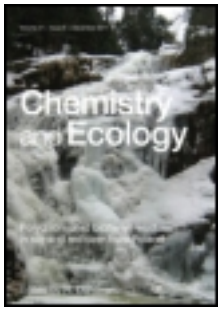

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Terrigenous nutrient and organic matter in a subtropical river estuary, Okinawa, Japan: origin, distribution and pattern across the estuarine salinity gradient

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Terrigenous nutrient and organic matter in a subtropical river estuary, Okinawa, Japan: origin, distribution and pattern across the estuarine salinity gradient

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Estuaries and their associated rivers are organically rich sites that play an important role in the removal and transformation of organic matter and nutrients derived from terrigenous and anthropogenic sources. This study conducted comprehensive field surveys from March 2010 to January 2011 to study the downstream pattern and distribution of nutrients (NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-}) and dissolved organic carbon (DOC) and particulate organic carbon (POC) and nitrogen (PN) in the surface waters of the Manko estuary, a subtropical estuary in Okinawa, Japan. Fatty acid (FA) analysis and POC–C/N ratios were used to further identify variations in the sources of particulate organic matter along the estuary during summer (July) and winter (January). The results suggest that the estuary contains high concentrations of dissolved inorganic (N and P) and organic (DOC) nutrients, which are largely influenced by terrestrial sources from the Kokuba and Noha rivers, and moderate levels of particulate nitrogen and carbon. In general, suspended particles and dissolved nutrients followed sedimentation and biotic uptake patterns common in other subtropical estuaries. Thus, an important fraction of terrestrial materials was rapidly sinking along the estuary and was replaced with estuarine and marine-derived materials at mid- to high-salinity along the estuary. The FA signatures suggested that bacteria, domestic and agricultural waste-derived organic matter were the dominant sources of suspended organic matter in the Manko estuary. Overall, despite the relatively high terrestrial and anthropogenic influences in the Manko estuary, effective processing of different sources and forms of terrigenous organic matter in the estuarine salinity gradient significantly reduces their signatures prior to export to the coastal ocean.

Keywords: terrigenous; nutrients; organic matter; river–estuary; salinity gradient

1. Introduction

Organic substances and nutrients in rivers provide important information on processes within the drainage basins and contribute terrestrial inorganic and organic material to the oceans. In most cases, riverine nutrients and organic matter are temporarily retained within estuaries prior to their discharge to coastal waters, the exception are large river systems that discharge terrestrial materials directly to the coastal ocean during periods of high flow. During this retention, terrestrial materials can be subjected to alteration through both biotic and abiotic processes including

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microbial respiration, photo-oxidation and flocculation or scavenging with subsequent removal to sediments [1].

In addition to terrigenous suspension, rivers carry many nutrients that make estuaries more productive than the coastal and adjacent water areas. This supply of terrigenous nutrients and organic substances is greater in estuaries that are situated near densely populated regions because of the entry of domestic and industrial waste, urban drainage and agricultural effluents [2]. Currently, >50% of the world's population resides either along or within 200 km of the coast, and it is predicted that 75% of the population will live in coastal regions by 2025 [3]. The increase in the human population along the coast has been accompanied by intensified agricultural production, together with increased urbanisation; as a result, more than half of the world's coastlines suffer from pressures of human activities such as increases in deliveries of sediment, inorganic nutrients and contaminants to estuarine and coastal sea ecosystems [4,5].

The target of this study, the Manko estuary, is located in the middle of dense urban and commercial areas in the southern part of Okinawa Island, Japan. The estuary is recognised as an important visiting and wintering area between South East Asia and Japan for migratory birds such as snipes and plovers. The Manko wetland area was added to the Ramsar Convention Register of Wetlands in 1999.

Although the Manko basin does not have major industrial activity, there are small-scale agricultural activities and residential areas that may affect water quality and the nature of organic carbon available to organisms in this system. The Manko estuary receives terrigenous inputs through the Kokuba and Noha rivers and through drainage and small canals that carry pollutants from untreated domestic run-off. The estuary also receives wastewater from livestock farms in rural upstream areas lacking appropriate treatment systems [6]. According to Okinawa Prefecture monitoring, biological oxygen demand regularly exceeds 10 $mg \cdot L^{-1}$ in river water just before it enters the estuary and has been associated with the agricultural activities upstream.

Inputs of these materials are expected to further increase in response to rapidly increasing land clearance for agriculture and coastal development in Okinawa. Thus, it is essential to establish and understand the current sources, patterns/distribution and fate of terrigenous materials in this estuarine ecosystem. To date, there have been only a few studies characterising sources of particulate and sedimentary organic matter in the estuary [7,8]. However, these studies were restricted to either mangrove or tidal flat areas. To our knowledge, this is the first study to investigate the sources, patterns/distribution and fate of terrigenous organic substances and nutrients in the water column of the Manko estuary. This study had the following main objectives: (1) to determine the downstream patterns/distributions of inorganic nutrients and organic matter across the Manko estuary; (2) to investigate seasonal variations in geochemical characteristics of the Manko estuarine organic matter and nutrients, and their possible controlling factors; and (3) to investigate the potential sources of organic matter in the water column particulate organic matter (POM) using fatty acid (FA) and elemental [particulate organic carbon (POC) and particulate nitrogen (PN)–C/N ratio] analysis. Because of their structural diversity and high biological specificity [9], FAs can assist in determining the source and fate of organic materials in particulate matter [3] and in sediment [10].

2. Materials and methods

2.1. *Study area*

The study was conducted in the Manko estuary (26°11′N, 127°41′E) (Figure 1) in Okinawa, Japan. This region has a subtropical climate; the lowest monthly mean temperature is 16.8 °C in February, and the highest monthly mean temperature is 28.8 °C in July. Precipitation exceeds

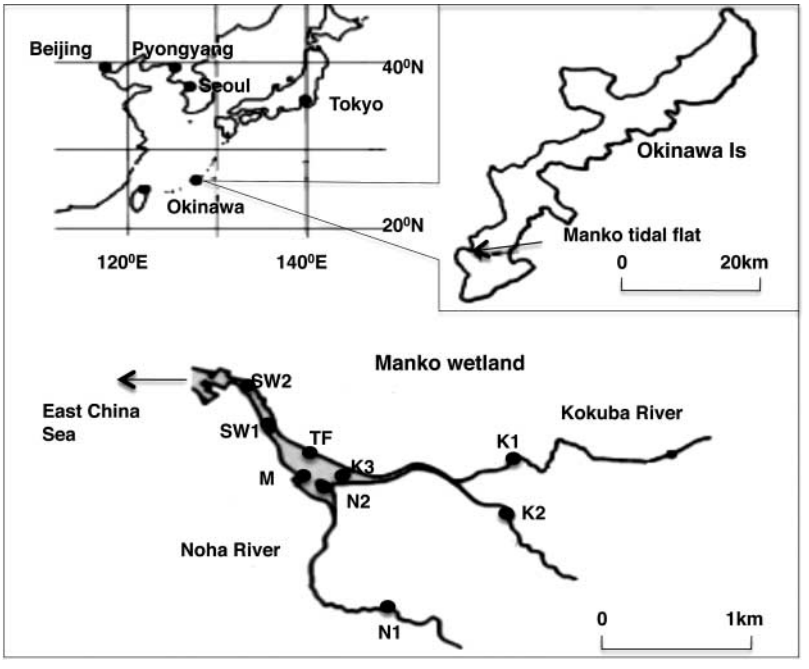

Figure 1. Location of Okinawa Island and sampled stations along the Manko estuary.

100 mm·month^{-1} throughout the year, and the average rainfall is 2086 mm·year^{-1}. The main water inputs to the estuary are through tidal influences and freshwater inputs derived from the Noha and Kokuba rivers. The water flow rates of the two rivers are relatively low (average, 1.3 to 1.8 m^3·s^{-1}) except during rainfall events, in which flow rates reach averages of 2.6 to 5.2 m^3·s^{-1} for the Noha and Kokuba rivers, respectively, according to Okinawa Prefectural monitoring for 2010.

Although end-member definition is important in any classic estuarine study, the approach of this study was different: our objective was to study the longitudinal variations in surface-water chemistry in the entire estuary at low tides. Nine sampling stations were selected to represent the salinity gradient of the Manko estuary (Figure 1). Station SW2 is at the mouth of the estuary and has a surface water salinity range of 25–33 psu. SW1 and TF stations are 400 and 900 m from SW2 and have salinity ranges of 23–25 and 12–22 psu, respectively. Station M is near a healthy stand of mangroves consisting mainly of *Kandelia obovata*, *Bruguiera gymnorrhiza* and a few patches of *Rhizophora stylosa*. The salinity at this station ranges from 9 to 22 psu. The following five stations represent the tidal freshwater zone along the Kokuba (K) and Noha (N) rivers: K3 (salinity: 5–18 psu), K2 and K1 (salinity: 2–6 psu), N1 (salinity: <2 psu) and N2 (salinity: 5–9 psu).

2.2. *Sample collection*

Surface-water samples were collected during low tide (2–3 h before high water; freshwater was targeted when tidal velocities were low) at all nine stations from March 2010 to January 2011. Water samples were collected for analyses of inorganic nutrients (NO_3^--N, NO_2^--N, NH_4^+-N and PO_4^{3-}), particulate organic matter (POC and PN), dissolved organic matter (DOC), total suspended material (TSM) and chlorophyll *a* (Chl-*a*). Enough samples were collected for four replicates for inorganic nutrients and DOC and three replicates for POC, PN, TSM and Chl-*a*. Surface water

samples from each station were collected using a plastic bucket and were transferred to new, sample-rinsed 5-L high-density polyethylene (HDPE) bottles and kept cold until further sample processing, within 5 h of collection. The samples were collected from the mid-channel with the exception of the M and TF stations, which were sampled along the shore (Figure 1).

