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Utilisation of different carbon sources in a shallow estuary identified through stable isotope techniques

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ABSTRACT

Organic carbon in estuarine sediments can have many different sources. Terrestrial, riverine, estuarine and marine C pools may all contribute to and influence the organic C (C_{org}) inventory of the estuarine sediments and the differing stable isotope signatures of the sources are reflected in the sediment's overall ^{13}C content. Ecological interpretations of sedimentary isotope data may, however, be limited by the fact the total C_{org} inventory of a sediment may not be an accurate representation of the fraction that is labile and being actively turned over by the sedimentary community. To gain a better understanding of sedimentary C_{org} dynamics in estuaries and the relationship between the sedimentary C pool and the C_{org} undergoing mineralisation, we studied three components of an estuarine system: (1) the sedimentary C_{org} inventory on a transect from the mouth to the upper end of the estuary, (2) temporal changes of sedimentary C_{org} at one station throughout a year, and (3) the $\delta^{13}C$ of respired CO_2 compared to the $\delta^{13}C$ of available source material and sedimentary C_{org} in a novel application of methods developed for soil science. Our experiments demonstrated that material of marine origin dominated the studied estuary. At the time-series station, material of marine origin dominated the sedimentary C_{org} throughout the 1-yr study period. $\delta^{13}C$ values of CO_2 released from the sediment differed significantly from the sedimentary C_{org} inventory at all study sites, but also clearly reflected differences between the main sections of the estuary. These results suggest that $\delta^{13}C$ measurements of respired CO_2 are promising as a tool to advance our understanding of C cycling in estuaries, and highlight that the sedimentary C_{org} pool alone may not be a satisfactory indicator of OM utilisation in estuarine sediments.

1. Introduction

Estuaries are amongst the most productive marine ecosystems on Earth, and it is here at the interface of the land and sea that C of terrestrial, riverine, estuarine and marine as well as anthropogenic origin meets and mixes (Middelburg and Nieuwenhuize, 1998). Carbon isotopes have been a major tool applied over the last few decades to obtain information on the sources and transformations of organic matter in estuaries (e.g. Fontugne and Jouanneau, 1987; Middelburg and Nieuwenhuize, 1998; Goñi et al., 2003). Organic material found in estuaries includes a range of dissolved, colloidal as well as particulate material (Thornton and McManus, 1994; Cook et al., 2004). Frequently, the $\delta^{13}C$ value of particulate and sedimentary organic material is used to investigate the importance of terrestrial vs. marine organic matter along a salinity gradient (Richard et al., 1997; Peterson, 1999; Augley et al., 2007), but has also been used to investigate what C is available and utilised by the benthic communities (e.g. Riera and Richard, 1996; Compton et al., 2008).

To date, studies on the utilisation of C using stable isotope tools have used either pulse-chase experiments with a labelled specific food source (e.g. Middleburg et al., 2000; Witte et al., 2003; Evrard et al., 2008) or the concept of trophic fractionation (a recognised stepwise enrichment of generally 1‰ for $\delta^{13}C$ between trophic levels) (Parsons and Lee Chen, 1995; Post, 2002) to elucidate food web structures and uptake of C. However, this concept of stepwise trophic enrichment has been criticised as recent studies have reported substantial variation in consumer enrichment (Aberle and Malzahn, 2007). The $\delta^{13}C$ content of the CO_2 released from a sediment, on the other hand provides an integrated measure of sedimentary C utilisation by the sediment community structure. Natural abundance $\delta^{13}C$ measurements of soil respiration are used in soil sciences studies to partition the respiration into either

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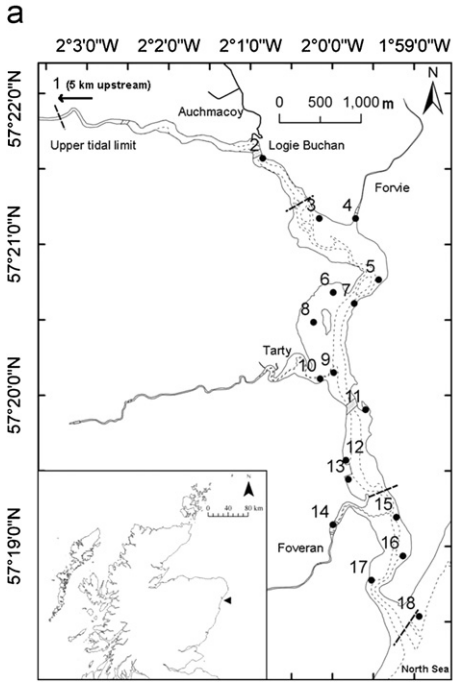
heterotrophic or autotrophic sources (Millard et al., 2010). Hence in this paper, we not only aim to (1) investigate the dominant sources of sedimentary C_{org} along the whole estuary, and (2) describe the temporal changes of $\delta^{13}C$ of sedimentary C_{org} throughout one year at a time-series station, but also to apply such a natural abundance approach for the first time to an estuarine system, to (3) measure the $\delta^{13}C$ of CO_2 released from the sediment and compare this with the C inventory.

2. Materials and methods

2.1. Study site

The Ythan Estuary is located approx. 20 km north of Aberdeen in north-east Scotland, United Kingdom (Fig. 1a) and is a shallow well-mixed estuary (Leach, 1969). Its upper tidal limit is reached approximately 10 km from the mouth of the estuary and with an average width of 300 m, its mean tidal prism volume (volume of water between mean high and low tide) has been estimated at 1.77×10^6 m^3 (Stove, 1978). The tidal component dominates the water volume in the estuary with the ratio of tidal prism to freshwater volume being 10:1 for a mean tidal period, increasing to 25:1 at spring tides (Leach, 1971). In addition to the Ythan river flow (contributing approx. 66% of all freshwater input (Stove, 1978)), four smaller tributary burns contribute to the estuary—the Auchmacoy, Forvie, Tarty and Foveran Burns (Fig. 1a). Flushing time estimates range from approximately one tidal cycle (Leach, 1969) to 5–12 days (Balls, 1994). Average daily river flow data ($m^{-3}\,s^{-1}$), collected upstream from the town of Ellon, for the sampling periods compared to the 10-yr annual average from 1998 to 2008 revealed a general tendency of above average flow rates in the late spring (April–June) and summer (July–September) months of 2007 and 2008 (Fig. 2); autumn (September–November) rates tended to be predominantly below average. Both October 2008 and April 2009, when samples for the sedimentary C_{org} inventories of the estuary were taken (see Section 2.2), showed below average monthly rates of 7.8 and 5.4 $m^{-3}\,s^{-1}$ compared to 9.4 and 9.2 $m^{-3}\,s^{-1}$ for 1998–2008, respectively.

