Biogeosciences Discussions, 2, 1617–1644, 2005
www.biogeosciences.net/bgd/2/1617/
SRef-ID: 1810-6285/bgd/2005-2-1617
European Geosciences Union

Biogeosciences Discussions is the access reviewed discussion forum of *Biogeosciences*

Bacterial carbon sources in coastal sediments: a review based on stable isotope data of biomarkers

S. Bouillon[1] and H. T. S. Boschker[2]

[1]Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[2]Netherlands Institute of Ecology (NIOO-KNAW), Centre for Estuarine and Marine Ecology, PO Box 140, 4400 AC Yerseke, The Netherlands

Received: 26 August 2005 – Accepted: 24 September 2005 – Published: 14 October 2005

Correspondence to: S. Bouillon (steven.bouillon@vub.ac.be)

Abstract

Coastal ecosystems are typically highly productive, and recieve organic matter from a variety of local and imported sources. To assess if general patterns are present in the origin of carbon sources for sedimentary bacteria and their relation to the origin of the sediment organic carbon pool, we compiled both literature and new data on $\delta^{13}C$ of bacterial biomarker PLFA (the phospholipid derived fatty acids $i+a15{:}0$) along with $\delta^{13}C$ data on sediment organic carbon ($\delta^{13}C_{TOC}$) and macrophyte biomass. Such data were collected from a variety of typical near-coastal systems, including mangroves, salt marshes (both C3 and C4-dominated sites), seagrass beds, and macroalgae-based systems, as well as unvegetated sediments. First, our $\delta^{13}C_{i+a15:0}$ data showed a large variability over the entire range of $\delta^{13}C_{TOC}$, indicating that in many settings, bacteria may depend on carbon derived from various origins. Secondly, systems where local macrophyte production is the major supplier of organic carbon for in situ decomposition are generally limited to organic carbon-rich, peaty sites (TOC>10 wt%) which are likely to make up only a small part of the global area of vegetated coastal systems. These carbon-rich sediments also provided a field based estimate of isotopic fractionation in bacterial lipid synthesis (−3.7±2.1‰), that is similar to the expected value. Thirdly, only in systems with low TOC (below ∼1 wt%), we consistently found that bacteria were on average selectively utilizing an isotopically enriched carbon source, which may be root exudates but more likely is derived from microphytobenthos. In other systems with between ∼1 and 10 wt% TOC, bacteria appear to show on average little selectivity and $\delta^{13}C_{i+a15:0}$ data generally follow the $\delta^{13}C_{TOC}$, even in systems where the TOC is a mixture of algal and macrophyte sources that generally are believed to have a very different degradability.

BGD

2, 1617–1644, 2005

1. Introduction

The coastal zone is widely recognized as a biogeochemically active region, where organic carbon inputs from a variety of sources undergo intense biogeochemical processing. It forms a significant component in the global oceanic carbon budget despite a relatively small areal extent (e.g. Gattuso et al., 1998; Borges, 2005; Middelburg et al., 2004; Duarte et al., 2004). The coastal zone is suggested to be responsible for about 20% of the oceanic primary production and the vast majority of oceanic organic carbon burial (Gattuso et al., 1998; Duarte et al., 2004), and the most recent data compilation indicates that, although not well constrained, benthic mineralization in coastal sediments amounts to 620 Tmol C y^{-1} or half of the total mineralization in marine sediments (Middelburg et al., 2004).

Various sources of organic matter enter the coastal zone, ranging from local primary production by phytoplankton or benthic microalgae, terrestrial inputs via river discharge to production by macrophyte systems such as seagrasses, macroalgal beds, mangroves and salt marshes. The sediment organic matter pool is therefore mostly derived from a mixture of source materials as a result of the intense mixing by currents. The identity and importance of the source materials that drive mineralization in sediments likely depends on a combination of their relative amounts and degradability. Carbon sources in coastal areas are characterized by a large variability in their composition and degradability, ranging from labile sources such as phytoplankton and benthic microalgae to less degradable sources such as macrophyte material and terrestrial C transported by rivers. Degradability can further be modified in time as less available fractions remain (Middelburg, 1989) or decreased by adsorption to clay minerals (Keil et al., 1994). Recent studies in estuaries have indicated that bacterial mineralization can be sustained both by aquatic primary production and by terrestrial C, and that it can have a large impact on the amount, composition, age, and lability of organic matter prior to its export into the coastal zone or ocean (e.g. Raymond and Bauer, 2001; McAllister et al., 2004; Boschker et al., 2005).