In the laboratory, water samples for dissolved nutrients were filtered through 0.45-μm fibreglass filters (Whatman, UK) to remove TSM, while samples for POC, PN and Chl-*a* were filtered through pre-weighed and pre-combusted glass fibre filters (Whatman GF/F, 0.7 μm) [11]. The filters with POM were stored at −20 °C until analysis. Filtrate was split into several portions for DOC and nutrient analysis. Four aliquots of the DOC filtrate (collected in 40-mL glass vials) were treated with 50 μL of concentrated HCl to remove inorganic carbon and prevent any bacterial development. The samples were stored in a refrigerator at 4 °C until analysis. Another four aliquots were collected in 15-mL HDPE tubes for inorganic nutrient analysis and kept at −20 °C to prevent changes in the samples' chemical composition [12] until analysis, which was conducted within 5 days of sampling.

Water for POM–FA analysis was collected during the low tide from six sites (K3, N1, M, TF, SW1 and SW2) in July 2010 (summer) and January 2011 (winter). In addition to these sites, residential/urban wastewater run-off flowing to the estuary from three canals and wastewater from livestock farms in the rural upstream were collected as references for later confirmation of potential terrigenous sources and sources that contributed more materials to the estuary. The water samples collected (4 L for each site and four replicates) were transported back to the laboratory, where they were filtered through pre-combusted (4 h at 450 °C) glass fibre filters (Whatman GF/F, 0.7 μm). Three filter papers were used for each replicate and were put into cold storage at −20 °C. The filter papers with POM were freeze-dried for 24 h before lipid extraction.

2.3. *Water analysis for environmental parameters*

Filters for POC and PN were washed with distilled water to eliminate salt and dried for 24 h at 60 °C. The dry weight of the collected suspension was used to calculate the total suspended matter. The POC and PN filters were placed in a desiccator in contact with HCl fumes overnight to decompose any inorganic carbon (e.g. calcium carbonate [$CaCO_3$]) and re-dried again at 60 °C [3]. POC and PN were quantified using a CHN analyser (NC-80 Model, Sumika Chemical Analysis, Tokyo, Japan) with a precision of <5%.

Concentrations of DOC were determined using a TOC analyser (TOC-5000A, Shimadzu, Kyoto, Japan) with a precision of 5%. Surface water samples for nitrates (NO_3^--N), nitrite (NO_2^--N), ammonium (NH_4^+-N) and phosphate (PO_4^{3-}) were analysed using an automatic water analyser (Autoanalyzer-3, QuAAttro, Bran + Luebbe GmbH, Norderstedt, Germany) with a precision of 5%. Reagents (for $NO_2^- + NO_3^-$, NH_4^+ and PO_4^{3-} assay) and mixed standards ($NaNO_2$, KNO_3 $(NH_4)_2SO_4$, and KH_2PO_4 for NO_2^-, NO_3^-, NH_4^+ and PO_4^{3-}, respectively) were run throughout the sample analysis to check the precision of nutrient analysis. The detection limits and precision of analysis were provided in the Autoanalyzer-3 manual (ACCE-6, Bran + Luebbe GmbH).

The filters for Chl-*a* measurements were extracted in 8 mL of 90% acetone, and pigment analysis was carried out spectrophotometrically using the trichromatic method [13]. Salinity (psu), temperature (°C), and pH readings were measured *in situ* using a multiparameter meter (for salinity, pH, and temperature; 556 MPS, YSI, Yellow Springs, OH, USA).

2.4. *Lipid extraction and analysis*

GF/F filters with particulate matter were cut into small pieces, and ∼3 g dry weight was used for extraction of lipid and FAs using a modified method of Bligh and Dyer [14]. A total of 1 mL of

nonadecanoic acid methyl ester solution was added to each sample as an internal standard (1 mg in 10 mL of chloroform). Separation and purification of FA methyl esters (FAMEs) was carried out according to Mfilinge et al. [15]. Lipid fractions were separated using thin-layer chromatography (TLC) on two Merc plates coated with Kieselgel 60 silica (Darmstadt, Germany). A developing solution was prepared by mixing dry hexane, diethyl ether, and acetic acid (70:30:1) in a TLC chamber. One of the plates was used for FAME band position estimation [16]. Bands containing FAMEs were scraped off and collected in a mixture of chloroform/methanol (2:1, v:v) at 40 °C for 60 min. FAMEs were then separated and quantified on a Shimadzu 14B gas chromatograph equipped with a flame ionisation detector. Separation was performed with a free FAs phase polar capillary column (30 × 0.32 mm internal diameter, 0.25-μm film thickness) with helium as a carrier gas. The peaks of FAMEs were identified by comparing their retention times with those of authentic standards (Supelco Inc., Bellefonte, PA, USA).

2.5. *Statistical analysis*

Linear regression and Pearson's correlation coefficient test were run to explore the relationships between variables (POC, PN, DOC, inorganic nutrients, TSM and Chl-*a*) and between nutrients and individual/groups of FA markers in suspended POM.

3. Results

3.1. *Inorganic nutrient variability through space and time*

Spatial and seasonal patterns of inorganic nutrients along the estuary (Figure 2) show that concentrations of all inorganic nutrient species decreased from tidal freshwater zone stations (K1, K2, K3, N1 and N2) to seaward stations (M, TF, SW1 and SW2) with the exception of PO_4^{3-}, which was generally higher at station M during March and July (40 and 30 $\mu mol \cdot L^{-1}$, respectively); NO_3^-, which showed some peaks at station TF in March and July (82 and 53 $\mu mol \cdot L^{-1}$, respectively); and NH_4^+, which also showed a relative increase at seaward stations SW1 and SW2 in September (68 and 49 $\mu mol \cdot L^{-1}$, respectively). NH_4^+ and NO_3^- were the most abundant of the measured inorganic nutrients at all nine sites throughout the year, with concentrations ranging from 30 to 132 and 9.4 to 121.1 $\mu mol \cdot L^{-1}$, respectively, during March. The concentration ranges of these dominant nutrients during May and July were 14–51 and 31–136 $\mu mol \cdot L^{-1}$ for NH_4^+ and 3–55 and 10–101 $\mu mol \cdot L^{-1}$ for NO_3^-, respectively. In September, the concentrations of NH_4^+ and NO_3^- ranged from 22 to 59° and 8 to 129 $\mu mol \cdot L^{-1}$, respectively. Concentration ranges of 9–86 $\mu mol \cdot L^{-1}$ for NH_4^+ and 3–74 $\mu mol \cdot L^{-1}$ for NO_3^- were recorded in January.

Diagrams for the relationships between salinity and nutrients (inorganic nutrients, DOC, and bulk particles parameters) are shown in Figure 3 for March following the dry winter period (January–February) and in Figure 4 for July following the rainy period (May and June) in Okinawa. NO_2^-, NO_3^- and NH_4^+ decreased linearly in March ($r = -0.972$, $p = 0.00$; $r = -0.815$, $p = 0.007$; and $r = -0.680$, $p = 0.044$, respectively), whereas PO_4^{3-} was less linear ($r = 0.240$, $p = 0.533$), showing several data points below the predicted mixing line.

The NO_2^- mixing behaviour tended to be more nonlinear in July ($r = -0.034$, $p = 0.932$) with a slight decrease in concentrations between salinities of 4 and 5 psu. NO_3^- showed relatively more linear decreases in July ($r = -0.900$, $p = 0.001$). However, Figure 4 suggests that there was a mid-estuarine removal of NO_3^- because several data points were below the mixing line and that there was a subsequent mid-estuarine input of NH_4^+ in July. PO_4^{3-} showed less conservative decreases along the salinity gradient ($r = -0.506$, $p = 0.164$).

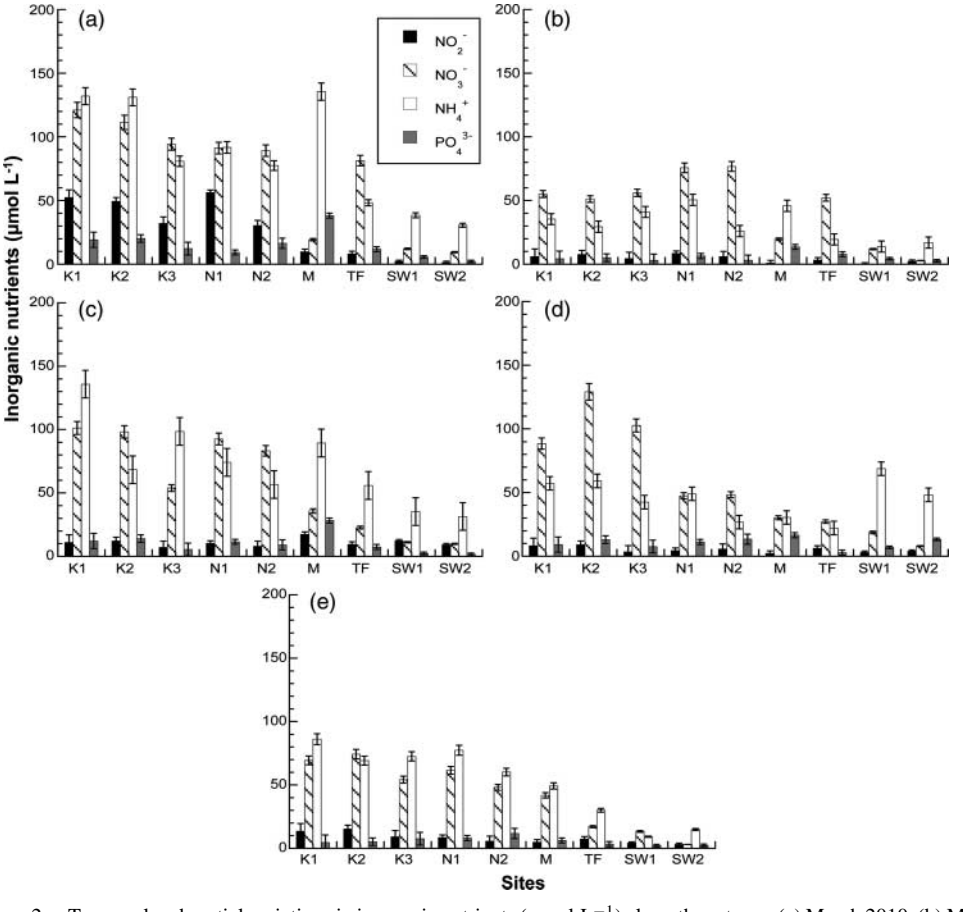

Figure 2. Temporal and spatial variations in inorganic nutrients (μmol·L^{-1}) along the estuary. (a) March 2010, (b) May 2010, (c) July 2010, (d) September 2010, (e) January 2011. Values are shown as means $\pm$1 SE.

