggests that organic matter added to decaying leaves originated from living microflora, but was incorporated into the detritus through humification processes (Rice & Hanson, 1984).

For some of the data, both $\delta^{13}C$ or $\delta^{15}N$ were outside the range defined by the organic sources used in Figure 6. In particular, the strikingly negative $\delta^{13}C$ (<28%) could be explained by two mechanisms. It is possible they could have resulted from greater degradation of proteinaceous and carbohydrate components compared with lipid and lignin fractions. This occurs often in both algal and vascular plant material (e.g. Hatcher & Spiker, 1988; Benner *et al.*, 1990). Since lipid and lignin fractions have more negative $\delta^{13}C$ than proteins and carbohydrates (Benner *et al.*, 1987), the selective loss of the latter fractions would result in lighter carbon isotope ratios. Furthermore, the $(C:N)_a$ would increase correspondingly. Following this line of argument, however, the ^{13}C-depleted samples (see Figure 6) could not have originated from vascular plant material because the $(C:N)_a$ decreased instead of increasing.

Earlier, it was argued that the utilization of ^{13}C-depleted CO_2 by algae was not the principal source of organic matter with $\delta^{13}C$ similar to terrestrial plant material. The most negative $\delta^{13}C$ ($-29{\cdot}9$), however, was observed at 14.00 h during the tidal study, and coincided with distinct maxima in chl *a* and dissolved oxygen [see Figure 2(a,b,f)]. Although this explanation can not be confirmed with a measurement of the $\delta^{13}C$ of CO_2, it appeared that, in this particular case, a burst of productivity did occur resulting in isotopically light $\delta^{13}C$ and the relatively low $(C:N)_a$. Alternatively, the chl *a* maximum could be explained by advection of chlorophyll-rich water from a highly productive upstream location, but this is not consistent with the lack of a corresponding minimum in salinity [Figure 2(a)]. Finally, it is unlikely that tidal scouring of benthic algae resulted in this pulse of chl *a* because SPM decreased [Figure 2(b)], and this process would not necessarily increase O_2.

Implications of tidal variations

The original paradigm for tropical estuarine ecosystems was that detrital particulate matter is a potentially important energy source to complex food webs (Odum & Heald, 1972). This hypothesis has been tested more recently by characterizing SPM isotopically, which has been sampled with either plankton nets or by filtration. In turn, the value is considered to be representative of detrital material in food-web analyses. One of the main concerns of food-web studies is the significant spatial and temporal variations of isotopic ratios in SPM (Mariotti *et al.*, 1984 Cifuentes *et al.*, 1988; Horrigan *et al.*, 1990; Fogel *et al.*, 1992). This is particularly true in systems with large tidal fluctuations where, depending on the stage of the tide, quite different isotopic measurements can result (Rezende *et al.*, 1990).

Although the tidal range is 2 m in the Churute Estuary, the salinity variation at the present sampling location during the tidal cycle was small (1·7). The variations in $\delta^{13}C$ and $(C:N)_a$, however, were substantial, abrupt and occurred at the end of low slack tide [Figure 7(a,b)]. There were also small, but significant, changes in $\delta^{15}N$ at concurrent salinities [Figure 7(c)]. Owing to increased clarity of the water during slack flood tide [Figure 2(b)], it was suggested earlier that increases in chl *a* [Figure 2(a)] and O_2 [Figure 2(b)] resulted from a burst of algal production. Cell counts documented an increase in