BGD
2, 1617–1644, 2005

In coastal ecosystems, movement of carbon across ecosystem boundaries complicates budgeting studies, as mineralization may be partially sustained by non-local sources, necessitating the use of proxies to take these sources into account (e.g. Bouillon et al., 2004). For a given system, it is not always straightforward to assess the origin of carbon driving benthic mineralization. The importance of non-local sources has been demonstrated both with stable isotope techniques (e.g. Boschker et al., 1999; Holmer et al., 2004) and based on mass balance considerations – i.e. when benthic mineralization rates considerably exceed rates of local primary production (e.g. Barrón et al., 2004).

Stable carbon isotope signatures (δ^{13}C) of the various carbon inputs are often different, and despite some overlap between different sources, can be powerful tracers of carbon inputs in various ecosystem components (Fry and Sherr, 1984). Although bulk stable isotope measurements have been possible for several decades, the introduction of compound-specific δ^{13}C analyses as a tool to include microbial communities has only started a decade ago (Freeman et al., 1990). PLFA (phospholipid derived fatty acids) in particular have become popular biomarkers for stable isotope studies since they are representative of live microbial biomass (fast degradation of phospholipids occurs after cell death), are suitable for gas chromatography – isotope ratio mass spectrometry (GC-IRMS) analysis after a derivatization procedure which introduces only one additional C atom, and because various PLFA can be linked to specific microbial groups (Boschker and Middelburg 2002).

In this study, we have compiled 339 data on both bulk sedimentary biogeochemical parameters (total organic carbon content (%TOC) and the stable isotope composition or organic C, i.e. $\delta^{13}C_{TOC}$) and PLFA proxies for the isotope composition of sedimentary bacteria ($\delta^{13}C_{i+a15:0}$) from a variety of coastal ecosystems in order to identify general patterns in the sources of sedimentary carbon and their use by microbial communities across and within these coastal ecosystem types. To determine the origin of carbon supporting in situ bacterial populations, we selected iso- and anteiso-branched 15:0 (i+a15:0) PLFA because (i) these branched fatty acids have been well

BGD
2, 1617–1644, 2005

demonstrated to be of bacterial origin, (ii) they show no chromatographic interferences with other compounds under the analytical conditions used, (iii) they are ubiquitous in coastal marine sediments in concentrations suitable for $\delta^{13}C$ analysis, and (iv) fractionation data are available in the literature (Boschker et al., 1999). The extensive dataset in this meta-analysis is used to demonstrate general trends in carbon sources used by bacteria in near-coastal sediments.

2. Materials and methods

2.1. Data sources

Overall, our dataset contains 187 data from our own published work, 23 data from other literature sources, and 129 new observations (see http://www.biogeosciences.net/bgd/2/1617/bgd-2-1617-sp.pdf). An overview of the various sampling locations and data sources is presented in Table 1. Only data where both $\delta^{13}C_{TOC}$ and $\delta^{13}C_{i+a15:0}$ was measured on sediment horizons in the 0 to 10 cm depth range were retained. Due to some missing data on other parameters (e.g. %TOC, $\delta^{13}C_{plant}$), the number of data points for each ecosystem in some of the graphs may differ slightly.

Data from mangrove systems have been gathered in the following locations: (i) a lagoonal mangrove system in southwest Sri Lanka (Pambala, see Bouillon et al., 2004a), (ii) estuarine mangrove sites in southeast India (Pichavaram and Chunnambar, see Bouillon et al., 2004a), (iii) various sites in an estuarine mangrove system with adjacent seagrass beds in southeast Kenya (Gazi Bay, see Bouillon et al., 2004b), and (iv) riverine mangrove forests along the Tana river (northeast Kenya) and estuarine mangrove sites in the Tana delta (northeast Kenya) collected in April 2004 (this study). For the latter sites, it is worth mentioning that the organic matter transported by Tana river contains a significant amount of C4-derived carbon, with river and mangrove creek particulate organic carbon having $\delta^{13}C$ values of ~−20‰ (S. Bouillon, unpublished data).