3.2. *Dissolved and particulate organic matter variability*

DOC concentrations measured along the estuary (Figure 5) ranged from 3.1 to 88.4 mg·L^{-1} and were higher during the rainy season in May than in March (Figure 5a,b). DOC concentrations were generally higher at the freshwater stations than at the seaward stations (mean concentration of 5–88.4 mg·L^{-1} for freshwater stations and 2.5–45 mg·L^{-1} for seaward stations) with the exception of station SW1, which had higher DOC concentrations during May and January (38.8 and 45 mg·L^{-1}, respectively). In both March and July, the DOC concentrations decreased as salinity increased, as anticipated (Figures 3 and 4). However, the decrease in July was significantly more linear ($r = -0.905$, $p = 0.001$) and less variable along the mixing line than that during March ($r = -0.601$, $p = 0.087$).

There was a strong gradient in POC and PN concentrations (mg·g^{-1} of TSM) during March and July (Figure 6a,b), with highest concentrations occurring at the mid-estuary (M and TF) and seaward (SW1 and SW2) stations. By contrast, POC and PN concentrations did not show much variation between the stations in September 2010 and January 2011 (Figure 6c,d). Unlike DOC, the POC and PN concentrations increased significantly as salinity increased ($r = 0.842$, $p = 0.004$ and $r = 0.771$, $p = 0.015$ for March, and $r = 0.913$, $p = 0.001$ and $r = 0.913$, $p = 0.001$ for July, respectively) (Figures 4 and 5). However, the downstream changes were generally below

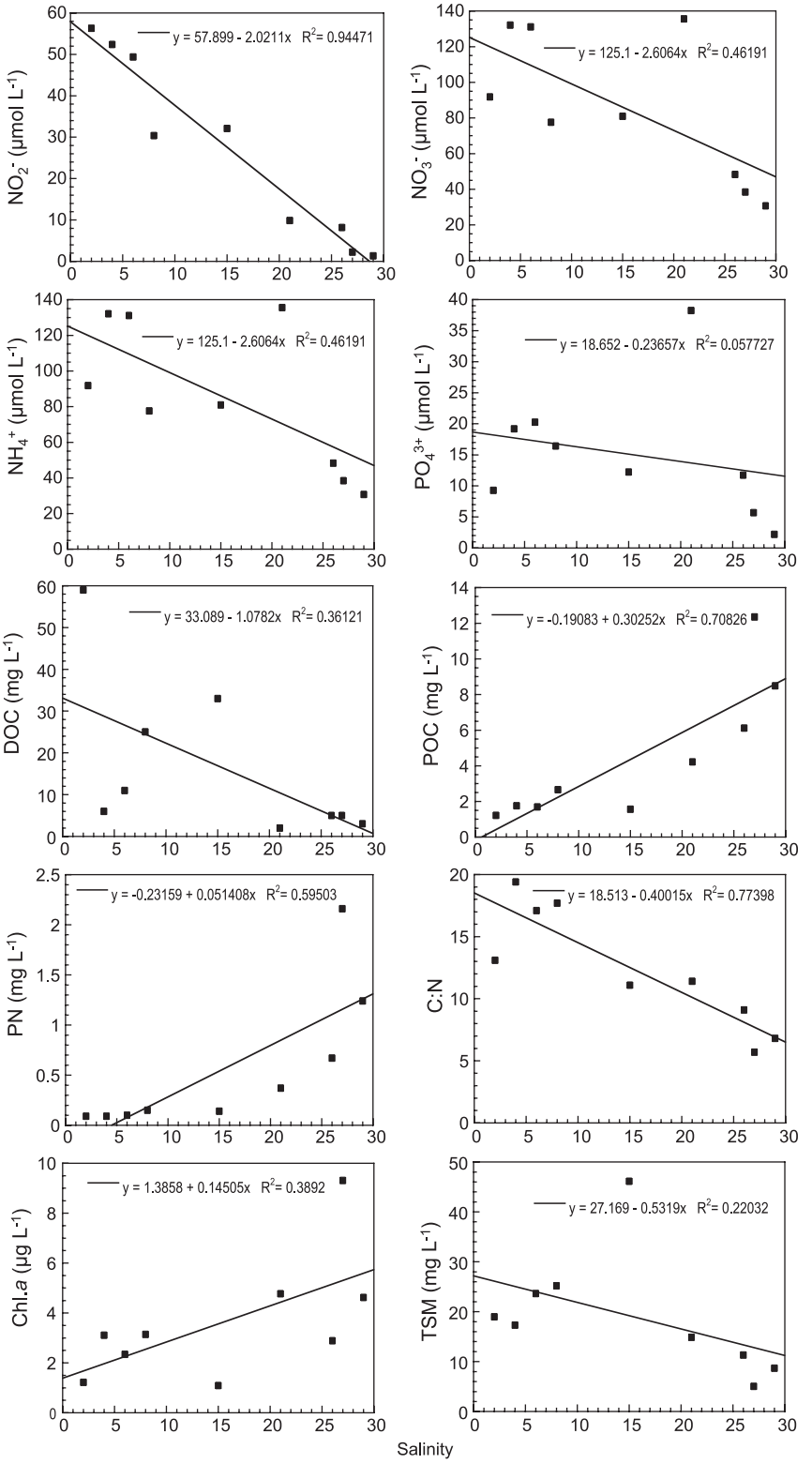

Figure 3. Mixing plots of surface water constituents along the river estuary during March 2010.

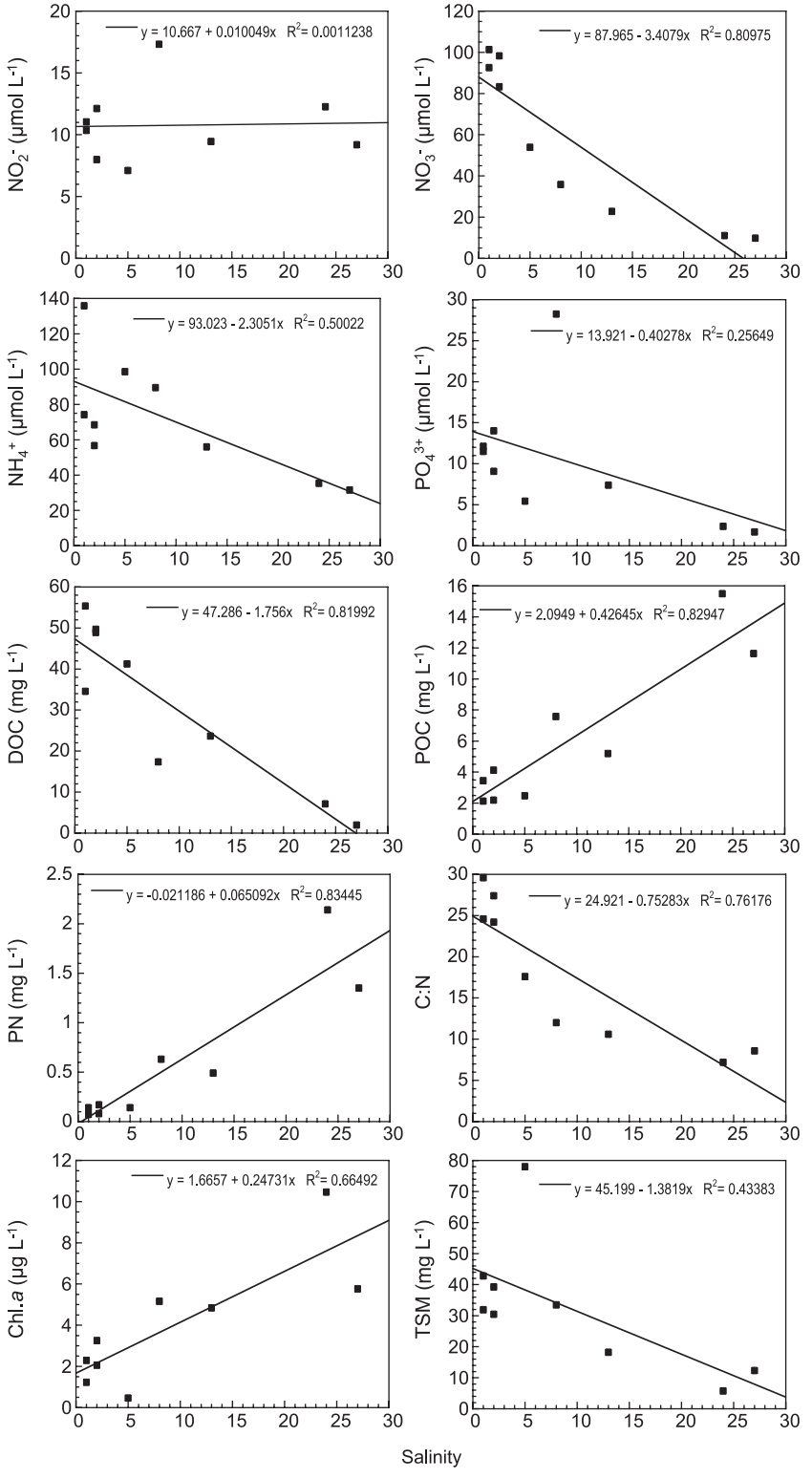

Figure 4. Mixing plots of surface water constituents along the river estuary during July 2010.