The estuary is home to a variety of habitats including intertidal mud- and sandflats, mussel beds, salt marshes and reedbeds, creating an intertidal area totalling 185 ha (Leach, 1969). The estuary was divided into upper (Machamuir to Auchmacoy), middle (Machamuir to Inch) and lower (Inch to mouth) reaches by Leach (1969), based particularly on the salt water intrusion patterns; this division has been adopted for this study.



b

St. No.	$\delta^{13}C$	± S.E.
1	-28.29	0.19
2	-25.86	0.37
3	-22.01	0.27
4	-27.30	0.11
5	-21.46	0.2
6	-22.45	0.16
7	-21.51	0.26
8	-21.31	0.08
9	-21.56	0.45
10	-19.74	0.21
11	-21.08	0.32
12	-21.43	0.52
13	-21.63	0.09
14	-23.88	0.60
15	-20.42	0.29
16	-20.22	0.39
17	-20.76	0.42
18	-19.51	0.52
19	-20.68	0.51
20	-20.80	0.38

Fig. 1. (a) Location of sampling stations on the Ythan Estuary in north-east Scotland with the dotted line indicating lower tide limits. The dashed lines demarcate the boundaries between the tidal limit, upper, middle and lower reaches of the estuary according to Leach (1969). Not plotted is Station 1, located approximately 5 km upstream from the tidal limit near Ellon, and Stations 19 and 20, which are located approx. 3.5 and 4.6 km offshore near Stonehaven. (b) Average $\delta^{13}C$ values and respective standard errors measured for each of the stations given in (a).

2.2. Sedimentary C_{org} inventory

$\delta^{13}C$ of sedimentary C_{org} was determined for 18 stations along the Ythan (Fig. 1a). At each of the stations, triplicate surface scrapes of sediment to a depth of ~1 cm were obtained at low tide. Sampling was undertaken over a 2-d period at the beginning of October 2008 and repeated in early April 2009. Offshore samples were not available directly from the shelf at the mouth of the estuary, instead samples obtained from two sites near Stonehaven, 33 and 40 km south of the Ythan, were used as representative of a marine coastal sediment $\delta^{13}C$ value. Station 19 (57°1'N, 2°5'W) located approximately 3.5 km offshore, is a muddy–sandy sediment and Station 20 (56°57'N, 2°7'W), approximately 4.6 km offshore, is dominated by sands. All samples were dried (60 °C), acidified using 1 M HC1 to eliminate calcareous C ensuring all remaining C in the sample was in the form of C_{org}, re-dried and milled prior to analysis (see Section 2.6).

2.3. Temporal variability at the time-series station

Approximately every 2 weeks, sediment samples were obtained from Station 9 over a 1-yr period from the end of May 2007 to the beginning of May 2008. Three replicate cores were taken using acrylic tubes (inner diameter 3.6 cm; sampling depth 15–20 cm) at low tide and sliced at 1 cm intervals. The top 1-cm slice was dried, acidified, milled and analysed for stable isotopes and organic C and N content as described in Section 2.6. Further cores ($n=4$) taken at the same time in the field were dried, pooled and milled; a 5–7 mg subsample was weighed out and analysed using a NA1500 NCS Elemental analyser (Fisons Instruments, Manchester, UK) to obtain total carbon (TC) content of the sediment. Total nitrogen contents were below the instrument's detection limit, which, based on a 5 mg sample, was 0.09%, and are therefore not reported. In this instance we did not remove the inorganic C component and have clearly distinguished this in the Section 3.

2.4. Sediment respiration

Over a 2-d period in early June 2009, five replicate sediment cores (diameter 10 cm) were collected at each of the following four sites at low tide: 2, 9, 10 and 17 (Fig. 1). All sites are fully exposed at low tide with Stations 2 and 10 being muddy sites (porosity φ for Station 2 = 0.7; fraction < 63 μm at Station 10: 66%); Station 9 a sandy site ($\varphi=0.4$) and Station 17 having a mixed sand/mud substratum ($\varphi=0.4$). Two stations were sampled each day (Stations 2 and 17 on 02 June 2009, Stations 9 and 10 on 03 June 2009) obtaining ten cores in total each day which were immediately returned to the laboratory. In the laboratory, the cores were placed in gas-impermeable 5-L Tedlar® bags fitted with a Kynar® 2-in-1 valve (Keika Ventures, Chapel Hill, NC, USA). Enclosed air was removed with a pump and bags refilled with CO_2-free air, produced

