

## Variability in the origin of carbon substrates for bacterial communities in mangrove sediments

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### Abstract

Organic carbon in mangrove sediments originates from both local sources (mangroves, microphytobenthos) and tidal inputs (e.g. phytoplankton, seagrass-derived material). The relative inputs of these sources may vary strongly, both within and between different mangrove sites. We combined elemental (TOC/TN) and bulk  $\delta^{13}\text{C}$  analysis on sediment cores from various mangrove sites with  $\delta^{13}\text{C}$  data of bacteria-specific phospholipid fatty acids (PLFA) in order to identify the dominant carbon substrates used by in situ bacterial communities.  $\delta^{13}\text{C}$  values of each of these markers showed a range of 10‰ or more across the different sites and sampling depths, but generally followed the  $\delta^{13}\text{C}$  trend observed in bulk organic carbon. Several sediment cores show a strong vertical gradient in PLFA  $\delta^{13}\text{C}$ , suggesting a selectivity for algal-derived carbon in the surface layers. Our data demonstrate that the origin of bacterial carbon substrates varies widely across different mangrove sites, and imply that data on mineralization of organic matter cannot be directly incorporated in ecosystem carbon budgets without an estimation of the contribution of various sources.

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### 1. Introduction

Mangroves are known as highly productive ecosystems (global litterfall of 100 Tg C  $\text{y}^{-1}$  [1]) and recent estimates that as much as 11% of the total organic C inputs across the land–ocean interface (i.e. through riverine transport) is of mangrove origin show that the carbon fixed by mangroves is potentially significant in the carbon budget of the coastal zone [1]. Such estimates are not well constrained, however, and our understanding of the ecological fate of these large quantities of organic matter is far from complete. It has become clear that a strong interaction exists between the inter-

tidal zone and the adjacent aquatic environment, i.e. both import (of terrestrial material, phytoplankton, seagrasses, etc.) and export (of mangrove-derived organic matter) which are expected to have major consequences for the carbon dynamics in both compartments [2–4]. The imported carbon sources have an important trophic role in sustaining macro-invertebrate communities in the intertidal zone [5] – at least in estuarine systems where tidal import is significant. Mineralization, however, could represent a major fate for exported organic matter [6,7], but its role remains insufficiently quantified. Similarly, the few available estimates [8–11] indicate that intense mineralization takes place in intertidal mangrove sediments. However, no data are currently available that directly identify the carbon sources sustaining these microbial communities. In

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temperate salt marsh ecosystems, however, a direct link between local vascular plant production and bacterial communities does not necessarily exist, and selectivity for imported phytodetritus and local microphytobenthos has been demonstrated in some systems [12]. We have previously found that a continuous range of mangrove ecosystem types exists, from ‘retention’ systems where sediments are rich in organic carbon which is almost entirely of local origin, to ‘flow-through’ systems with mineral sediments (i.e. relatively low in organic carbon) where the organic matter can be dominated by imported sources [3].

Compound-specific stable isotope analysis is a major analytical tool enabling the study of microbial processing of organic matter [13]. The analysis of PLFA (phospholipid-derived fatty acids) in particular has found wide applications (reviewed in [14]). It has, for example, been used to study the sources of carbon assimilated by microbial communities [12,15,16] and to assess the involvement of specific microbial groups in biogeochemical processes [17]. As PLFA are cell membrane components that are rapidly degraded after cell death, they can be considered representative of the ‘living’ microbial biomass. The paucity of data on  $^{13}\text{C}$  fractionation between specific PLFA and the natural carbon substrates under different environmental conditions still limits a more quantitative use of PLFA  $\delta^{13}\text{C}$  data in delineating substrate utilization by bacteria [13]. Nevertheless, the natural abundance of  $^{13}\text{C}$  in PLFA across gradients of available substrates is a powerful approach to study the assimilation of different substrates under natural conditions [12,14,18].

Thus, we aimed at verifying to what extent the dominant carbon substrates for sedimentary bacterial communities vary across different mangrove sites, by comparing  $\delta^{13}\text{C}$  data of bacteria-specific PLFA with bulk properties ( $\delta^{13}\text{C}$  and TOC/TN) of sediment organic matter from a number of mangrove sites in the Indian subcontinent, varying in the degree of retention of local vascular plant production and tidal import of aquatic carbon sources.

## 2. Materials and methods

### 2.1. Study sites and sample collection

Sediment cores were collected in November and December 2002 in three mangrove systems: (1) Pambala–Chilaw lagoon, western Sri Lanka (07°35′N–79°47′E), (2) the estuarine mangroves of Pichavaram, south-east India (11°27′N, 79°17′E), and (3) a small fringing riverine mangrove stand along the Chunnambar river (11°53′N, 79°48′E), south-east India.