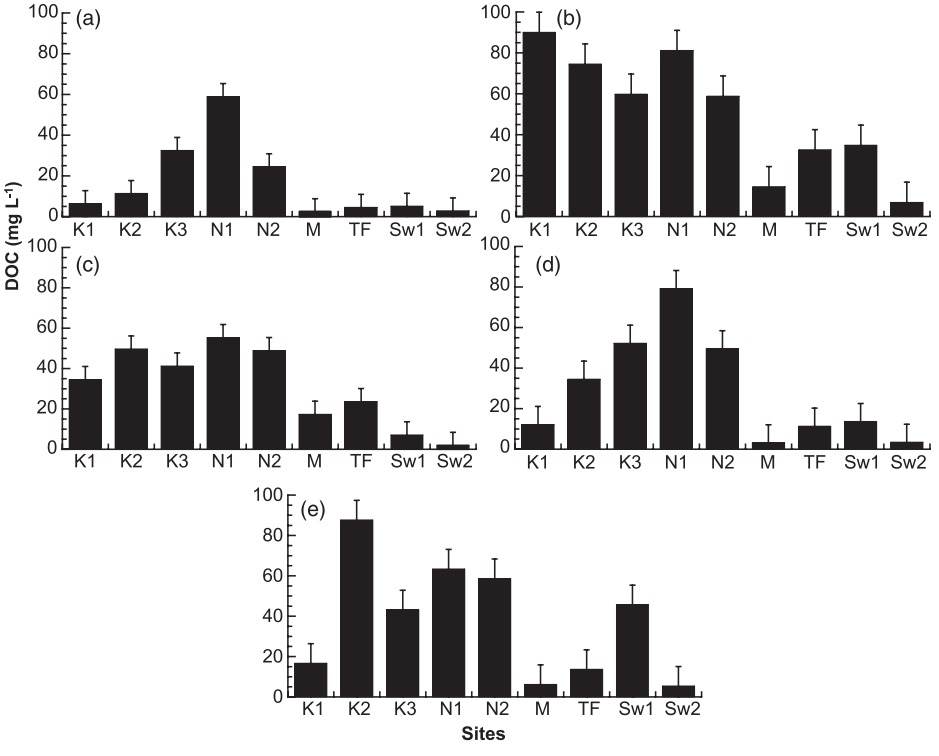

Figure 5. Temporal and spatial variations in dissolved organic matter (DOC) along the river estuary. (a) March 2010, (b) May 2010, (c) July 2010, (d) September 2010, (e) January 2011. Values are shown as means ±1 SE.

the mixing line in March compared with July. The C/N ratios of surface POM ranged between 6 and 30 (Figure 6e,f) along the estuary, with the highest ratios recorded in July and September and occurring at the freshwater stations.

Figure 6g–j shows the spatial and temporal variation in TSM and Chl-*a* along the estuary. TSM ($mg{\cdot}L^{-1}$) followed a predictable change from the particle-rich riverine environment to the depleted seaward stations. The highest concentrations of TSM (22–111 $mg{\cdot}L^{-1}$) were recorded in May (not shown in the figure), whereas the lowest particle concentrations were recorded in January 2011 (7–25 $mg{\cdot}L^{-1}$) (Figure 6j). By contrast, Chl-*a* ($\mu g{\cdot}L^{-1}$) showed the opposite trend, with a substantial increase in concentration at a salinity range of 6–27 psu and subsequent decrease at a salinity of >28 psu (SW2); this trend was observed over the course of the study. Interestingly, Chl-*a* did not show much variation between the months sampled. However, there was an overall increase in Chl-*a* concentrations throughout the freshwater stations in January compared with other months, and a very sharp increase in the mid-estuary stations as shown in Figure 6j.

3.3. *FA composition and abundance*

3.3.1. *FAs in POM along the estuary*

The FA compositions of suspended organic matter in surface water (Figure 7; Tables S1 and S2, available online only) showed seasonal and spatial variation. The total FAs given in absolute dry weight ($\mu g{\cdot}g^{-1}$) followed the organic carbon concentration trend, with the highest concentrations recorded at the mid-estuary stations (M, TF and SW1) in July (128.42, 147.44 and 121.41 $\mu g{\cdot}g^{-1}$, respectively) and in January (68.50. 83.75 and 85.12 $\mu g{\cdot}g^{-1}$, respectively) (Figure 7a). The major

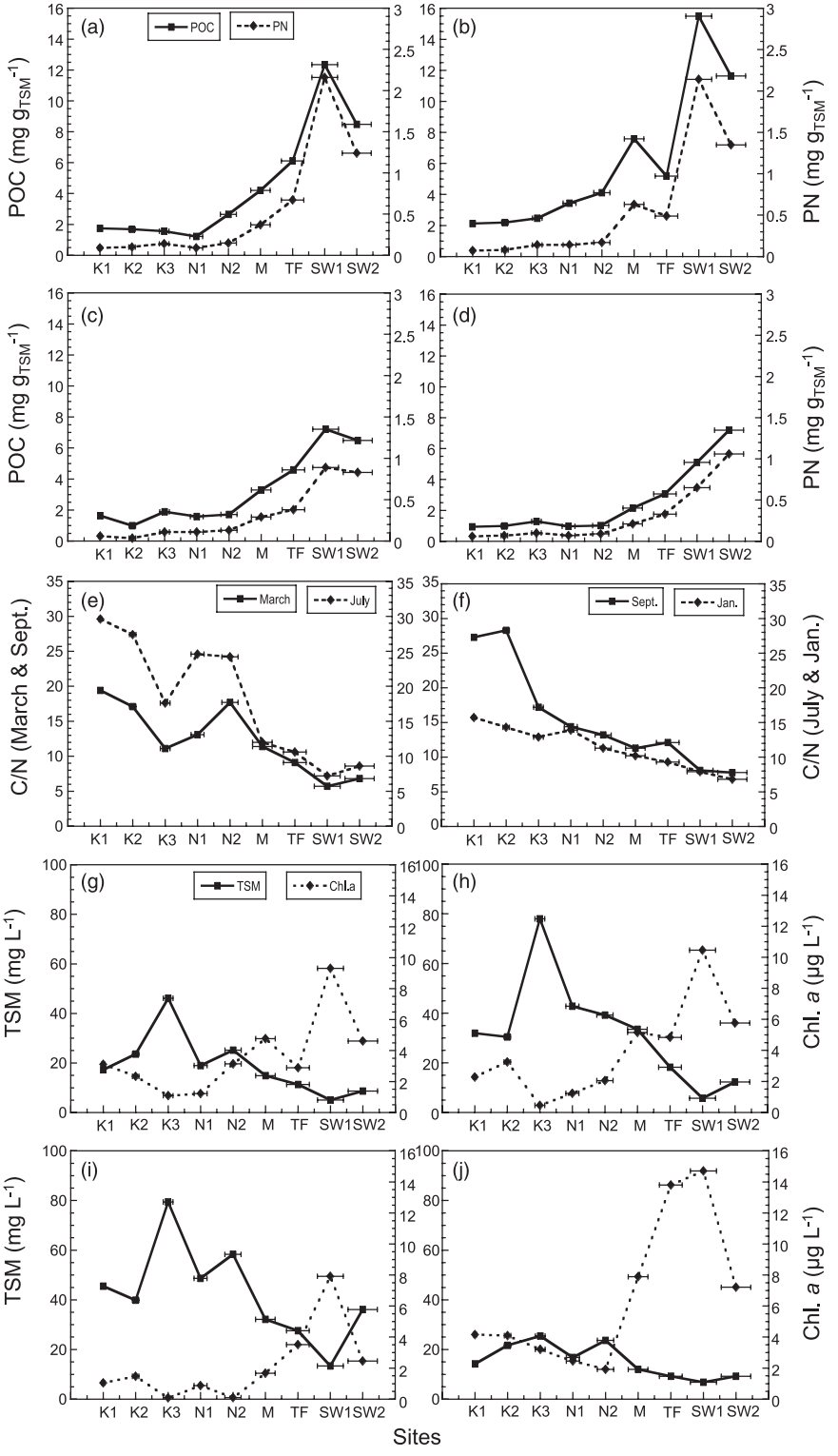

Figure 6. Surface water particulate organic carbon, nitrogen, and their C/N ratios; total suspended matter; and chlorophyll *a* concentration along the estuary. (a) March 2010, (b) July 2010, (c) September 2010, (d) January 2011, (e) March and July 2010, (f) September 2010 and January 2011, (g) March 2010, (h) July 2010, (i) September 2010, (j) January 2011. Error bars represent the SE for the dependent variable.