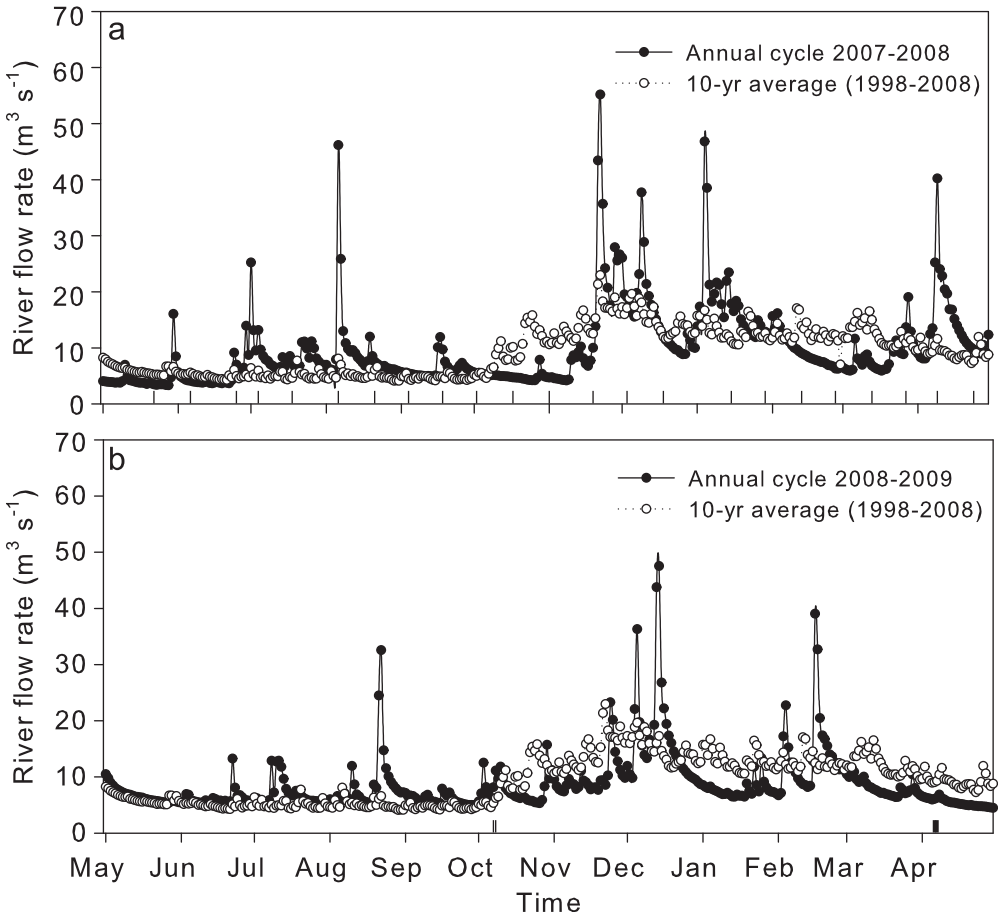


Fig. 2. The Scottish Environmental Protection Agency (SEPA) maintains a calibrated gauging station upstream of Ellon (Station 10003; grid reference 38 (NJ) 947303) for the Ythan River. River flow rates measured for the Ythan River have been averaged for the period of 1998–2008 and are compared to the annual daily flow rate averages (m^{-3} s^{-1}) for (a) 2007–2008 and (b) 2008–2009. Inward tick marks on the time axis denote sampling events during these 2 years.

by pumping air through self-indicating soda lime. This was repeated six times before cores were left to incubate in a temperature controlled room set at *in situ* temperature (14 ± 1 °C). After 2.5 h the concentration of accumulated CO_2 derived from respiration was measured with an infrared gas analyser system (EGM-4, PP Systems, Hitchin, Hertfordshire, UK). Gas samples were then collected and stored in pre-evacuated 12 mL septum-capped exetainers (Labco (UK) Ltd, High Wycombe, UK) and analysed to determine the ^{13}C content (see section below). Sediment samples (scrapes) were taken from the top 1-cm layer and at 5-cm depth of each core and analysed for ^{13}C content.

2.5. Source material

Possible sources of organic material to the estuary were also sampled to determine the ^{13}C content. This included samples of two of the macroalgal species predominantly found in the estuary (*Enteromorpha* spp. and *Fucus* spp.). Strands of macroalgae were cleaned of epibionts, rinsed with 1 M HCl and Milli-Q water, dried (60 °C), milled and then analysed (see below). Furthermore, parts of the leaf and roots of the Common reed (*Phragmites australis*), the main species comprising the reedbeds found in the upper reaches of the estuary, were obtained, cleaned, rinsed, dried and milled, prior to isotope analysis.

2.6. Stable isotope analysis

Dried milled solid samples were analysed for both their C and N contents and ^{13}C content using a Flash EA 1112 Series Elemental Analyser connected via a Conflow III to a Delta^{Plus} XP isotope ratio mass spectrometer (all Thermo Finnigan, Bremen, Germany). The C_{org} and N content of the samples was measured using the mass spectrometer which had been calibrated against National Institute of Standards and Technology (NIST) standard reference material 1547 Peach Leaves analysed with every ten samples. The isotope ratios were calculated with respect to CO_2 and N_2 reference gases injected with every sample and traceable to International Atomic Energy Agency (IAEA) reference materials USGS40 and USGS41 (both L-glutamic acid). The $\delta^{13}C$ signatures are expressed as $\delta^{13}C_{V\text{-}PDB}$, where $\delta^{13}C = (R_{Sample}/R_{V\text{-}PDB} - 1) \times 1000$ (‰), and R_{Sample} and $R_{V\text{-}PDB}$ are the $^{13}C/^{12}C$ of the sample and reference material V-PDB, respectively. Long term precision for quality control standards (milled flour) were: total C 40.3 ± 0.42% and $\delta^{13}C$ -25.5 ± 0.29‰ (mean ± SD, $n=200$).

The carbon isotope ratios of the CO_2 in gas samples were analysed using a Gas-bench II connected to a Delta^{Plus} Advantage isotope ratio mass spectrometer (both Thermo Finnigan, Bremen, Germany). The C isotope ratios were calculated with respect to a CO_2 reference gas injected with every sample and traceable to IAEA reference material NBS 19 TS-Limestone. Repeated analysis of a quality control standard gas indicated that the precision of the gas bench for analysing $\delta^{13}C$ of CO_2 at a concentration of 450 ppm in exetainers was -35.03 ± 0.24‰ (mean ± SD, $n=65$).

2.7. Statistical analysis

Differences in $\delta^{13}C$ value were analysed with one-way analysis of variance (ANOVA) with subsequent multiple pairwise comparisons

being carried out with the Student–Newman–Keuls (SNK) test. Where assumptions of normality and/or homogeneity of variance failed, non-parametric tests were applied as data distributions did not lend themselves to transformations. Given that a significant result with non-parametric testing places greater emphasis on there actually being a difference, this approach was deemed valid. Where Kruskal–Wallis tests were applied and treatment groups were of unequal size, post-hoc testing employed Dunn's method. Analysis of data was carried out using SigmaStat® 4 incorporated into SigmaPlot® 11.0 (Systat Software Inc., USA) with a level of significance of $p < 0.05$.

3. Results

3.1. Sedimentary C_{org} inventories along the estuary

$\delta^{13}C$ of sedimentary C_{org} in the Ythan Estuary are summarised in Fig. 1b for both months ($n=6$) as no statistically significant difference was found between the 2 months, although the $\delta^{13}C$ content tended to be more depleted in the samples taken in April compared to those from October. Values ranged from −28.3‰ upstream of the tidal limit to −25.9‰ in the upper reaches and up to −20.8 at Station 17 in the mouth of the estuary. However, excluding Station 2 and other riverine samples, the range of $\delta^{13}C$ in the estuary itself was restricted to a much smaller range from −22.1‰ to −19.7‰. In general, a pattern of enrichment was observed moving towards the more marine dominated areas downstream (Fig. 3a). Stations 1, 2, 4 and 14 were pooled together as so-called "riverine" samples. Their depleted $\delta^{13}C$ content predominantly reflected their location within the Ythan River or one of the tributary burns, or in the case of Station 2 a location proximal to reedbeds whose dominant component, *P. australis*, had a signature of −27.5‰. Samples from offshore Stonehaven (Stations 19 and 20) as well as at the very outer margin of the estuary mouth's sandbars (Station 18) were also pooled to represent "truly" marine values. Subsequently, comparisons made between the different parts of the estuary—the riverine, middle and lower reaches, and the marine end-members, revealed significant differences ($H=62.04$, df=3, $P<0.001$). The riverine signals were distinctly different (-26.3 ± 1.9‰) to all other parts of the estuary (Dunn's post-hoc test) (Fig. 3a). Excluding these riverine signals, the sedimentary C_{org} of the middle reaches (pooled mean -21.4 ± 0.9‰) were more depleted than further down the estuary in the lower reaches (-20.5 ± 0.7‰) and than the marine values (-20.6 ± 1.1‰). This is also reflected when a simple isotope mixing model (Harji et al., 2010) is applied: terrestrial contribution $(\%)=(\delta^{13}C_{marine}-\delta^{13}C_{sample})/(\delta^{13}C_{marine}-\delta^{13}C_{terrestrial})*100$, where $\delta^{13}C_{marine}$ is the marine end member of the estuary (−19.5‰) and $\delta^{13}C_{terrestrial}$ the terrestrial end member (−28.3‰). The calculated terrestrial contribution at each site clearly supports the differences along the estuary's length (Fig. 3b).