Pambala lagoon can be characterized as a low-tidal amplitude system (tidal variations rarely exceeds 0.2 m

and much of the forest is rarely inundated [19]), with  $\sim 3.5\text{ km}^2$  of fringing mangroves. Surface water salinity varies strongly both seasonally and spatially, between 0 and 55‰. At this site, one sediment core was taken in each of five different forest zones, each characterized by a different dominant mangrove species, i.e. *Rhizophora mucronata*, *Bruguiera gymnorhiza*, *Avicennia officinalis*, *Excoecaria agallocha*, and *Lumnitzera racemosa*. One additional core was taken at a recently converted site, which was formerly an unvegetated mudflat but is now dominated by ( $\sim 10$  yr old) *R. mucronata*. The Pichavaram mangroves are located  $\sim 250$  km south of Chennai (Tamil Nadu, India) in the estuaries of the Vellar and Coleroon. Tidal range at this site is reported to be 0.5–1.0 m. One sediment core was collected at a site dominated by *A. officinalis*, and one at a mixed species stand close to a creek border. A sediment core was also collected at a small stand of fringing mangroves ( $< 10$  m width) along the Chunnambar river (between Pondicherry and Cuddalore, Tamil Nadu), with mostly *A. officinalis* and *R. mucronata*. We have no precise data on the tidal range at this site, but expect it to be similar to that in the nearby Pichavaram mangroves (i.e. 0.5–1 m). Unfortunately, data on mangrove biomass or productivity are lacking for all the mangrove sites studied here.

All cores from Pambala and Pichavaram were subsectioned at 0–1, 1–2, 2–4, and 4–10 cm. For the site in Chunnambar, only three intervals were taken: 0–1, 1–3, and 3–5 cm. Samples were stored frozen and transported on dry ice back to the home laboratory where they were freeze-dried and stored frozen until further processing.

### 2.2. Analytical procedures

Sediments were ground to a fine powder using a mortar and pestle. Subsamples for elemental (TOC/TN, atom) and bulk stable isotope composition were acidified with dilute (5%) HCl before analysis to remove carbonates. Concentrations of organic carbon and total nitrogen, and TOC/TN ratios were determined by combusting preweighed samples in a ThermoFinnigan Flash1112 elemental analyzer; acetanilide (Merck) was used for calibration.  $\delta^{13}\text{C}$  analysis of TOC was performed with the aforementioned elemental analyzer, linked with a ThermoFinnigan delta +XL via a ConFlo III interface, with a typical reproducibility of  $\pm 0.15\%$ .

Extraction and derivatization of PLFA for compound-specific  $\delta^{13}\text{C}$  analysis followed the general outline of [12]. Briefly, 2–6 g of sediment (dry weight) were subjected to a modified Bligh and Dyer extraction [20] using chloroform/methanol/water at a 1:1:0.9 ratio. Total lipids retrieved in the chloroform phase were partitioned on silica gel columns by sequential elution with chloroform, acetone, and methanol. The polar fraction was subjected to mild alkaline transmethylation (using methanolic Na) at room temperature to form

FAMES (fatty acid methyl esters).  $\delta^{13}\text{C}$  values of PLFA were determined on a HP6890 GC coupled to a ThermoFinnigan delta+XL via a GC/C III interface. All samples were run in splitless mode and at an injector temperature of 250 °C, using a HP-5 column or BPX-70 column (30 m, 0.32 mm ID) with a He flow rate of 2 ml/min. Temperature was programmed at 50 °C for 2 min, then at 40 °C min<sup>-1</sup> to 130 °C, and at 3 °C min<sup>-1</sup> to 240 °C. FAMES are identified based on their retention times (with 12:0 and 19:0 as internal standards) and quantified based on their peak areas using 19:0 as the quantitative internal standard. The  $\delta^{13}\text{C}$  values of FAMES are corrected for the addition of the methyl group by simple mass balance, and using a measured  $\delta^{13}\text{C}$  value of -40.3‰ for the methanol used for derivatization. The reproducibility of the PLFA  $\delta^{13}\text{C}$  analysis on well resolved compounds is typically 0.3‰, but can be lower ( $\pm 0.6\%$ ) for major PLFA considered from natural samples. All stable isotope ratios are expressed relative to the conventional standard (VPDB limestone) as  $\delta$  values, defined as

$$\delta^{13}\text{C} = \frac{X_{\text{sample}} - X_{\text{standard}}}{X_{\text{standard}}} \times 10^3 \text{ [‰]}$$

where  $X = {}^{13}\text{C}/{}^{12}\text{C}$ . The typically negative  $\delta^{13}\text{C}$  values for biological compounds indicate that they have less  ${}^{13}\text{C}$  than the reference material; compounds with more negative  $\delta^{13}\text{C}$  values are referred to as being 'depleted' in  ${}^{13}\text{C}$ , less negative  $\delta^{13}\text{C}$  values indicate a relative 'enrichment' in  ${}^{13}\text{C}$ . PLFA data were calibrated using a mix of fatty acid methyl esters in hexane, for which  $\delta^{13}\text{C}$  values were measured using both traditional combustion (with a ThermoFinnigan Flash 1112 elemental analyzer coupled to a delta+XL) and using the GC/C under conditions identical to those for environmental samples.

Fatty acid notation [21] contains the number of C atoms, followed by a colon and the number of double bonds. The position of the first double bond (from the aliphatic end of the fatty acid) is indicated after the ' $\omega$ '. Prefixes 'i' (iso) and 'a' (anteiso) refer to the location of a methyl group 1 or 2 carbons from the aliphatic end, respectively. Methyl branches in other positions are indicated by the prefix 'Me' (e.g. 10Me16:0), and the 'cy' prefix indicates a cyclopropane fatty acid. 'c' and 't' refer to the cis or trans geometry of double bonds.