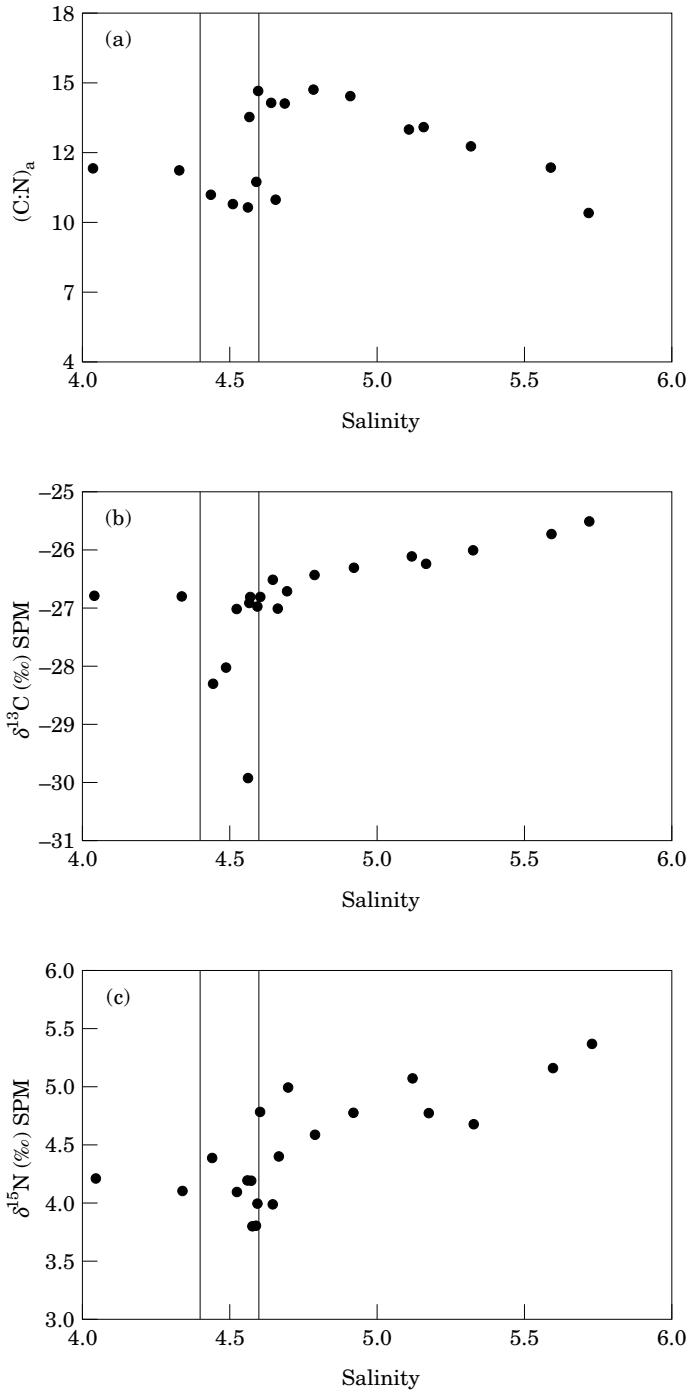

Figure 7. (a) Atomic carbon:nitrogen ratio [$(C:N)_a$], (b) Stable carbon isotope ratio $\delta^{13}C$) of suspended particulate matter (SPM) and (c) Stable nitrogen isotope ratio ($\delta^{15}N$) of SPM *vs.* salinity for the tidal station in the Churute River Estuary (C2).

cyanobacteria during this tidal stage, which would be consistent with decreases in $\delta^{13}C$, $(C:N)_a$ and $\delta^{15}N$. Although the authors can not provide an unequivocal explanation for the observed phenomenon, it is clear that food-web analysis based on a few isotopic measurements in this type of environment could lead to erroneous conclusions.

Conclusions

The majority of stable carbon and nitrogen isotope measurements made in temperate estuarine systems suggest that *in situ*, algal production dominates the pool of SPM (Mariotti *et al.*, 1984; Sigleo & Macko, 1985; Cifuentes *et al.*, 1988; Horrigan *et al.*, 1990; Coffin *et al.*, 1994 and references therein). Samples of particulate organic matter from tidal creeks and estuaries dominated by salt marshes or seagrass beds exhibit signatures of allochthonous organic inputs (Peterson & Fry, 1987). Also, when samples are taken along the boundaries of larger estuarine systems, the isotopic data often reflect that of the organic matter from marine origin. In contrast, the importance of allochthonous organic matter throughout the Guayas River Estuary system was evident in the isotopic analyses. Although some of the intertidal region has been transformed from natural mangrove forests into shrimp farms, organic matter released from these farms had only a local effect. In fact, it appeared that detritus from mangroves and other allochthonous sources supplied much of the organic matter to the system. Microbial activity, however, altered the nitrogen content of the original material. Finally, in one of the stations sampled, elemental and isotopic data taken over a tidal cycle varied significantly. Insufficient characterization of the isotopic ratio of end-members and samples in these systems will lead to significant errors when isotopic measurements are used in models that estimate contributions of different sources of organic matter or in food-web studies.

Acknowledgements

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