Organic matter input to the estuary can include a wide range of source material, from terrestrial plants to marine algae of varying influence on sedimentary C_{org}. The $\delta^{13}C$ content of *Phragmites*, irrespective of whether measured from the leaves or roots (−27.3‰ and −27.7‰, respectively), is clearly different to that of both macroalgal species tested (Fig. 6). Both *Enteromorpha* sp. and *Fucus* sp. exhibit similar values with -16.7 ± 0.5‰ and -17.3 ± 0.4‰, respectively, and fall within the wide range previously measured by in the Ythan estuary or generally found in the literature.

3.2. Annual variability

The C isotope value of sedimentary C_{org} at the sandflat (Station 9) displayed temporal variability throughout the 1-yr sampling period. Seasonal differences existed ($F=13.65$, df=4, $P<0.001$), but this

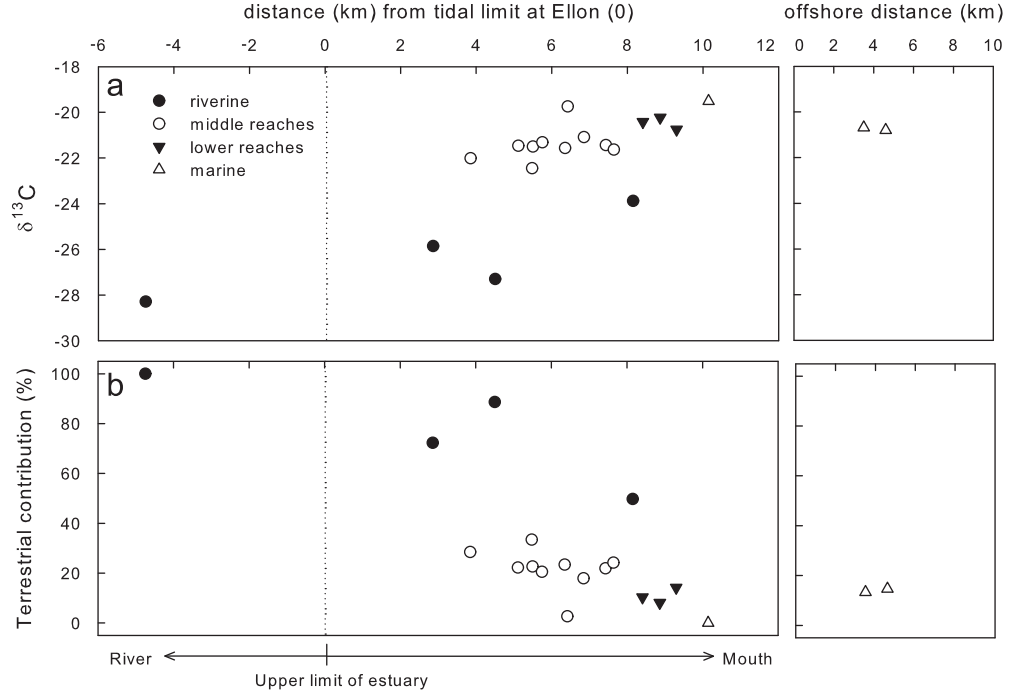


Fig. 3. (a) Measurements of $\delta^{13}C$ of sedimentary C_{org} are given with distance from the estuary's upper tidal limit near Ellon. Standard errors are given in Fig. 1, where the order of the station numbers follows the order of their distance along the estuary (left panel). Measurements of $\delta^{13}C$ for Stations 19 and 20 are presented separately (right panel) due to their location off shores near Stonehaven. All samples are differentiated into riverine sites, those of the middle and lower reaches of the estuary and marine samples. (b) The amount of terrestrial contribution (in %) to each estuarine station's $\delta^{13}C$ measurement of sedimentary C_{org} has been calculated by using a simple isotope mixing model with a marine end member ($\delta^{13}C_{marine}=-19.5$‰) and a terrestrial end member ($\delta^{13}C_{terrestrial}=-28.3$‰) for the estuary (left panel) and the offshore stations (right panel).

predominantly stemmed from the enriched values observed in the spring (April–June) 2007 (-18.1‰) compared to the rest of the year where seasonal means ranged from -19.0‰ in autumn (September–November) to -19.6‰ in winter (November–April) (Fig. 4). In general, $\delta^{13}C$ was linearly correlated to the average daily river flow obtained for each sampling occasion (Spearman's rank correlation, $r=-0.46$, $P=0.03$). It was also correlated to the sediment surface TC content (Spearman's rank correlation, $r=-0.42$, $P=0.04$), indicating that more deplete $\delta^{13}C$ values were not only associated with higher rates of river run-off but also with higher TC in the sediment. No correlation was observed with the tidal height at mean low water or the actual water content of the sediment.