Estimates of bacterial carbon were derived from the combined abundance of selected bacterial PLFA (i14:0, i15:0, a15:0, i16:0, and 18:1 $\omega$ 7c), by assuming that these represent an average of  $28 \pm 4\%$  of the total bacterial PLFA pool and taking an average PLFA concentration in bacteria of 0.056 g PLFA carbon per g bacterial C, i.e. the average taken for aerobic and anaerobic bacteria (see [22] for references). A second approach was to assume that the majority of PLFAs were of bacterial origin (as microalgal markers were only detectable in significant amounts in two sites, see Section 4), and by

using the total PLFA concentration as a proxy for bacterial C, and as in the previous approach assuming a conversion factor of 0.056 between total PLFA-derived carbon and bacterial C (both in mg/g). A comparison of both approaches (i.e. using total PLFA concentrations versus specific PLFA concentrations) gave an excellent correlation ( $R^2 = 0.98$ ) with a slope of 1.15, i.e. the 'total PLFA concentration' approach gave only slightly higher estimates, and this mostly in samples with high microbial abundances. Therefore, all bacterial C estimates given below are based on the sum of selected bacterial PLFA concentrations. Although these approaches have some inherent uncertainties, they should provide us with a consistent comparative measure of bacterial carbon stocks.

Statistical relationships between  $\delta^{13}\text{C}$  of bulk TOC and specific bacterial PLFAs were examined using bivariate least squares (BLS) regression, which takes into account the different variability of both parameters. For other parameters, Spearman's rank order correlation analysis, BLS regression or multiple linear regression analysis was performed using the Statgraphics software package.

### 3. Results

Sedimentary organic carbon content (% dry weight basis) varied from 0.4% to 37.2%, being lowest in the cores taken at the riverine site at Chunnambar ( $0.5 \pm 0.1\%$ ,  $n = 3$ ) and highest in several of the forest patches at the lagoonal site, i.e. Pambala ( $33.0 \pm 3.6\%$ ,  $26.2 \pm 8.3\%$ , and  $25.1 \pm 6.5\%$  for the *Rhizophora*, *Bruquiera* and *Avicennia* stand, respectively). Intermediate values were found in the two estuarine sites in Pichavaram ( $1.1 \pm 0.1\%$  and  $5.4 \pm 2.0\%$ ), at the afforested *Rhizophora* site in Pambala ( $12.3 \pm 4.5\%$ ) and in the *Excoecaria* patch in Pambala ( $3.9 \pm 1.3\%$ ), the latter occurring at slightly elevated, sandier sites than the other mangrove species (Fig. 1, Table 1). In most sediment cores, TOC decreased stepwise with depth (Table 1), although in two cores the 4–10 cm section showed higher TOC than the 2–4 cm layer. Overall, the reduction in TOC between the top layer (0–1 cm) and the deepest layer sampled (typically 4–10 cm) averaged  $42 \pm 23\%$  of the top layer concentration. Elemental ratios (TOC/TN, atom) of sediments similarly showed a wide range of values, ranging from 7.5 to 21.3, but without consistent depthwise trends (Table 1). Lowest TOC/TN ratios were generally associated with sediments poor in organic carbon at the riverine and estuarine sites, while high TOC values coincided with high TOC/TN ratios in the lagoonal sites (Spearman's rank correlation:  $p < 0.0001$ ; Fig. 1, Table 1). Overall, bulk organic matter  $\delta^{13}\text{C}$  values ranged between -28.6 and -22.4‰, with more enriched values in the low-organic

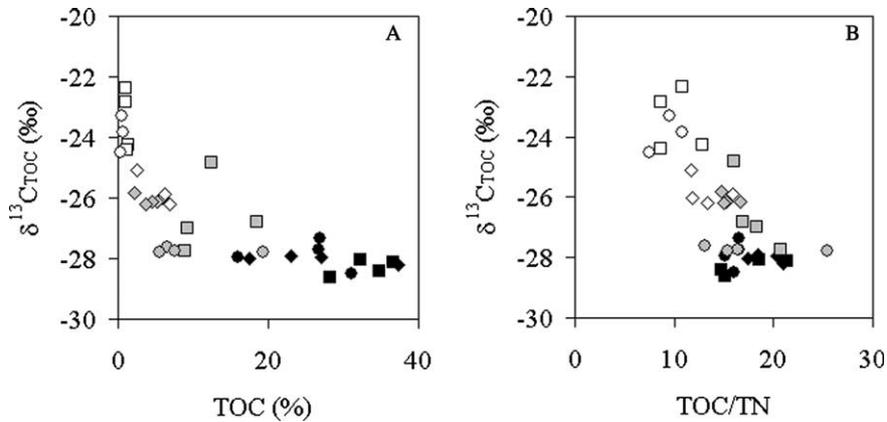


Fig. 1. Plot of (A) sedimentary organic carbon content (in % DW) versus  $\delta^{13}\text{C}$  of bulk organic carbon, and (B) sedimentary TOC/TN ratios versus  $\delta^{13}\text{C}$  of bulk organic carbon for sediment cores from different mangrove sites. Each datapoint represents one depth section of a sediment core. Black squares: Pambala, *Rhizophora* site. Black diamonds: Pambala, *Bruguiera* site. Black circles: Pambala, *Avicennia* site. Grey squares: Pambala, planted *Rhizophora* site. Grey diamonds: Pambala, *Excoecaria* site. Grey circles: Pambala, *Lumnitzera* site. Open squares: Pichavaram, mixed species stand. Open diamonds: Pichavaram, *Avicennia* site. Open circles: Chunnambar.

carbon sites, and more depleted values close to those of mangrove biomass in organic-rich sediments (Spearman's rank correlation:  $p < 0.0001$ ; Fig. 1, Table 1).