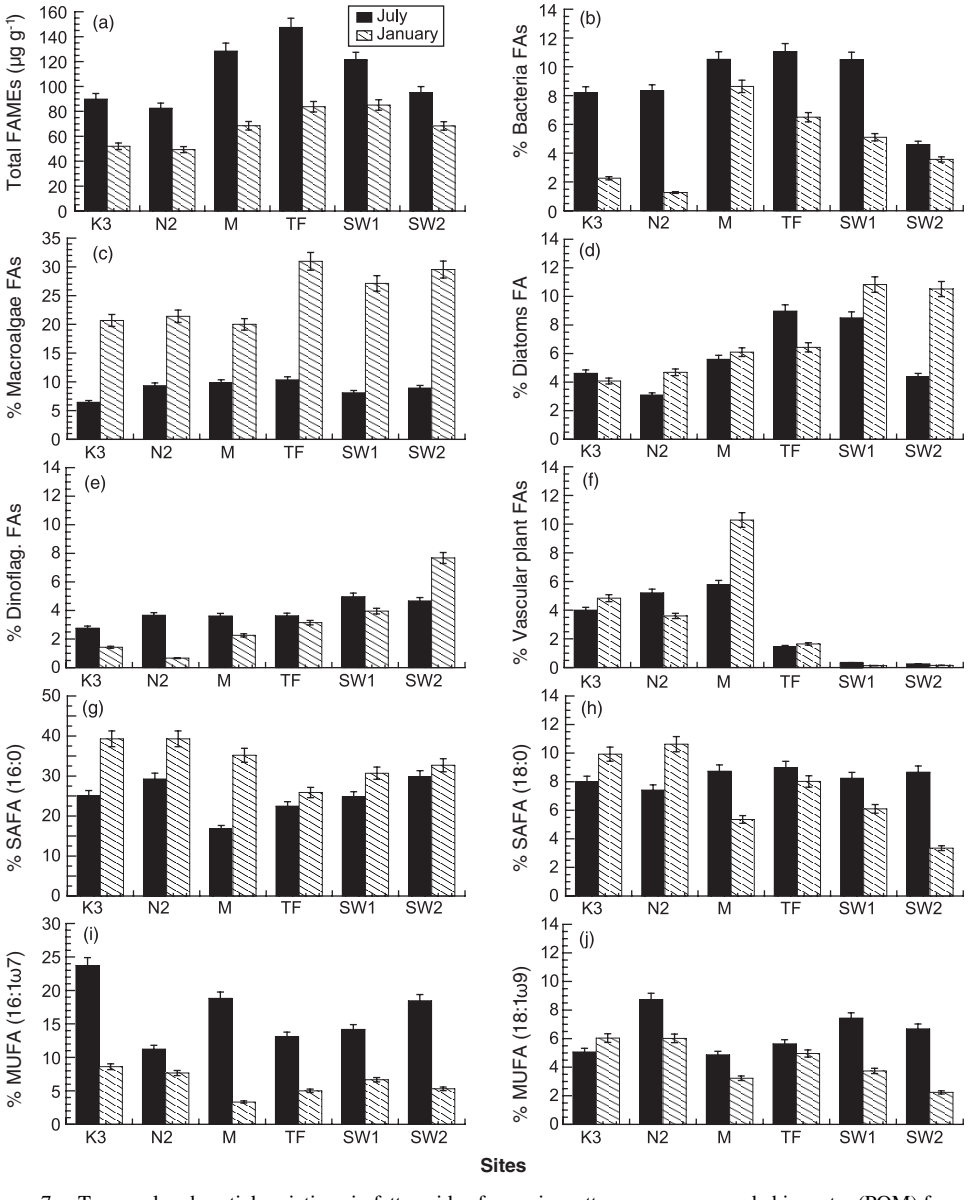

Figure 7. Temporal and spatial variations in fatty acids of organic matter sources suspended in water (POM) from six locations in the Manko estuary during July 2010 and January 2011. (a) Total fatty acid methyl esters (FAMEs); (b–j) contribution of selected fatty acid biomarkers: (b) bacterial FA, (c) macroalgal FA, (d) diatom FA, (e) dinoflagellates FA, (f) vascular plants, (g) saturated fatty acid (16:0), (h) saturated fatty acid (18:0), (i) monounsaturated fatty acid (16:1ω7), (j) monounsaturated fatty acid (18:1ω9). Values are shown as means ± SE.

FA ($\geq$ 5%) constituents made up 50.7–71.32% and 62.6–80.3% of the total FAs present in the water sample during July and January, respectively. Of these FAs, ubiquitous 16:0 contributed 16.8–29.8% in July and 25.9–39.3% in January (Figure 7g). Other FAs that frequently constituted at least 5% of the total were 18:0, 16 : 1ω7 and 18 : 1ω9, recorded at all sampled stations (Figure 7h–j).

Branched FAs (C_{15}–C_{17} iso and anteiso) and vaccenic acid (18 : 1ω7), markers of bacteria, exhibited temporal variability; they were found in higher proportions throughout the estuary in

Table 1. Results of Pearson's correlation coefficient (r) test among inorganic nutrients, dissolved and particulate matter, and the fatty acids markers (combined data for both season, summer and winter).

	DOC	POC	PN	SPM	Chl.*a*	NO_2^-	NO_3^-	NH_4^+	PO_4^{3-}	Bet	M.alg	20:5ω3	16:0	16:1ω7	18:1ω9	18:0	22:6ω3	LCFA
DOC	1.000																	
POM	**−0.618**	1.000																
PN	**−0.626**	**0.974**	1.000															
SPM	0.541	−0.599	**−0.654**	1.000														
Chl.a	−0.492	**0.922**	0.875	−0.580	1.000													
NO_2^-	−0.008	0.349	0.186	0.122	0.504	1.000												
NO_3^-	**0.734**	**−0.607**	**−0.683**	**0.728**	−0.535	0.217	1.000											
NH_4^+	0.481	−0.411	−0.531	**0.841**	−0.294	0.540	**0.759**	1.000										
PO_4^{3-}	0.144	−0.119	−0.277	0.296	0.004	**0.751**	0.391	**0.641**	1.000									
Bct	−0.299	0.394	0.269	−0.128	**0.610**	0.591	−0.129	0.220	0.424	1.000								
M.alg	−0.238	−0.106	−0.008	−0.555	−0.089	−0.443	−0.564	**−0.688**	−0.228	**0.734**	1.000							
20:5ω3	−0.393	0.534	0.568	**−0.636**	**0.616**	−0.104	−0.719	−0.578	−0.216	0.530	**0.804**	1.000						
16:0	−0.379	**0.605**	0.568	−0.519	**0.719**	0.083	−0.430	−0.361	−0.298	**0.628**	−0.046	**0.679**	1.000					
16:1ω7	−0.196	0.492	0.331	0.153	0.583	**0.674**	−0.089	0.411	0.418	**0.756**	−0.472	0.277	0.448	1.000				
18:1ω9	−0.128	0.587	0.453	−0.150	**0.747**	**0.686**	0.005	0.172	0.199	**0.776**	−0.387	0.312	**0.697**	**0.731**	1.000			
18:0	−0.169	0.429	0.287	−0.098	**0.667**	**0.728**	−0.143	0.272	0.385	**0.871**	−0.220	0.382	**0.610**	**0.833**	**0.857**	1.000		
22:6ω3	−0.606	**0.961**	**0.924**	−0.564	**0.874**	0.258	−0.573	−0.420	−0.233	0.431	−0.136	0.548	**0.728**	0.524	**0.660**	**0.664**	1.000	
LCFA	0.179	−0.331	−0.483	0.559	−0.247	0.454	**0.666**	**0.735**	**0.762**	0.375	−0.423	−0.377	−0.257	0.316	0.141	0.166	−0.341	1.000

Notes: Bct, group fatty acids for bacteria markers; M.alg, green macroalgae biomarkers; LCFA, long chain fatty acids. A correlation is significant at 0.05 level (two-tailed), and significant values are shown in bold.

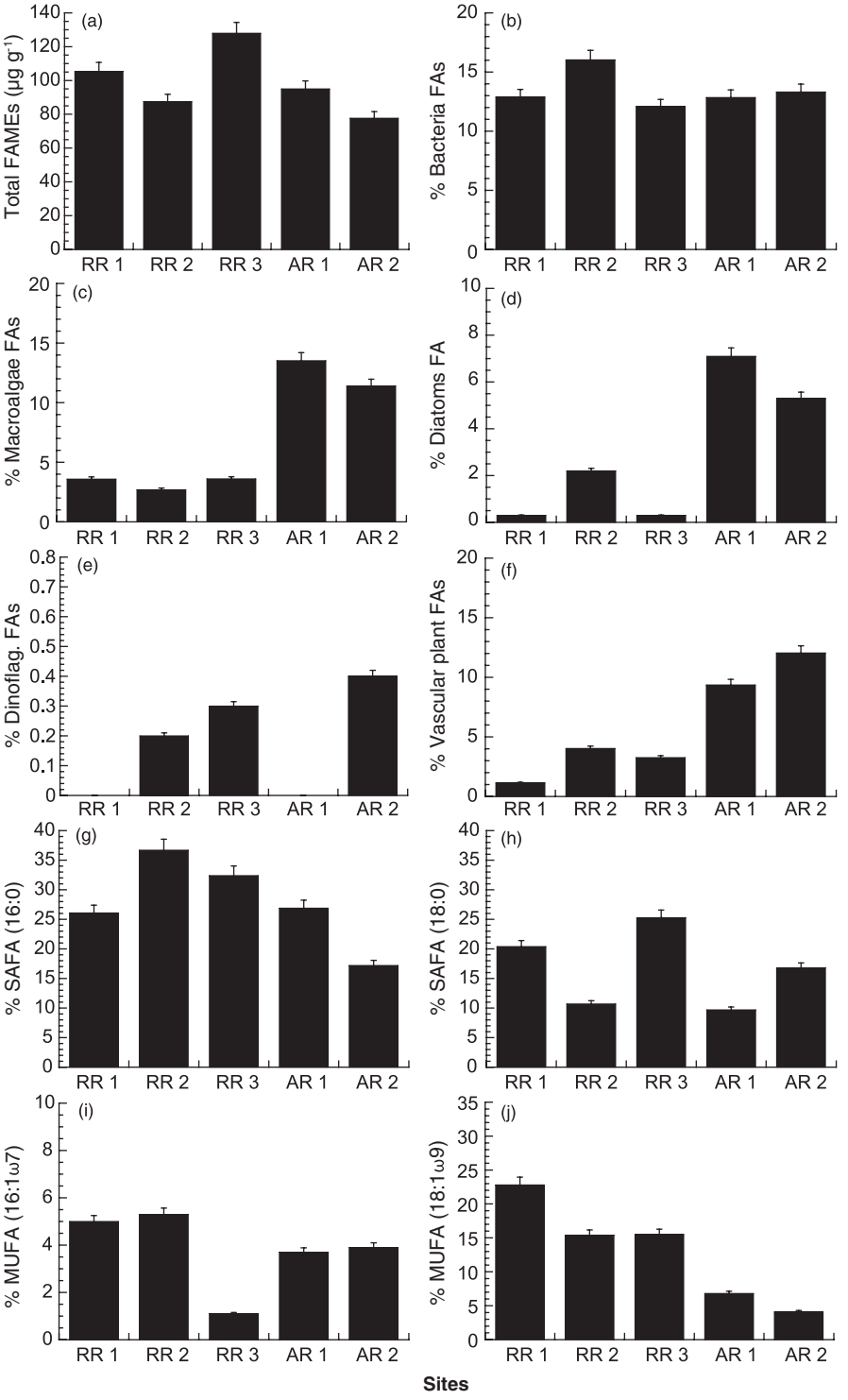

Figure 8. Relative fatty acid composition in organic matter suspended in residential (RR) and agricultural (AR) run-off. (a) Total fatty acid methyl esters (FAMEs); (b–j) contribution of selected fatty acid biomarkers: (b) bacterial FA, (c) macroalgal FA, (d) diatom FA, (e) dinoflagellates FA, (f) vascular plants FA, (g) saturated fatty acid (16:0), (h) saturated fatty acid (18:0), (i) monounsaturated fatty acid (16:1ω7), (j) monounsaturated fatty acid (18:1ω9). Values are shown as means ± SE.

summer than in winter (Figure 7b). Pearson's correlation coefficient test from combined data for both seasons showed a significant correlation between groups of FA bacteria markers and 16:1ω7, 18:1ω9 and 18:0 ($r = 0.756$, $p = 0.004$; $r = 0.776$, $p = 0.003$; and $r = 0.871$, $p = 0.000$, respectively) (Table 1).