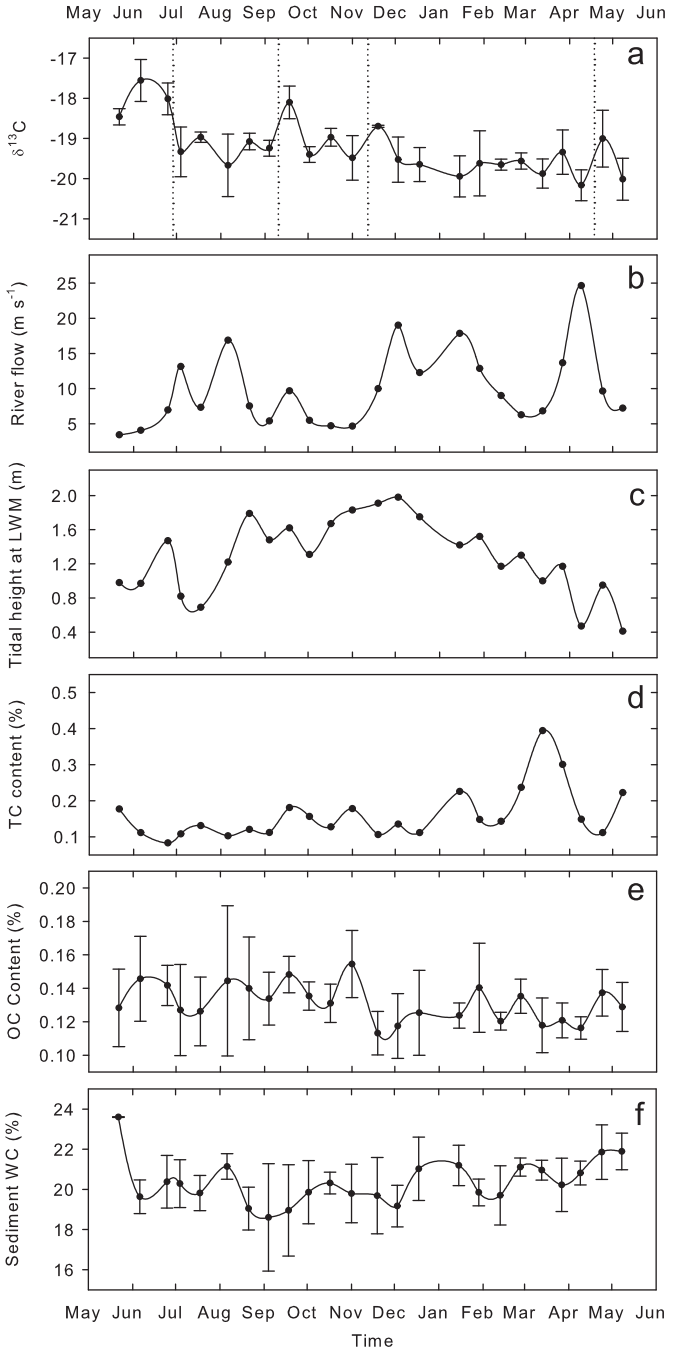


Fig. 4. Measurements for samples taken over a 1-yr period at Station 9 in the middle reaches of the estuary for: (a) $\delta^{13}C$ content of the surface sedimentary C_{org} (mean $\pm$ SD), (b) daily river flow rates, (c) tidal height at mean low water (MLW), (d) total carbon (TC) content, (e) organic carbon (OC) content (mean $\pm$ SD) and (f) sediment water content (WC) (mean $\pm$ SD) (SD=standard deviation). The vertical dashed lines in (a) mark the boundaries of the seasons (spring 2007, summer, autumn, winter and spring 2008).

3.3. Sediment respiration

Fig. 5 compares the $\delta^{13}C$ content for surface sedimentary C_{org} with the $\delta^{13}C$ of CO_2 released from the sediments. The $\delta^{13}C$ for sedimentary C_{org} differed significantly between the four sites ($H=16.07$, df=3, $p=0.001$). Using multiple pairwise comparisons procedures (SNK-test), the only two sites not significantly different were Stations 10 and 17. As expected for a site closest to terrestrial inputs, signatures at Station 2 were the lowest (Fig. 5a). Interestingly, despite their spatial proximity, the $\delta^{13}C$ content at Stations 9 and 10 were significantly different, with a mean of -18.4 ± 0.9‰ and -20.9 ± 0.8‰, respectively. This difference was also observed in the measurements of respired CO_2 with -14.9 ± 0.5‰ compared to -16.7 ± 1.2‰, respectively (Fig. 5b).

For all four sites, significant differences were seen between the sediment C inventory and the community respired CO_2. In addition, all four sites differed in the respired CO_2 measurements ($F=41.22$, df=3, $P<0.001$), except between Stations 9 and 17 (SNK post-hoc test). The most enriched site for the sedimentary C_{org} was Station 9, whereas Station 17 displayed the most enriched signal for sediment-respired CO_2. The difference between the averaged sedimentary C_{org}-values and that of the respired CO_2 per site varied from 6.1‰ at Station 17 to 4.3‰ at Station 10, to 3.5‰ at Station 9 and 3.2‰ at Station 2. Except for Station 2, the sedimentary $\delta^{13}C$ measured at a depth of 5 cm in the cores was always lower than at the surface, albeit this difference was not statistically significant for any of the sites.

4. Discussion

To elucidate C utilisation in sediments, ecologists have frequently investigated the two ends of this biophysical spectrum—C in the sediment and C in the organisms. For this, both pulse-chase tracer experiments and the application of trophic fractionation structures have been employed. Significant inter- and intraspecific variability in enrichment processes have been described in recent studies making this approach complex (Aberle and Malzahn, 2007). In recognition of the problems associated with the aforementioned methodology, a third approach is applied in this study where the actual process linking these two end-members is investigated.

4.1. A marine-dominated estuary

Analysis of sedimentary C_{org} along the whole estuary revealed that, apart from the riverine sites, sediments along most of the estuary's length display very similar $\delta^{13}C$ signatures, falling within a narrow range of values never more depleted than -23‰. The estuary can, therefore, be described as being dominated by C of marine origin, which typically has relatively enriched $\delta^{13}C$ values of -24‰ to -18‰ (modal value of approximately -21‰) (see Gearing et al., 1984; Middelburg and Nieuwenhuize, 1998; Bianchi, 2006). This confirms the work by Leach (1969) who noted the marine contribution of particulate organic C to the estuary to be at least an order of magnitude greater than that of freshwater. He further mentions that a large part of the terrestrial input is confined to the main channel and therefore deposition of this material on the intertidal areas is restricted. The samples taken in the Ythan River and Forvie burn, display $\delta^{13}C$ values previously observed for terrestrial organic matter around -28‰ to -26‰ (Dauby et al., 1992; Middelburg and Nieuwenhuize, 1998). The proximity of the Foveran burn to the mouth of the estuary, and thus an area where