Total sedimentary PLFA concentrations ranged between 2.3 and 352.9  $\mu\text{g g}^{-1}$  (DW), and were consistently highest in the top layer, except in the mixed species zone in Pichavaram where no consistent depthwise variations were observed (Table 1). In most cases, PLFA concentrations decreased stepwise with depth, but in some cores there was a slight increase again in the deepest layer (replanted site and *Bruguiera* site in Pambala) or in the 2–4 cm layer (*Excoecaria* site, Pambala). The overall decrease in total PLFA concentrations between the upper 1 cm and the 4–10 cm section averaged  $70 \pm 16\%$  of the upper layer concentration (excluding the data from the mixed species core in Pambala). Bacterial carbon stocks, estimated based on the combined abundance of i14:0, i15:0, a15:0, i16:0, and 18:1 $\omega$ 7c, ranged between 0.1 and 4.5  $\mu\text{g C g}^{-1}$  DW, and showed a similar relative decrease with depth ( $75 \pm 13\%$  between the upper 1 cm and the deepest layer considered, excluding the data from the mixed species site in Pichavaram) as observed in the total PLFA concentration data (Table 1). The depthwise decrease in total PLFA and bacterial carbon stocks generally accompanies the depthwise reduction of TOC and TN concentrations in these sediments (Fig. 2A and B, Spearman's rank order correlation:  $p < 0.0001$ ), although the decrease in PLFA concentration was also noted in the case where TOC values did not decrease markedly with depth (i.e. the natural *Rhizophora* stand in Pambala). The contribution of bacterial C to the TOC pool decreased significantly with increasing TOC/TN ratios (Spearman's rank order correlation:  $p = 0.0026$ ; Fig. 2C). PLFA profiles (two typical examples are presented in Fig. 3) generally show high concentrations of 10Me16:0, and in particular cy19:0.

$\delta^{13}\text{C}$  values of selected bacterial PLFA varied widely between different sites. i+a15:0 (both PLFAs combined – although peak overlap was minimal, we combine these for reasons of comparison with fractionation factors given by Boschker et al. [12]) ranged between  $-33.9$  and  $-23.6\%$ , and markers specific for sulfate-reducing bacteria such as 10Me16:0 and i17:0 [23] showed similar ranges ( $-37.5$  to  $-24.9\%$  and  $-33.5$  to  $-24.1\%$ , respectively). In general, an overall linear relationship between bacterial PLFA  $\delta^{13}\text{C}$  values and bulk organic matter  $\delta^{13}\text{C}$  values was observed (BLS regression:  $p < 0.001$  for the examples in Fig. 4A–C,  $p < 0.05$  for cy19:0 shown in Fig. 4D), with a slope typically between 1.1 and 1.5, albeit in most cases not significantly different from unity (see regression formulas in Fig. 4). In two sites (Chunnambar and the *Lumnitzera* site in Pambala), however, large deviations in the slope of this relationship were observed (see Fig. 4), with much larger depthwise variation in the  $\delta^{13}\text{C}$  of bacterial markers than in the bulk sediment organic carbon (Table 1).

## 4. Discussion

### 4.1. Sediment organic matter: stocks and origin

The range of TOC, TOC/TN, and  $\delta^{13}\text{C}$  values found for bulk sedimentary organic matter is similar to that reported earlier in a comparative analysis of data from various mangrove systems [3]. The inverse relationship between  $\delta^{13}\text{C}_{\text{TOC}}$  and the TOC content or TOC/TN ratios is consistent with the simple two-source mixing model proposed in [3]. Briefly, mangrove litter and aquatic suspended matter (which can be composed of various sources e.g. phytoplankton, upriver terrestrial matter, seagrass litter) are considered to be the main

Table 1

TOC content, TOC/TN ratios (atom), total PLFA concentrations, calculated bacterial C stocks, the contribution of bacterial C to the TOC pool, and the carbon isotope composition of bulk TOC and the bacterial PLFA  $i+a15:0$  (corrected for fractionation according to [12]) for all sediment cores examined