The polyunsaturated FAs (PUFAs) 18:2ω6, 18:3ω3 and 18:3ω6, which are macroalgae markers, were generally found in higher proportions along the estuary in January (Figure 7c) than in July; however, relatively higher proportions were recorded particularly from stations TF, SW1 and SW2. These FAs were significantly correlated with groups of FA bacteria markers ($r = 0.734$, $p = 0.006$) (Table 1).

The FAs 20:5ω3 (diatom marker), 18:4ω3 and 22:6ω3 (dinoflagellate markers) appeared to be more abundant in samples from TF, SW1 and SW2 than in riverine stations during both July and January sampling (Figure 7d,e). Significant correlation was found between 20:5ω3 and the groups of FA macroalgae markers ($r = 0.804$, $p = 0.001$) (Table 1). However, no significant correlation was found between 22:6ω3 and any group of FA markers with the exception of individual FA 16:0 ($r = 0.728$, $p = 0.005$) (Table 1).

The proportion of long-chain FAs (LCFAs) C_{24}–C_{28}, which are vascular plant markers, was 0.5–9% in July and 0.1–10% in January. Significantly higher proportions were recorded from station M, followed by riverine stations (K3 and N1) and seaward stations (Figure 7f). There was no significant correlation between LCFAs and other groups of FA markers or individual FAs for the combined season data (Table 1). However, significant correlations were found between LCFAs and 16:0 and 22:6ω3 FAs ($r = -0.809$, $p = 0.001$ and $r = 0.864$, $p = 0.001$, respectively) in July, and between LCFAs and 16:1ω7 ($r = -0.763$, $p = 0.002$) in January (correlation tables for July and January are not shown).

3.3.2. *FAs in POM from wastewater run-off*

The FA compositions of residential and agricultural wastewater run-off are shown in Figure 8 and Table S3 (available online only). Total FAs from residential run-off were 105, 87.5 and 128.0 $\mu g \cdot g^{-1}$ for channels 1, 2 and 3, respectively, while those from agricultural run-off were 95 and 77.7 $\mu g \cdot g^{-1}$ (Figure 8a). In residential run-off, the dominant FAs in addition to the ubiquitous 16:0 and 18:0 FAs were 18:1ω9, bacterial FAs (18:1ω7 and branched FAs), and 16:1ω7, which made up 15.4–22.8%, 12.0–14.1% and 1.2–5.4% of the total FAs present in the water sample, respectively (Figure 8b,i,j). The PUFAs 18:2ω6, 18:3ω3 and 18:3ω6 (macroalgae markers) were also detected in residential run-off, contributing between 2.3 and 4.5% of the total FAs (Figure 8c).

The FA compositions and abundance for agricultural wastewater run-off were similar to those found in residential wastewater run-off, with higher contributions from 16:0, 18:0 (Figure 8g–h) and bacterial FAs (18:1ω7) (Figure 8b). However, unlike domestic wastewater run-off, there was a significant contribution of macroalgae FAs (18:2ω6 and 18:3ω6) and diatom FA (20:5ω3) to the total FAs detected in the agricultural wastewater run-off (11.3–13.2% and 5.3–7.1%, respectively) (Figure 8c,d). The component LCFAs (vascular plant markers) were also abundant in the agricultural run-off, contributing 9.1–12.0% of the total FAs (Figure 8f).

4. Discussion

4.1. *Inorganic nutrient pattern and behaviour along the river estuary*

Inorganic nutrient species showed variations in pattern and behaviour along the estuarine gradient associated with seasonal and subsequent differences in the river flow rate. In general, nutrients

were relatively high at low-salinity stations and rapidly decreased as salinity increased. Moreover, the strong negative correlation between these nutrients and salinity that was observed in this study (Figures 3 and 4) suggests freshwater influx, which is considered to be the main source of these nutrients in coastal waters [2]. The riverine end-members for dissolved inorganic nitrate (sum of NO_2^-, NO_3^- and NH_4^+) and dissolved inorganic PO_4^{3-} concentrations recorded in a previous study [17] were very high (DIN = 388 and 296 $\mu mol \cdot L^{-1}$ and DIP = 31 and 26.2 $\mu mol \cdot L^{-1}$ for the Kokuba and Noha rivers, respectively). This observation further supports the idea of upstream sources of these nutrients. Seasonally, the nutrient concentrations were lower throughout the estuary in May than in March and July. Heavy rains in May and June on Okinawa Island result in high river flow, which may have diluted the nutrients from the upstream sources.

Considering the pattern and behaviour of individual nutrients, non-conservative behaviour dominated the mixing pattern of PO_4^{3-}. Although the general trend of higher freshwater and lower saltwater concentrations was usually retained, there was a mid-estuary (station M) increase in PO_4^{3-} concentrations, especially during March and July (Figures 2–4). This increase was probably associated with the release of nutrients from suspended particles during early estuarine mixing [18] and/or release of PO_4^{3-} from decomposition of organic matter in the water column or from reducing sediments, which has also been identified as a source of PO_4^{3-} to the water column [19]. Given that this trend (mid-salinity increase in PO_4^{3-} followed by a seaward decrease) has been identified as a unifying aspect of PO_4^{3-} distribution in unperturbed estuaries [20], it is unlikely that the increase in PO_4^{3-} found in this study was mainly caused by its release from suspended particles. Moreover, in carbonate-dominated sediments typical of tropical and subtropical estuaries, authigenic fluorapatite formation appears to be an important permanent sink for PO_4^{3-}, while refractory organic detritus (e.g. mangrove) may be a long-term sink for PO_4^{3-} [21]. It seems likely, therefore, that the mid-estuarine increase was mainly linked to the decomposition of organic matter during and shortly after the blooms in late January through March, as noted in other studies [8,22].

The nutrients NO_3^-, NO_2^- and NH_4^+ also exhibited complex pattern distributions but had strong negative correlations with salinity (with the exception of NO_2^- in July), indicating no major addition or removal within the estuary. However, the frequent scatter of data points for NO_3^- and NH_4^+ along the mixing line (Figure 3) might suggest the occurrence of local addition and removal. An interesting trend was observed in July: there was a slight decrease in the concentration of NO_3^- and a subsequent increase in NH_4^+ concentration at salinity levels between 5 and 14 psu. This observation may be attributed to dissimilatory NO_3^- reduction to NH_4^+ [18] or changes associated with a catchment 'events' during the time of sampling. However, it has been reported that reduction of NO_3^- to NH_4^+ is not a major mechanism for generating NH_4^+ in water columns as it is in sediment [18]. Given that this particular estuary has higher algae carbon, which provides a better substrate (NH_4^+) for heterotrophic bacteria [8], it seems reasonable that denitrification does occur within the estuary; however, such a pattern is less distinct because of high nutrient concentrations frequently supplied by rivers. This suggestion is also supported by the occurrence of higher contributions of bacterial FAs and macroalgae markers within the estuary (see section 4.3).

4.2. *Dissolved and particulate organic matter along the river estuary*

The DOC distribution in surface water over the course of the study (Figure 5) and its dilution diagrams for March and July (Figures 3 and 4) provide information about recycling patterns and the behaviour of dissolved organic matter along the estuary. Higher concentrations were more frequently recorded at riverine than at mid-estuary and seaward stations, indicating the influence of terrestrial- and/or riverine-derived organic matter in the estuary. In May (heavy rain season), DOC concentrations were generally higher and less variable along the estuary than in other

months. This is because the freshet period delivers larger water volumes and is more influenced by run-off from surface soils and organic matter, resulting in dilute nutrient concentrations, high DOC levels, and peak turbidity [23]. This idea is well supported by the high TSM ($mg \cdot L^{-1}$) and low concentration of inorganic nutrient data obtained during the same time.

The plot of the DOC content against salinity (Figure 4) was essentially linear in July, indicating no major addition or removal within the estuary, while deviation from linearity was evident in March (Figure 3). Removal occurred at a salinity range of 3–6 psu, and apparent addition at a salinity range of 8–16 psu. The removal was probably due to abiotic sorption and transformations of DOC into other compounds or by microbial uptake [24]. In the Manko estuary, TSM could be as high as 48–120 $mg \cdot L^{-1}$ at a salinity of 3–9 psu (24-h monitoring, 2009, unpublished data). This condition appears to be favourable for adsorption of DOC into suspended particulate matter and their subsequent removal from the water column. Mid-estuary addition of DOC was probably due to *in situ* production from marine plankton during the bloom, which occurred in late January to March. Several authors have already reported that DOC concentrations increase 3–4 weeks after the start of the bloom in response to biological activity [25–27]. Although this observation is not well supported by our salinity-mixing graph for Chl-*a*, the overall seaward increases in Chl-*a* concentrations in this study (Figure 6g–j) and the high contributions from bacterial biomarker compounds (see section 4.3) within these stations support the above idea.

Suspended materials (POC, PN, TSM and Chl-*a*) in the Manko estuary followed patterns commonly found in other estuaries. TSM ($mg \cdot L^{-1}$) rapidly declined seaward, accompanied by increases in water transparency, POC and PN, and Chl-*a*. Several differences are apparent in the behaviour of TSM along the salinity gradient of the Manko estuary. The high TSM concentration in the estuary during the May survey may be linked to variations in the rate of freshwater supply and in the resuspension of bottom sediments, which also appear to limit primary production in the freshwater zone of the estuary.

The strong gradients in POC and PN in July (Figure 6b) following the heavy rain season (May–June) suggest strong freshwater influences in the estuary, an idea that is also supported by relatively high C/N ratios (10–30) (Figure 6e), which are usually associated with the dominance of terrigenous organic matter. Contrary to our result, organic load is generally expected to decrease as suspended particles decrease because the organic load concentrations depend primarily on the amount of suspended matter and then on the origin and age of the particulate material [28]. The seaward increase in POC and PN along the estuary in this study corresponded with both increased colonisation by marine organisms with increasing production found mid-estuary and the settling of estuarine materials. This is strongly supported by the low C/N ratios of 6–11, a typical range for fresh phytoplankton [29], found at mid- and high-salinity waters in this study. The C/N ratios are used as a tool to indicate the quality of the carbon and have been used along with FA analysis to infer sources and cycling of organic matter in aquatic systems [30]. Although not yet conclusive, the suspended material results generally suggest that an important fraction of terrestrial suspended matter with its associated organic load is rapidly sinking in the estuary and being replaced with marine plankton, which is abundant at mid- to high-salinity ranges (15–30) along the estuary.