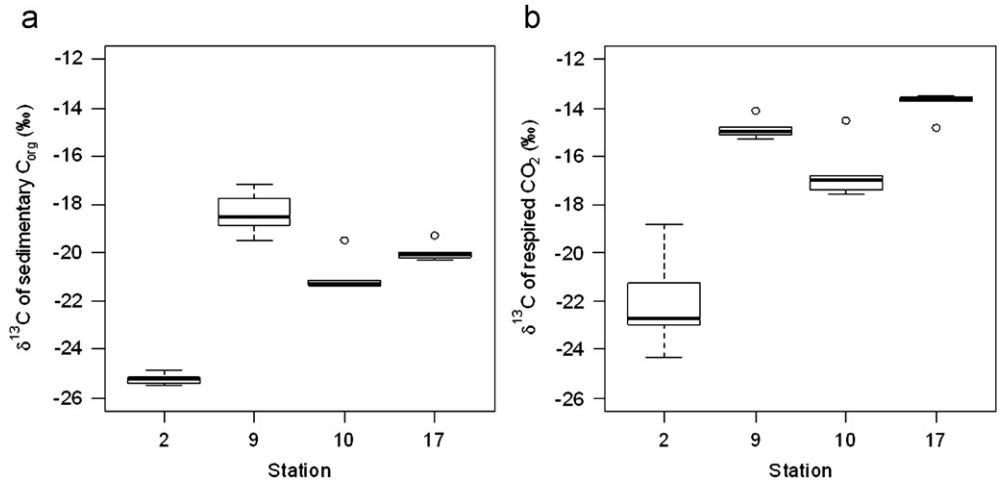


Fig. 5. Boxplots are given for $\delta^{13}C$ content of (a) surface sedimentary C_{org} and (b) respired CO_2 from sediment cores taken at Stations 2, 9, 10 and 17 in June 2009. The boxes indicate 50% of the data and include the median line, the whiskers the 10th–90th percentile and the open circles outliers.

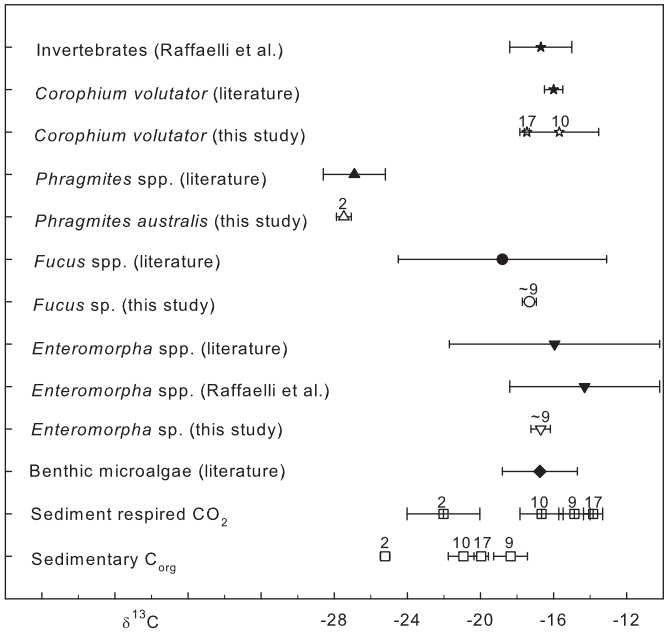


Fig. 6. Typical ranges of $\delta^{13}C$ signatures found in the literature, including unpublished data by Raffaelli et al. for the Ythan Estuary (filled symbols), and values measured for the Ythan in this study (open symbols) are given for various sources of organic material and consumers. Also given are the $\delta^{13}C$ signatures of the surface sedimentary C_{org} and respired CO_2 measured at the four stations (2, 9, 10 and 17). Literature values are sourced from: (a) Riera and Richard (1996); (b) Adin and Riera (2003); (c) Gearing et al. (1984); (d) Riera and Hubas (2003); (e) Doi et al. (2005); (f) Yokoyama et al. (2005); for benthic microalgae (a, d–f), *Enteromorpha* spp. (b, d, f), *Fucus* spp. (a, b, d), *Phragmites* spp. (e–f) and *Corophium* sp. (c), respectively.

tidal influx of marine material would be expected to be strong, is reflected in its enriched value compared to the other two riverine sites. Here, the calculated terrestrial contribution also reaches around 50% compared to 70–90% for the other two sites (Fig. 3b). Leach (1969) concluded that the particulate organic material input at Logie Buchan (Fig. 1) was primarily of riverborne detritus. However, even at this Station (2) the relatively enriched sediment (-25.9 ± 0.9‰) compared with both the river C_{org} and with the main source material of *P. australis* (-27.5‰) suggests some influence of non-terrigenous material (72% terrestrial contribution). The tidal limit is approx. 2.8 km upstream of this site which is therefore likely to experience a moderate tidal influence, especially during low river flow conditions. The approach taken with a simple isotope mixing model, allowing the dominance of marine inputs to be visualised and quantified (Fig. 3b), is sensitive enough to detect the contribution of terrestrial C in a marine dominated estuary and non-terrigenous inputs where only terrestrial sources would be expected, but is still limited with regard to considering the relevance of autochthonous C sources (micro- and macroalgae).

Numerous studies have been undertaken on estuarine systems dominated by terrestrial organic material (e.g. Sackett and Thompson, 1963; Shultz and Calder, 1976); where strong enrichment along the estuary occurs from around -27‰ at the freshwater end to around -19‰ at the marine end. Frequently, estuaries studied in the past have long residence times and are much larger in size compared to the Ythan Estuary studied herein (e.g. Cai et al., 1988). In contrast, the Ythan is a short, shallow estuary with a complex hydrodynamic regime (Stove, 1978). Calculations on its flushing time have been made on several occasions, ranging from 1 tidal period (Leach, 1969) and 11–60 h (Gillibrand and Balls, 1998) to 61/2 days (Stove, 1978) and 5–12 days (Balls et al., 1995). All characterize the Ythan as an estuary with a short flushing time. In addition, it has been suggested that long-term net deposition of material brought in by marine and Aeolian processes takes place (Stove, 1978) and that contributions from outside the estuary of primary organic material dominate (Leach, 1969). The dominance of marine processes in the estuary, which these physical parameters suggest, is confirmed not only by the mixing model (Fig. 3b) but also by measured C/N ratios averaging around 6–7 in the middle reaches and downstream (Fig. 7), within the generally accepted range for marine material of 6–8 (Richard et al., 1997; Andrews et al., 1998). Terrestrial material has more variable C/N ratios, generally >12 (Thornton and McManus, 1994). Although none of our ratios were observed in this range, the values determined for the riverine sites clearly are tending towards higher values compared to the rest of the estuary (Fig. 7). Although the tidal prism volume to freshwater is generally 10:1 (Leach, 1969), river flow still influences some material flow in the estuary. River flow in October was below the 10-yr average but followed an above-average summer and early autumn, which combined with the below average winter flows may explain the lack of a significant difference between the two sampling events. However, the generally more depleted ^{13}C content of sedimentary C_{org} in April may be explained by the longer term influence of the winter river input on the surface C_{org}. Sedimentary C_{org} tends to integrate annual organic matter sources (Middelburg and Nieuwenhuize, 1998) and summer flows were