Site/zone	Depth	TOC (%)	TOC/TN	Total PLFA ( $\mu\text{g g}^{-1}$ )	Bacterial C ( $\text{mg g}^{-1}$ )	Bacterial C/TOC (%)	$\delta^{13}\text{C}_{\text{TOC}}$	$\delta^{13}\text{C}_{i+a15:0}$
Pambala, Rhizophora	0–1	34.8	14.7	352.9	3.38	0.97	–28.4	–25.3
	1–2	28.2	15.1	194.3	1.69	0.60	–28.6	–26.0
	2–4	32.3	18.6	124.9	0.96	0.30	–28.1	–26.5
	4–10	36.6	21.3	105.6	0.95	0.26	–28.1	–27.8
Pambala, Bruguiera	0–1	37.2	21.0	243.5	2.84	0.76	–28.2	–25.4
	1–2	27.1	20.4	148.4	1.46	0.54	–27.9	–25.9
	2–4	17.5	17.5	55.4	0.57	0.32	–28.0	–27.1
	4–10	23.0	18.4	60.5	0.61	0.26	–27.9	–26.9
Pambala, Avicennia	0–1	31.0	16.0	148.2	1.76	0.57	–28.4	–24.8
	1–2	26.9	16.6	134.3	1.75	0.65	–27.4	–25.8
	2–4	26.7	16.5	75.3	0.83	0.31	–27.7	–26.5
	4–10	15.9	15.1	49.6	0.65	0.41	–28.0	–26.4
Pambala, planted Rhizophora	0–1	18.5	17.0	70.6	0.97	0.52	–26.8	–21.5
	1–2	12.5	16.0	66.8	0.80	0.64	–24.8	–20.9
	2–4	9.2	18.4	25.8	0.27	0.29	–27.0	–21.9
	4–10	8.9	20.8	31.4	0.24	0.27	–27.7	–24.0
Pambala, Excoecaria	0–1	5.2	16.7	9.2	0.12	0.22	–26.1	–23.8
	1–2	4.6	15.2	5.0	0.05	0.10	–26.1	–25.2
	2–4	3.7	15.0	9.1	0.10	0.28	–26.2	–24.6
	4–10	2.2	14.8	5.5	0.06	0.25	–25.8	–25.5
Pambala, Lumnitzera	0–1	19.3	25.44	229.8	2.71	1.41	–27.8	–20.3
	1–2	6.5	13.03	62.1	0.54	0.82	–27.6	–25.5
	2–4	7.5	16.43	36.7	0.26	0.35	–27.8	–20.3
	4–10	5.5	15.41	28.7	0.15	0.28	–27.8	–25.6
Pichavaram, mixed species	0–1	1.3	12.8	12.3	0.10	0.75	–24.3	–20.2
	1–2	1.2	8.6	16.7	0.15	1.28	–24.4	–19.2
	2–4	1.1	8.6	13.8	0.12	1.16	–22.8	–19.5
	4–10	1.0	10.8	11.9	0.13	1.28	–22.4	–20.0
Pichavaram, Avicennia	0–1	6.9	13.3	168.9	1.95	2.82	–26.2	–21.8
	1–2	5.8	11.8	131.8	1.40	2.40	–26.0	–22.4
	2–4	6.3	15.8	47.3	0.52	0.83	–25.9	–22.2
	4–10	2.5	11.7	25.1	0.25	1.01	–25.1	–21.1
Chunnambar	0–1	0.6	9.5	12.1	0.13	2.32	–23.3	–17.5
	1–3	0.6	10.8	3.9	0.04	0.62	–23.8	–21.7
	3–5	0.4	7.5	2.3	0.03	0.71	–24.5	–23.2

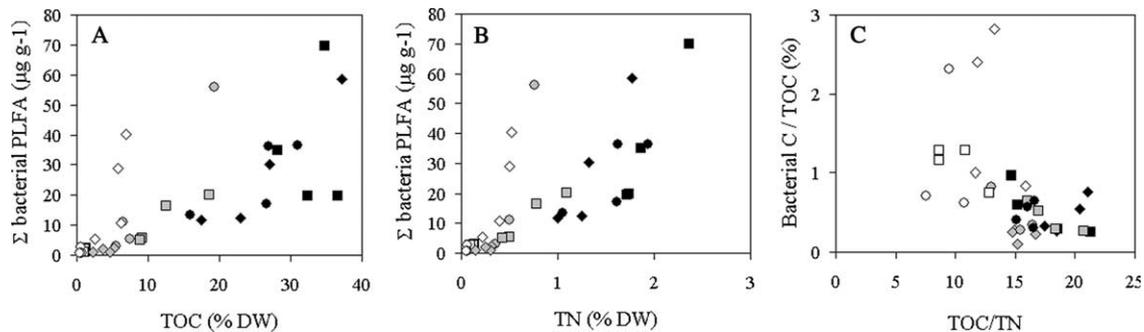


Fig. 2. Sum of selected bacterial PLFA concentrations used to estimate bacterial C stocks (see text for details) versus (A) total sedimentary organic carbon (in %, DW), (B) total nitrogen (% DW), and (C) the contribution of bacterial C to the total organic carbon pool versus the elemental (TOC/TN, atom) ratio of sediments. Each datapoint represents one depth section of a sediment core. Symbols as in Fig. 1.