4.3. *Sources of suspended organic matter*

A wide range of FAs along the estuary and the seasonal differences observed in this study indicate the contribution of a variety of organic matter sources and their relative contributions to the estuary. Specifically, 16:0, 18:0 and 16:1ω7, ascribed to a mixed planktonic source (i.e. phytoplankton, zooplankton and bacteria); 18:1ω7, usually synthesised by bacteria; and 18:1ω9, primarily derived from phytoplankton [31,32], appeared to be important contributors of particulate organic matter from almost all sampled stations along the estuary, especially during summer (July) (Figure 7).

However, these FAs, in addition to the PUFA 18:2ω6, have also been associated with domestic wastewater discharge, particularly during high river flow following periods of heavy rain [7,33]. Concurrently, 16:0, 18:0 and 18:1ω9 were recorded at pronounced levels in residential run-off (26.1–36.7%, 10.7–25.3% and 15.4–22.8%, respectively) (Figure 8g,h,j). According to Okinawa Prefectural Government data [6], domestic discharges account for ∼40% of the biological oxygen demand in the Manko estuary. Thus, relatively high levels of these FAs in suspended particulate matter along the estuary suggest that domestic wastewater is among the major contributors to the POM pool in the Manko estuary.

The contributions of PUFAs (macroalgae, diatom and dinoflagellate markers) associated with POM showed some variations along the estuarine salinity gradient, with relatively high-percentage contributions at mid- and high-salinity stations (TF, SW1 and SW2) in January (Figure 7c–e; Tables S1 and S2, available online only), which may also explain the high Chl-*a* concentrations and low C/N ratios observed at these particular stations (section 4.2). However, although Chl-*a* and C/N ratios suggest minimal algal inputs at the freshwater zone stations, ∼26–28% of the total FAs associated with POM were composed of PUFAs diagnostic for algal sources in January. These FAs are generally labile in nature [34] and are therefore not expected to be preserved in their original abundance. Their presence indicates a fresh input of algae, diatom and dinoflagellate sources along the salinity continuum [3]. This observation suggests that lipid biomarkers may be more sensitive than bulk organic matter characteristics (e.g. Chl-*a* and C/N ratios) that provide a weighted average measure of organic matter composition [30].

The considerable contributions (20–30.5%) of PUFAs during winter, particularly of the green macroalgae markers 18:2ω6 and 18:3ω3 [15] (Figure 7c), were not surprising because of the abundance of green macroalgae (*Ulva pertusa* and *Enteromorpha intestinalis*) throughout the estuary at this time of the year. However, the significant contribution of these biomarkers in summer (7.2–10.1%), although a smaller amount than in winter, indicates overenrichment [8] in the Manko estuary. The sum of these FAs ($\sum$18:2ω6 and 18:3ω3) has been used as an indicator of the relative importance of terrestrial material in marine environments [35]. Concurrently, macroalgae markers (18:2ω6 and 18:3ω3 FAs) were also recorded in relatively high amounts in agricultural wastewater run-off (11.5–13%) (Figure 8c). Moreover, the strong positive correlations between $\sum$18:2ω6 and 18:3ω3 and 18:0 in summer ($r = 0.944$, $p = 0.001$; table not shown) further support the contribution of terrestrial input into the estuary. This observation reinforces the possibility of input from agricultural products [8] and domestic wastewater [33] into the estuary. Although there were no significant differences between summer and winter in PUFA contributions, the FAs 20:5ω3 (biomarker for diatoms) and 22:6ω3 (biomarker for dinoflagellates) tended to be more highly represented at seaward than at riverine stations. In general, low abundance of PUFAs at riverine stations may suggest that the autochthonous signal at these stations is likely diluted with relatively high contributions from terrestrial organic matter sources.

Consistent with the above discussion, the contribution of LCFAs, which are markers of terrigenous vascular plants [36], was relatively higher at riverine stations and station M in both summer and winter (Figure 7f). The high contribution of these FAs at station M, which is near the mangrove forest, indicates the influence of mangrove organic matter to the water column. However, their presence at riverine stations with or without mangrove trees suggests that sources other than mangrove detritus are entering the estuary. Plausible sources of this terrigenous-derived organic matter to the estuary could be agricultural and domestic wastewater run-off, as this is also indicated in FA results for the agricultural wastewater, which showed relatively higher contributions of LCFAs (Figure 8f). The negative correlation between LCFAs and salinity ($r = -0.734$, $p = 0.007$) and the strong positive correlation between LCFAs and inorganic nutrients in summer ($r = 0.762$, $p = 0.004$; $r = 0.883$, $p = 0.000$; and $r = 0.811$, $p = 0.001$ for NO_3^-, NH_4^+ and PO_4^{3-}, respectively) (table not shown) further confirm the terrigenous sources of organic matter in the estuary. The depletion of this terrigenous signature downstream along the estuary, tracking

the trend of terrigenous organic carbon load (see section 4.2), is likely due to flocculation and sedimentation that provide a depositional sink for terrigenous organic matter [30].

The MUFA 18:1ω7 and odd-branched chain FAs (C_{15}–C_{17} iso and anteiso) are typically ascribed to bacterial contribution of the stock of organic matter associated with particles from estuarine and marine environments [36,37]. Consistent with the results of an earlier study [7], the contributions of these FAs were relatively higher in summer than in winter throughout the estuary (Figure 7b). Temporal variability in the abundance of bacterial FAs may reflect the availability of labile organic matter and/or processing of bioavailable organic matter by heterotrophic bacteria [3]. However, this should have been obvious in winter when there was a high contribution from labile algal organic matter throughout the estuary (Figure 8b). Therefore, the high contributions of bacterial FAs in summer might have been influenced by not only by the bioavailability of organic matter, but also the high summer temperature, which is associated with a high level of organic matter degradation by bacteria; this degradation may also enhance community metabolism and stimulate the recycling of nutrients [8,38].

5. Synthesis and concluding remarks

Comparison of nutrient and organic matter distributions between the Manko estuary and other reported estuarine systems (Table 2) suggest that the Manko estuary contains high amounts of dissolved inorganic (N and P) and organic (DOC) nutrients, which are largely influenced by terrestrial sources from the Kokuba and Noha rivers, and a moderate level of particulate nitrogen and carbon. Concentrations of dissolved inorganic nitrate (NO_2^-, NO_3^- and NH_4^+) and dissolved inorganic PO_4^{3-} were comparable with those of the Don estuary, the catchment of which is under intensive agriculture [20], but were much higher than those of the eutrophic Pearl and Loire estuaries. Because of substantial differences in the size (catchment area, km^2) and hence the water discharge rate between the estuaries reported in Table 2 and the Manko estuary, parallel

Table 2. Comparison of nutrients and organic matter distribution between Manko estuary and other estuarine systems across different climatic zones

Climate zone	River-estuary	Catchment area (km^2)	DIN ($\mu mol \cdot L^{-1}$)	DIP ($\mu mol \cdot L^{-1}$)	DOC ($mg \cdot L^{-1}$)	POC ($\mu g \cdot L^{-1}$)	PIN ($\mu g \cdot L^{-1}$)	Ref
Tropical	Tsengwen, Taiwan	1177	22–279	0.2–2.7	1.44–8.16	504–7800	–	[39]
	Cochin, India	256	13.4–40.9	1.25–2.61	1.58–34.2	65–146	–	[40]
Sub-tropical	Pearl, China	2100	50–110	0.2–1.2	0.56–25.7	120–5760	–	[41,42]
	Manko Okinawa	58	45–300	2.0–39.8	3.1–98.4	2.4–15.9*	0.1–2.3	This study
Arctic	Mackenzie, Canada	284	5.1–7.2	0.01–0.1	2.76–4.92	480–1920	42–112	[23]
Temperate	Ythan, UK	523	10–600	0.1–5	–	–	–	[20]
	Don, UK	1273	8–311	0.5–7.2	–	–	–	[20]
	The Loire, France	100	11.6–87.7	0.4–1.47	2.4–7.8	2620–30,000	–	[43,44]
	Great Bay, USA	44	4–250	0.1–1.3	3.24–8.52	–	–	[45]
	York, USA	~4350	0.4–12.5	0.1–6	0.3–8.6	50–250*	1.9–32*	[3,46]

Notes: *Measured in $mg \cdot g_{TSM}^{-1}$. DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphate; DOC, dissolved organic carbon; PIN, particulate inorganic nitrogen; POC, particulate organic carbon.

comparisons may be easily drawn from small island estuarine systems. However, there is a general absence of studies of these systems within the peer-reviewed literature.

In conclusion, the distribution of nutrients and organic matter in the Manko estuary seemed to be influenced by terrestrial sources from domestic and agricultural wastewater. However, most of these materials are largely transformed and decreased in amount because of flocculation and removal to sediment, microbial degradation and/or dilution by other organic matter sources from mid- and high-salinity production. Further studies to quantify the amount of terrestrial materials delivered to the coastal waters and their relative contribution to the East China Sea are expected to provide more insight into the effectiveness of estuarine processes in filtering such materials.

Collectively, our study contributes detailed information about nutrients and organic matter sources and distribution along the estuarine gradient in a subtropical river–estuary ecosystem. Such information and knowledge is useful in addressing basic questions related to the ecology and management of these ecosystems.