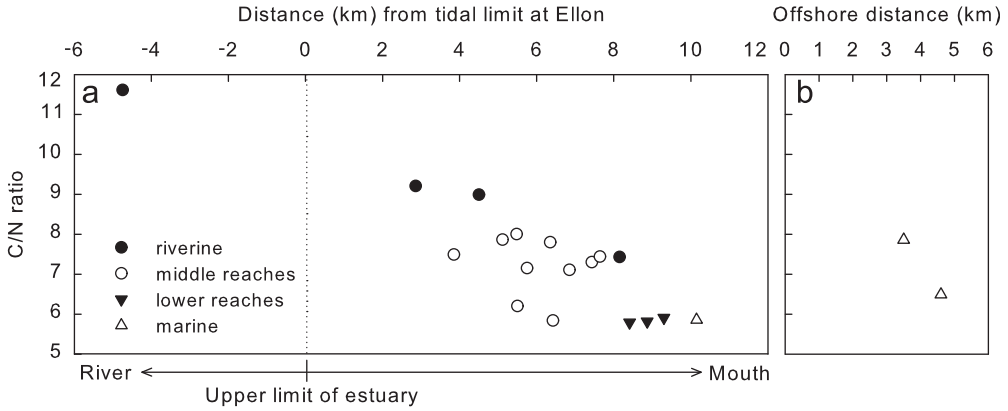


Fig. 7. (a) Measurements of C/N ratios along the estuary from the estuary's upper tidal limit near Ellon to the mouth following the order of station numbers given in Fig. 1. (b) Measurements of C/N ratios for stations 19 and 20 located off shores near Stonehaven. The samples have been coded depending on their classification as riverine samples, those of the middle and lower reaches of the estuary or marine sites.

still only about half those of what was observed in winter. The river flow data show periods of high run-off throughout the winter (Fig. 2), bringing with it terrestrial material that would deplete the ^{13}C content of the sedimentary C_{org}. The terrestrial contribution to the sediment ^{13}C content of samples was up to 30% higher in April.

4.2. Temporal changes in sedimentary C_{org}

Our high frequency of sampling for the time series station allowed us to observe temporal changes in the sedimentary C_{org} for a sandflat in the middle reaches of the estuary over a 12-month period. Tidal inflow of material appears dominant at this station, but the observed correlation between the river flow and the $\delta^{13}C$ values indicates that river inputs are still influential. Although, the river flow varied substantially over the measurement period from <5 to $>25\ m^3\ s^{-1}$ (Fig. 4), the depletion of isotope values at high flows compared to those at lower flow rates, suggests more deposition of terrestrially derived organic C during the periods of high flow.

Seasonal differences in $\delta^{13}C$ of particulate matter have been observed for several estuaries (Cifuentes et al., 1988; Richard et al., 1997). Often this is the result of seasonal changes to the source material, both terrestrial and marine. For living material this may depend on growth rate, light and temperature, whilst seasonal changes in river flows and current conditions may affect more the detrital material of the system (Megens et al., 2001; Faganeli et al., 2009). Temporal differences of $\delta^{13}C$ observed in this study, showed a distinctly more enriched $\delta^{13}C$ signal in the spring of 2007 compared to the rest of the year, possibly the result of the previous dry winter months (below average) in combination with the appearance of source material known to have enriched signals such as benthic microalgae (Riera and Richard, 1996). River flow is important at this time of the sampling period; the observed depletion of the $\delta^{13}C$ values follows closely the increase in river flow rates in late June. However, the influence of terrestrial material overall, despite temporal fluctuations is generally small with $\delta^{13}C$ values never falling below -20.6‰ during the 1-yr period; the dominance of marine material is verified by C/N ratios largely remaining between 5 and 7 (unpubl. data). This contrasts the general tendency for sedimentary C_{org} to largely reflect terrestrial organic matter, as labile fractions are preferentially utilised, leaving the refractory terrestrial material to accumulate (Middelburg and Nieuwenhuize, 1998) and reveal increases in C/N ratios to >12 (Richard et al., 1997). Instead, refractory material of marine origin may be accumulating; particulate organic material in winter may include larger amounts of degraded marine phytoplankton decreasing values of $\delta^{13}C$ in the range seen for this study (Megens et al., 2001). Higher and more variable values in spring to autumn on the other hand, indicate the availability of other sources such as, for example, macro- and microalgae with enriched $\delta^{13}C$ values (Fig. 6).

4.3. The source of sedimentary C used in community respiration

In our study, distinct enrichment is seen in the $\delta^{13}C$ values of the respired CO_2 compared to that of the sedimentary C_{org}. At Station 2, no macroalgal species such as *Enteromorpha* or *Fucus* were observed, yet the $\delta^{13}C$ signatures of both the sedimentary C_{org} and the respired CO_2 were clearly enriched compared to that of the terrestrial organic matter expected to be the main source (*P. australis* or other riverborne detritus); this may be attributed to superficial patches of unidentified macroalgal filaments. Variability between the sedimentary C_{org} of the five different cores at this site is minimal (standard deviation (SD)=0.3), however, the respired CO_2 signals show much greater variability between cores (SD=2.1) compared to any of the other sites. These distinct differences observed between individual cores are reflecting the presence of the algal filaments on the sediment surface. The more algal material was present (visual observations of sediment surface), the more enriched the CO_2 was, the difference being as much as 5.5‰ between a core with no visible algal layer (-24.3‰) to one with nearly 100% algal cover (-18.8‰). Similarly, at Station 9, patches of benthic microalgae were observed and the $\delta^{13}C$ signature of respired CO_2 of a core taken directly from such a patch compared to cores without visible signs of microalgae was enriched by more than 1.5‰ (unpubl. data). This suggests the utilisation of this algae but should also consider the respiration of the algae itself. The benthic communities readily degrade fresh organic matter of macro- or benthic microalgal origin which demonstrates that sedimentary C_{org} only provides a limited picture of the active C cycling within this system highlighting the value of measuring directly the actual CO_2 efflux.