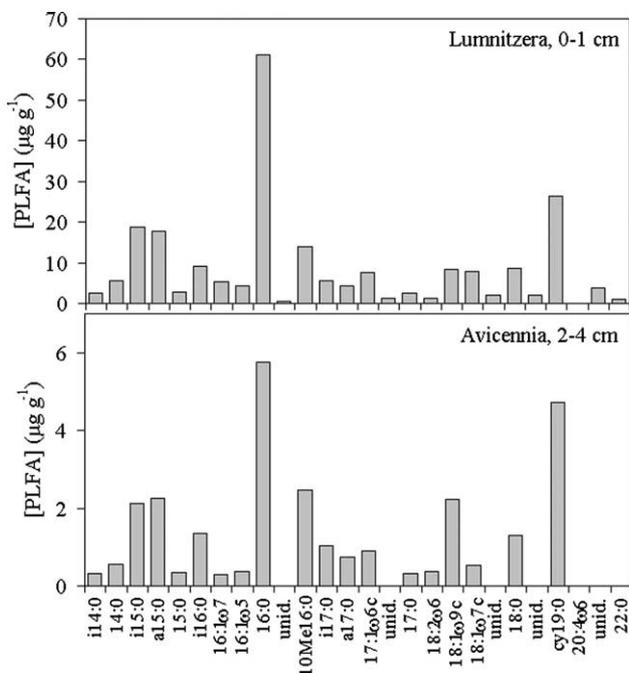


Fig. 3. Typical example of PLFA concentration profiles for two selected samples: (A) surface layer (0–1 cm) of the *Lumnitzera racemosa* site in Pambala, Sri Lanka, and (B) 2–4 cm depth section of the *Avicennia marina* site in Pambala, Sri Lanka.

end-members. Mangrove litter is characterized by a high C content ( $\sim 45\%$ ), high C/N ratios (25–50), and low  $\delta^{13}\text{C}$  ( $\sim -28\text{‰}$ ), whereas tidally imported material has a low organic C content (0.5–3%), lower C/N ratios (6–12), and a higher but variable  $\delta^{13}\text{C}$  signature ( $-28$  to  $-20\text{‰}$ , note that all ranges given here are indicative). Despite these obvious simplifications and the variability in input parameters, the variations in bulk sediment C and N concentrations and  $\delta^{13}\text{C}$  generally reflect the variable inputs of local mangrove litter and imported carbon sources from the aquatic environment. From the observed relationships, we can classify most of the forest zones in the lagoonal system of Pambala (low tidal amplitude, much of the mangrove zone rarely inundated) as ‘retention’ sites where mangrove carbon is the dominant contributor to the sediment organic carbon pool, whereas the sediments from Pichavaram and Chunnambar (tidal range up to 1 m) reflect a balance between local inputs (possibly including microphytobenthos) and carbon imported from the water column during high tide. Cores from the afforested and natural *Rhizophora* sites in Pambala, however, differed strikingly in their organic carbon content (average  $\pm 1$  s.d. for the 4 depth sections are  $12.3 \pm 4.5\%$  versus  $33.0 \pm 3.6\%$  for the afforested and natural site, respectively), and slightly more enriched  $\delta^{13}\text{C}$  values ( $-26.6 \pm 1.2\text{‰}$  versus  $-28.3 \pm 0.3\text{‰}$ ) but similar TOC/TN ratios ( $18.0 \pm 2.1$  versus  $17.4 \pm 3.1$ ). This difference was expected, as the afforested site was previously a bare mudflat (i.e. which typically have much lower concentrations of organic

carbon, and less inputs from the mangrove vegetation), which has been planted in 1993. Depthwise, a fairly consistent trend of decreasing TOC concentrations was found in all sediment cores, except in the natural *Rhizophora* stand from Pambala. When comparing TOC concentrations in the top layer (0–1 cm) with those in the deepest layer sampled, a relative decrease of  $42 \pm 23\%$  in organic carbon content is observed. For TOC/TN ratios and bulk  $\delta^{13}\text{C}$  ratios, no consistent trends with depth can be discerned. The most likely explanation for the depthwise decrease in TOC could be the fact that the main inputs of TOC in the sediment layers considered is from aboveground processes (i.e. litterfall and tidal deposition) rather than from belowground inputs (i.e. root exudation and root turnover), with subsequent mineralization resulting in a decrease in TOC over time, and thus, with depth. Nevertheless, it should be kept in mind that our data only consider the top 10 cm of the sediment, and that inputs from roots may only be evident in deeper layers.

#### 4.2. Abundance patterns of bacterial populations: estimates based on PLFA profiles

In all cores considered, except in the mixed species stand in Pichavaram, bacterial C stocks decreased consistently with depth, with an average relative reduction by  $75 \pm 13\%$  between the top layer (0–1 cm) and the deepest layer sampled (usually 4–10 cm). This pattern clearly identifies the sediment surface layer as the dominant site of bacterial activity, and is therefore consistent with the abovementioned interpretation that the depthwise decrease of TOC would result from mineralization. As the decrease in bacterial C with depth is more pronounced than the decrease in TOC with depth, the contribution of live bacterial biomass to the total organic carbon is highest at the sediment surface layer (see Table 1). Alongi [8] noted that bacterial densities in mangrove sediments are related to a number of factors, such as organic C and N content, and particle size density – but no depthwise distribution was studied. In our dataset, bacterial C stocks were correlated with both the total stocks of organic carbon and total nitrogen (see Fig. 2A and B), the elemental ratios (TOC/TN, see Fig. 2C), and depth. The latter is evident from the observation that (i) the TOC decrease with depth averaged  $42 \pm 23\%$ , which is markedly smaller than the  $75 \pm 13\%$  decrease in bacterial C stocks, and (ii) a similar decrease in total PLFA and bacterial C is observed in the data from the natural *Rhizophora* stand in Pambala, where TOC and TN concentrations do not show a consistent variation with depth. Alongi [8] – the only estimate in the literature to our knowledge – mentions that bacterial C stocks represent  $\sim 5\%$  of the total organic carbon, and therefore that mangrove sediments harbor much higher densities of bacteria than other benthic environments,