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References

[1] J.I. Hedges and R.G. Keil, *Organic geochemical perspectives on estuarine processes: Sorption reactions and consequences*, Mar. Chem. 65 (1999), pp. 55–65.
[2] J.I.N. Kumar, B. George, R.N. Kumar, P.R. Sajish, and S. Viyol, *Assessment of spatial and temporal fluctuations in water quality of a tropical permanent estuarine system-tapi, west coast India*, Appl. Ecol. Environ. Res. 3 (2009), pp. 267–276.
[3] R.E. Countway, E.A. Canuel, and R.M. Dickhut, *Sources of particulate organic matter in surface waters of the York River, VA estuary*, Org. Geochem. 38 (2007), pp. 365–379.
[4] R.W. Howarth, J.R. Fruci, and D. Sherman, *Inputs of sediment and carbon to an estuarine ecosystem: Influence of land use*, Ecol. Appl. 1 (1991), pp. 27–39.
[5] J.E. Cloern, *Our evolving conceptual model of the coastal eutrophication problem*, Mar. Ecol. Progr. Series 210 (2001), pp. 223–253.
[6] Y. Tashiro, A. Takemura, H. Fujii, K. Takahira, and Y. Nakanishi, *Livestock wastes as a source of estrogens and their effects on wildlife of Manko tidal flat, Okinawa*, Mar. Pollut. Bull. 47 (2003), pp. 143–147.
[7] A. Sakdullah and M. Tsuchiya, *The origin of particulate organic matter and the diet of tilapia from an estuarine ecosystem subjected to domestic wastewater discharge: Fatty acid analysis approach*, Aquat. Ecol. 43 (2008), pp. 577–589.
[8] I.S.S. Mchenga and M. Tsuchiya, *Nutrient dynamics in mangrove crab burrow sediments subjected to anthropogenic input*, J. Sea Res. 59 (2008), pp. 103–113.
[9] R.J. Parkes, *Analysis of microbial communities within sediments using biomarkers*, in: *Ecology of Microbial Communities*, M. Fletcher, T.R.G. Gray and J.G. Jones, eds., Cambridge University Press, Cambridge, 1987, pp. 147–178.
[10] T. Meziane and M. Tsuchiya, *Organic matter in a subtropical mangrove-estuary subjected to wastewater discharged: Origin and utilization by two macrozoobenthic species*, J. Sea Res, 47 (2002) pp. 1–11.
[11] L. Guo, J.K. Lehner, D.M. White, and D.S. Garland, *Heterogeneity of natural organic matter from the Chena River, Alaska*, Water Res. 37 (2003), pp. 1015–1022.
[12] Md.S. Islam, S. Shokita, P.L. Mfilinge, and M. Tsuchiya, *Status of organic and inorganic nutrients in waters and sediments at the habitat of the mangrove Sesarmid Crabs from the Ryukyu Island, Japan*, Bull. Facult. Sci. Univ. Ryukyus 78 (2004), pp 327–356.
[13] J.D.H. Strickland and T.R. Parsons, *A Practical Handbook of Seawater Analysis*, Fisheries Research Board of Canada Bulletin, Ottawa, Canada, 1972, 167 pp.
[14] E.G. Bligh and W.J. Dyer, *A rapid method of total lipid extraction and purification*, Can. J. Physiol. Pharmacol. 37 (1959), pp. 911–917.
[15] P.L. Mfilinge, T. Meziane, Z. Bachok, and M. Tsuchiya, *Litter dynamics and particulate organic matter outwelling from a subtropical mangrove in Okinawa Island, South Japan*, Est. Coast. Shelf Sci. 63 (2005), pp. 301–313.
[16] H. Yamashiro, H. Oku, H. Higa, I. Chinen, and K. Sakai, *Composition of lipids, fatty acids, and sterols in Okinawan Corals*, Comp. Biochem. Physiol. 122 (1999), pp. 397–407.

[17] D.J. Shilla, I., Mimura, K.K. Takagi, and M. Tsuchiya, *Preliminary survey of the nutrient dischsrge characteristics of Okinawa River, and their potential effects on inshore coral reefs*, in Proceedings of the 2nd Asia-Pacific Coral Reef Symposium, Phuket, Thailand, June 20–24, 2010 (in press).
[18] A.D. Tappin, *An examination of the fluxes of nitrogen and phosphorus in temperate and tropical estuaries: Current estimates and uncertainties*, Est. Coast. Shelf Sci. 55 (2002), pp. 885–901.
[19] M.E.Q. Pilson, *Annual cycles of nutrients and chlorophyll in Narragansett Bay, Rhode Island*, J. Mar. Res. 43 (1985), pp. 849–873.
[20] P.W. Balls, *Nutrient inputs to estuaries from nine Scottish east coast rivers; influence of estuarine processes in inputs to the North Sea*, Est. Coast. Shelf Sci. 39 (1994), pp. 329–352.
[21] R.W. Howarth, H.S. Jensen, R. Marino, and H. Postma, *Transport to and processing of P in near-shore and oceanic water*, in: *Phosphorus in the Global Environment*, H. Tiessen, ed., Wiley, Chichester, 1995, pp. 323–345.
[22] K.K. Satpathy, A.K. Mohanty, G. Sahu, M.V.R. Prasad, R. Venkatesan, U. Natesan, and M. Rajan, *On the occurrence of Trichodesmium erythraeum (Her.) bloom in the coastal waters of Kalpakkam, east coast India*, Ind. J. Sci. Technol. 1(2) (2007), pp. 1–8.
[23] C.A. Emmerton, F.W.L. Lesack, and F.W. Vicent, *Nutrient and organic matter patterns across the Mackenze River, estuary and shelf during the seasonal recession of sea-ice*, J. Mar. Syst. 74 (2008), pp. 741–755.
[24] D.J. Allan and M.M. Castillo, *Stream Ecology: Structure and Function of Running Waters*, Springer, Dordrecht, 2008, 436 pp.
[25] B. Norman, U.L. Zweifell, C.S. Hopkinson, and B. Fray, *Production and utilization of dissolved organic carbon during experimental diatom bloom*, Limnol. Oceanogr. 40 (1995), pp. 898–907.
[26] L. Bodineau, G. Thoumelin, and M. Wartel, *Fluxes and seasonal changes in composition of organic matter in the English Channel*, Cont. Shelf Res. 19 (1999), pp. 2101–2119.
[27] H. Fukuda, H. Ogawa, R. Sohrin, A. Yamasaki, and I. Koike, *Sources of dissolved organic carbon and nitrogen in Otsuchi Bay on the Sanriku ria coast of Japan in the spring*, Coast. Mar. Sci. 31 (2007), pp. 19–29.
[28] G. Cauet and I. Sidorov, *The biogeochemistry of Lena River: Organic carbon and nutrients distribution*, Mar. Chem. 53 (1996), pp. 211–227.
[29] J.C. Goldman, D.A. Caron, and M.R. Dennet, *Regulation of gross growth efficiency in bacteria by substrate C:N ratio*, Limnol. Oceanogr. 32 (1987), pp. 1239–1252.
[30] S.L. McCallister, J.E. Bauer, H.W. Ducklow, and E.A. Canuel, *Sources of estuarine dissolved and particulate organic matter: A multi-tracer approach*, Org. Geochem. 37 (2006), pp. 454–468.
[31] P.A. Cranwell, *Lipids of aquatic sediments and sedimenting particulates*, Prog. Lipid Res. 21 (1982), pp. 271–308.
[32] S.D. Killops and V.J. Killops, *Chemical composition of biogenic matter*, in *An Introduction to Organic Geochemistry*, S.D. Killops, ed., Langman Group UK Ltd, Harlow, 1993, pp. 22–62.
[33] M. Quemeneur and Y. Marty, *Fatty acids and sterols in domestic wastewater*, Water Res. 28 (1994), pp. 1217–1226.
[34] D.J. Smith, G. Eglinton, and R.J. Morris, *The lipid chemistry of an interfacial sediment from Peru continental shelf. Fatty acids, alcohols, aliphatic ketones and hydrocarbons*, Geochim. Cosmochim. Acta 47 (1983), pp. 2225–2232.
[35] S.M. Budge and C.C. Parrish, *Lipid biochemistry of plankton settling matter and sediments in Trinity Bay, New Foundland. II. Fatty acids*, Org. Geochem. 29 (1998), pp. 1547–1559.
[36] T. Meziane and M. Tsuchiya, *Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan*, Mar. Ecol. Prog. Series 200 (2000), pp. 49–57.
[37] E.A. Canuel, J.E. Cloern, D.B. Ringelberg, J.B. Guckert, and G.H. Rau, *Molecular and isotopic tracers used to examine sources of organic matter and its incorporation into the food webs of San Francisco Bay*, Limnol. Oceanogr. 40 (1995), pp. 67–81.
[38] T. Kaneda, *Iso- and anteiso- fatty acids in bacteria: Biosynthesis, function and taxonomic significance*, Microbiol. Rev. 55 (1991), pp. 288–302.
[39] J.J Hung and M.H. Huang, *Seasonal variations of organic-carbon and nutrient transport trough a tropical estuary (Tsengwen) in southwestern Taiwan*, Environ. Geochem. Health 27 (2005), pp. 75–95.
[40] G.V.M. Gupta, S.D. Thottathil, K.K. Balanchandran, N.V. Madhu, P. Madeswaran, and S. Nair, *CO_2 supersaturation and net heterotrophy in a tropical estuary (Cochin, India): Influence of anthropogenic effect*, Ecosystems 12 (2009), pp. 1145–1157.
[41] X.P. Huang, L.M. Huang, and W.Z. Yue, *The characteristics of nutrients and eutrophication in the Pearl River estuary, South China*, Mar. Pollut. Bull. 47 (2003), pp. 30–36.
[42] B. He, M. Dai, W. Zhai, L. Wang, K. Wang, J. Chen, J. Lin, A. Han, and Y. Xu, *Distribution, degradation and dynamics of dissolved organic carbon and its major compound classes in the Pearl River estuary, China*, Mar. Chem. 119 (2010), pp. 52–64.
[43] G. Abril, H. Etcheber, B. Deille, M. Frankignoulle and A.V. Borges, *Carbonate dissolution in the turbid and eutrophic Loire estuary*, Mar. Ecol. Prog. Series 259 (2003), pp. 129–138.
[44] M. Meybeck, G. Cauwet, S. Dessery, M. Somville, D. Gouleau, and G. Billen, *Nutrients (Organic C, P, N, Si) in the eutrophic River Loire (France) and its estuary*, Est. Coast. Shelf Sci. 27 (1988), pp. 595–624.
[45] C.W. Hunt and J.E. Salisbury, *Contrasting carbon dioxide inputs and exchange in three adjacent New England estuaries*, Est. Coast. 34(1) (2011), pp. 68–77.
[46] R.H. Condon, D.K. Steinberg, and D.A. Bronk, *Production of dissolved organic matter and inorganic nutrients by gelatinous zooplankton in the York River estuary, Chesapeake Bay*, J. Plankton Res. 32 (2010), pp. 153–170.