To the best of our knowledge, $\delta^{13}C$ of sediment-respired CO_2 has only been used once before for marine sediments whereby the utilisation of Orimulsion compared to control material containing material of different isotopic signature was successfully reflected in the $\delta^{13}C$ values of the respired CO_2 (Lapham et al., 1999). In soil science, the ^{13}C content of the soil surface CO_2 is determined more frequently as it is indicative of different C sources and able to distinguish autotrophic sources associated with the plant roots, from heterotrophic C associated with the turnover of soil organic matter (Ehleringer et al., 2000; Millard et al., 2010). In contrast to soils where these distinct end-members can be defined for isotope mixing models (respiration from roots and soil organic matter),

in intertidal sediments these end-members are more difficult to define. A range of organic matter, not restricted to macro- and microalgae, may be utilised by intertidal communities and further work would be necessary to quantify the various organic matter fractions present in sediment samples such as fractions of algae (chlorophyll *a*), invertebrates and terrestrial plant detritus (lignin).

Additionally, processes such as methanogenesis and sulphate reduction in intertidal sediments will potentially influence carbon speciation (Canfield et al., 2005; Kristensen et al., 2008). The isotope approach used herein relies on clearly defining the end-members of the isotope mixing model. This is arguably one of the most challenging aspects and where more work is needed. This future work might consider how best to incubate components of the sediment individually so that the overall efflux at each site could then be partitioned and compared across the estuary. Applying this isotope approach to an estuarine environment is still very new and this first attempt indicates exciting possibilities for future research. The $\delta^{13}C$ values of respired CO_2 clearly distinguished the four different sites across the estuary and were correlated with differences in the associated surface C_{org}. This indicates that labile C sources being utilised by the biological assemblages at each site vary across the estuary. It is also likely that this variation is in part due to differences in the community structure driven by available C. From this preliminary data it is, however, difficult to separate these two elements of the C cycling at this site and more work is needed. The differences between Stations 9 and 10, despite their proximity ($\sim$250 m), may be due to the stronger influence of the Tarty Burn on Station 10, its less dynamic environment and the known stronger enrichment for sandy sediments compared to muddy substratum (Augley et al., 2007). At both sites no macroalgae were observed directly on the flats, except along the shoreline several tens of meters away, where they had been deposited by the tide or grow attached to larger pebbles and rocks. Macroalgal species are known to provide significant contributions to members of the estuarine food web (Riera and Richard, 1996; Yoshino et al., 2006; Rossi, 2007) and predators may even consume a specific macroalgal species without trophic mediation (Adin and Riera, 2003). Live as well as detrital macroalgae can serve as food sources, are found on and buried within the sediment, and are easily transported by the tides in an estuarine setting (Riera and Richard, 1996; Riera and Hubas, 2003; Rossi, 2007).

Estuarine benthic communities are subject to strong spatial and temporal gradients with differences in sediment structure, environmental conditions and organic matter sources dictating the community compositions (Herman et al., 2001). Despite the potentially strong influence of large macrobenthic organisms such as, for example, the bioirrigator/-turbator *Arenicola marina* in coastal systems (Herman et al., 2001), microbial and meiofaunal communities can play a dominant role in sediment respiration (Dai et al., 2008; Riera and Hubas, 2003). Although sandy sediments, for example, are known to have high rates of microbial mineralisation (Hansen and Kristensen, 1998; Rusch et al., 2001), more information is needed on how much the various faunal components but also autotrophs of the system influence the respiration processes in relation to the evolved CO_2 that was measured in this study.

All macrofaunal organisms found at the four different sites are small in size, with Stations 10 and 17 being the only two sites where animals could be observed with the naked eye—the amphipod *Corophium volutator*. *C. volutator* burrows were numerous in cores from Station 17 and the organisms could be seen actively moving around the sediment once cores were sliced open. Assuming that signatures of *C. volutator* (Fig. 6) should reflect an enrichment of 1‰ in $\delta^{13}C$ and 3‰ in $\delta^{15}N$ (Peterson and Fry, 1987; Parsons and Lee Chen, 1995), a relationship between our measured source material and the predator is not clear. A similar conclusion was also reached by Raffaelli (1999) who referred to data of $\delta^{13}C$ measurements of *Enteromorpha* and invertebrates (*C. volutator* and *Nereis* sp.) whose values were similar to each other (Raffaelli et al., unpubl.) and also similar to ours (Fig. 6) for the Ythan Estuary.

However, Raffaelli and his colleagues also observed a high variability in values measured for *Enteromorpha* within a 30 m transect (-15‰ to -11‰), and given the wide range seen in the literature (Fig. 6), neither *Enteromorpha* nor other macroalgae should be ruled out as a possible food source. This is also suggested by the enriched $\delta^{13}C$ signatures of the respired CO_2 at these Stations (9, 10 and 17) and also applies to benthic microalgal species whose signatures tend to show similar ranges as those of macroalgal species (Fig. 6). Bacteria are known to preferentially utilise labile organic C derived from marine diatoms when a mixed C pool is available (Dai et al., 2008). Microphytobenthos (cyanobacteria, benthic diatoms and other eukaryotic algae) are an important source for benthic heterotrophs and can be rapidly transferred to them (Middelburg et al., 2000).

As it stands, CO_2 isotopic signatures differentiate the different parts of the estuary reflecting the same pattern seen in the surface sedimentary C_{org}. As we move towards the mouth of the estuary the signal becomes more enriched, reflecting the decreasing influence of terrestrial material. Measurements of CO_2 respiration at the air–sediment interface have shown the impact of benthic algal material for the sediment C budget (Hubas and Davoult, 2006) as confirmed by our CO_2 isotopic signatures. However, the use of stable isotopes on the respired CO_2 during the emersion period has not been undertaken before, and despite its limitations is a first step of encouragement that more emphasis should be placed on using such techniques in intertidal sediments, particularly given the extended periods of time sediments can be exposed to air in estuarine ecosystems. A better understanding of these areas crucial to C cycling processes by being at the interface of very different habitats and organic matter pools (riverine, estuarine, terrestrial and marine) is essential.

5. Conclusions

This study has investigated C cycling in a shallow estuary with short flushing time and dominant tidal components and is in marked contrast to many previous estuarine studies which have tended to concentrate on much larger systems. Organic material of marine origin dominated the C dynamics of the estuary, up to several kilometres upstream. The high frequency of sampling over time allowed us to see that both physical and biological processes are influencing the temporal variations seen in the $\delta^{13}C$ observations. Although material of marine origin dominates throughout the 1-yr period, river flow still appears to impact the observed values. The utilisation of C sources clearly varied between different parts of the estuary, reflecting different source material and biogeochemical processes taking place. A more interdisciplinary approach using methods such as the measurement of sediment-respired CO_2 should help us gain a better understanding of sediment characterisation and C utilisation in these complex systems.

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