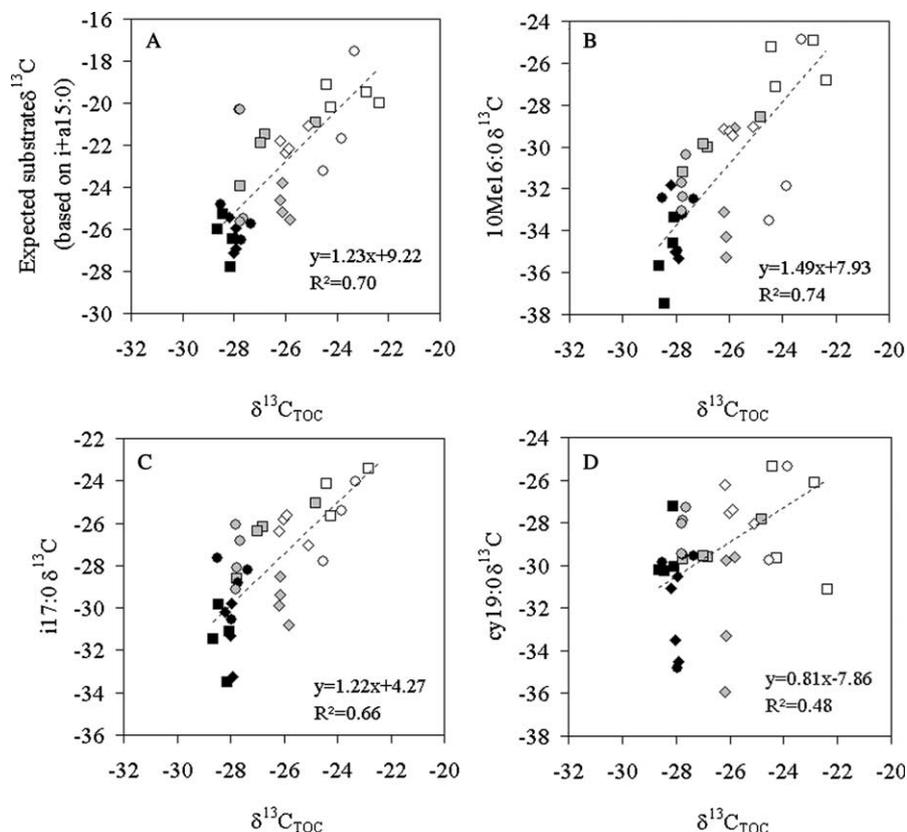


Fig. 4. Relationship between  $\delta^{13}\text{C}$  of selected bacterial PLFA and total sediment organic carbon  $\delta^{13}\text{C}$ . (A) combined i+a15:0, using a correction of  $-5.6\text{‰}$  as the average fractionation between the substrate used and these PLFA [12], (B) 10Me16:0, (C) i17:0, and (D) cy19:0; the latter three have no correction for fractionation associated with substrate assimilation applied. Each datapoint represents one depth section of a sediment core. Symbols as in Fig. 1.

the data from our study sites are typically lower (often below 1%, Table 1). Moreover, our data show that in all but two cases, the density of bacteria per unit of TOC is typically higher in the surface layers (Table 1).

When looking into the PLFA profiles, the ratio of 10Me16:0+i17:0 (both considered to be specific for sulphate-reducing bacteria [23]) to total PLFA or 16:0 (a general PLFA not linked to certain microbial groups) increased with depth in the majority of sediment cores. Although we are unable to reliably convert the concentrations of these markers into bacterial C stocks, this trend does indicate that the importance of sulphate reducers increases with depth, which is consistent with the anoxic conditions they require. In many cases, oxic respiration and sulphate reduction appear to be the dominant metabolic pathway of sediment organic matter degradation in mangroves [10,24–27], but precise interpretation of the relative importance of these two processes is difficult, as a discrepancy between rates of sulphate reduction and total  $\text{CO}_2$  fluxes is often observed (i.e. the former being larger than the latter). The most striking other pattern in the PLFA profiles is the prominence of cy19:0 in a large number of the sediment cores examined (with cy19:0 representing up to 15% of

the total PLFA carbon, see Fig. 3). This PLFA was found here in much higher relative abundances than in other estuarine/intertidal sediments [12,28,29], although it is the dominant PLFA in some terrestrial soils [15]. Although cy19:0 is known from both aerobic and anaerobic bacteria, high relative concentrations of this PLFA have been found to coincide with low oxygen levels or, more generally, with stressful conditions (for references see [30]). This PLFA has recently been suggested to increase the resistance of bacteria to low pH conditions [31] – a tempting hypothesis in view of the often low pH conditions that can be encountered in mangrove sediments [11]. Remarkably,  $\delta^{13}\text{C}$  signatures of cy19:0 also related in a more scattered pattern to those of TOC when compared to other bacterial PLFAs (see following section).

#### 4.3. $\delta^{13}\text{C}$ of PLFA: carbon substrates for natural microbial populations

To test our hypothesis whether the dominant carbon substrates used by bacterial communities in mangrove sediments varied in relation to differences in the relative importance of local versus imported carbon, we com-

pared  $\delta^{13}\text{C}$  values of bulk sediment organic matter with those in selected bacterial markers: i+a15:0 as relatively general bacterial markers (but found mostly in Gram+ bacteria, see [23]) and 10Me16:0 and i17:0 as markers considered specific for sulphate reducers [23]. Over the range of  $\delta^{13}\text{C}_{\text{TOC}}$  signatures encountered,  $\delta^{13}\text{C}$  values of these bacterial markers showed a wide range of values (generally  $>10\%$ ), but the  $\delta^{13}\text{C}$  values of the majority of bacterial markers followed the same overall trend as that observed in TOC (Fig. 4A–C shows the typical pattern, Fig. 4D presents data for the atypical cy19:0).

Relatively few data exist on the degree of isotope fractionation between the carbon substrate and bacterial PLFA. Fractionation appears to be more variable for even-numbered PLFA [12], and more pronounced under anaerobic than under aerobic conditions ([12,32], although it should be stressed that the latter considers the neutral FA pool). As much of the fractionation experiments [33,34] have been limited to single compounds or single bacterial strains, they may not be representative for natural situations. Boschker et al. [12] conducted experiments with a natural bacterial inoculum grown on cellulose and *Spartina* litter under both aerobic and anaerobic conditions. For i14:0 and i+a15:0, they found an average offset between  $-4$  and  $-6\%$  ( $-5.6 \pm 1.8\%$ ) compared to that of the carbon source, but much more variable carbon isotope fractionation (between  $+4$  and  $-8\%$ ) for even-numbered PLFA (14:0, 16:0, 18:0, 16:1 and 18:1). Burke et al. [13] even suggested that when microbial communities utilize a complex mixture of substrates in aerobic soils, little isotope fractionation may occur and average PLFA  $\delta^{13}\text{C}$  values could be a good direct indicator of microbial substrate use. A similar pattern has been suggested for terrestrial environments [18], but data from marine environments in the same study show larger and much more variable differences between the  $\delta^{13}\text{C}$  signatures of bulk organic carbon and microbial PLFA.

Despite the fact that converting  $\delta^{13}\text{C}$  data of specific PLFA to the  $\delta^{13}\text{C}$  signature of the carbon substrate is still associated with a relatively large uncertainty, the availability of data across a range of sediments enables us to draw more conclusions on the relative use of different substrates by microbial communities. First, a generally linear relationship is evident between  $\delta^{13}\text{C}$  of bacterial PLFA and  $\delta^{13}\text{C}_{\text{TOC}}$  (Fig. 4A–C) – suggesting that microbial communities largely assimilate the available carbon sources in proportion to their availability. Nevertheless, the slope of this overall relationship is consistently  $>1$  for all PLFA considered (even though not statistically significant at the 0.05 level) except for cy19:0 where the data are much more scattered (Fig. 4D) and where a slope of 0.71 is observed. This implies that there is no constant offset between the  $\delta^{13}\text{C}$  of PLFAs and of bulk sediment organic matter. A

similar pattern as that observed for nearly all PLFAs here can also be discerned in the data presented by Cifuentes and Salata [18] and Burke et al. [15]. Such a trend (i.e. the variable offset between  $\delta^{13}\text{C}$  signatures of TOC and PLFAs) could in our study be the result of (i) selective use of a more labile pool of organic carbon, which differs isotopically from the bulk sediment organic carbon pool, or (ii) higher isotope fractionation under the conditions found in the sediments with generally lower  $\delta^{13}\text{C}_{\text{TOC}}$  values – in our case these low  $\delta^{13}\text{C}$  values are found in organic-rich (and likely anoxic) sediments. In this stage, we cannot distinguish between these possible effects.

Secondly, for several individual cores (e.g. Chunnambar and the *Lummitzera* site in Pambala), the general trend between bacterial PLFA  $\delta^{13}\text{C}$  and bulk  $\delta^{13}\text{C}_{\text{TOC}}$  is quite different, with a much steeper slope, i.e. very large variations in the  $\delta^{13}\text{C}_{\text{PLFA}}$  data with much more enriched values in the top layer(s), despite only slight variations in the  $\delta^{13}\text{C}_{\text{TOC}}$  values with depth (see Fig. 4). As microphytobenthos is typically more enriched in  $\delta^{13}\text{C}$  (with  $\delta^{13}\text{C}$  values typically between  $-14$  and  $-20\%$  in mangroves, see [5] and references therein), this suggests that heterotrophic microbial populations in the surface layers show a marked selectivity for such labile carbon substrates, whereas below the surface the contribution by microphytobenthos rapidly fades. Although further confirmation is needed, these findings are in agreement with results from temperate salt marshes, where the contribution by local macrophytes as bacterial carbon substrates was dominant in some sites, but minimal in others where inputs by imported carbon sources and local microphytobenthos were significant [12]. Indirect evidence for a certain selectivity for more labile substrates also comes from the observation (Fig. 2C) that more labile substrates (i.e. with lower TOC/TN ratios) in general appear to support higher bacterial abundances for a given amount of organic carbon. In any case, our results imply that source identification should complement measurements of sediment mineralization rates, and are a crucial element in attempting to budget the fate of mangrove production or overall carbon dynamics.

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