BGD
2, 1617–1644, 2005

Data from marshes dominated by C4 plants (*Spartina* spp) were obtained from a number of sites on both sides of the Atlantic Ocean. Literature data from the estuarine Waarde and the Kattedijke marshes (The Netherlands), the Great Marshes (MA, USA, see Boschker et al., 1999), and from the Gulf of Mexico (Cifuentes and Salata, 2001) were included together with a large number of data from unpublished work on various marshes in the Netherlands, France and the USA (Table 1). Sampling sites for C4 marshes were chosen to cover a range of sediment organic matter content from very organic-poor and recently colonized sites on sand beaches to older, organic rich systems with silt or peat-rich sediments. Literature data from C3 marshes were very scarce (Waarde marsh, the Netherlands, see Boschker et al. (1999), and from the Gulf of Mexico, see Cifuentes and Salata, 2001), but a significant amount of new data have been gathered from the marsh on the island of Schiermonnikoog (Wadden Sea, the Netherlands) where an elevation and age gradient was sampled.

Finally, seagrass data were compiled from various temperate (Cifuentes and Salata, 2001; Boschker et al., 2000; Holmer et al., 2004) and tropical (Holmer et al., 2001; Jones et al., 2003; Bouillon et al., 2004b) systems. Both subtidal and intertidal seagrass beds from all climatic zones are represented in our data set. In addition to vegetated sediments, data from nearby unvegetated sites were also available for various marshes and seagrass beds. Many of the intertidal and subtidal unvegetated sediments were covered by benthic microalgae, mainly diatoms. Only a very limited number of data on macroalgae systems are available (Holmer et al., 2004), and in these cases the distinction between vegetated and unvegetated (i.e. mudflats and bare sub-tidal sediments) was not always straightforward, so no distinction was made and they were combined with the unvegetated sites.

It should be noted that for the data in Cifuentes and Salata (2001), we calculated some of the $\delta^{13}C_{i+a15:0}$ data as the average of tabulated $\delta^{13}C_{i15:0}$ and $\delta^{13}C_{a15:0}$ values. Since these show excellent correlation when sufficient chromatographic separation is achieved, any possible bias introduced by this procedure is likely irrelevant in the context of this meta-analysis. For all other sites, the more correct, concentration-

based average δ^{13}C is reported for the bacterial markers i15:0 and a15:0. Finally, we also considered the possibility of compiling data on the δ^{13}C composition of the diatom marker 20:5ω3 to evaluate the coupling between microphytobenthos and bacteria. Although such data were available from a limited number of sites (see Boschker et al., 2000; Holmer et al., 2004), the low concentrations of this marker in other systems did not allow us to reliably use the resulting δ^{13}C data or to unambiguously ascribe the marker data to benthic diatoms, since it has also been reported to occur in other algae (Volkman et al., 1989) and heterotrophic micro-eukaryotes such as ciliates (Harvey et al., 1997). In addition, the 20:5ω3 PLFA is only an indicator for living biomass and not for algal detritus which may be important in many of the studied systems.

2.2. Analytical techniques

For all new data in this study, sediment samples were collected with corers. The exact sediment horizons analyzed depends on the data set, but all samples presented here are from the 0 to 10 cm depth range. Samples for PLFA analysis were either directly transferred in the extraction solvents, or frozen, after which they were freeze-dried and stored frozen prior to extraction. Extraction and derivatisation of PLFA was performed using a modified Bligh and Dyer extraction, silica column partitioning, and mild alkaline transmethylation as described earlier (Boschker et al., 2004; Bouillon et al., 2004a). δ^{13}C of the resulting FAMEs (fatty acid methyl esters) were determined on a ThermoFinnigan Delta type of GC-IRMS (gas chromatograph – isotope ratio mass spectrometer) in various configurations. All samples were run in split-less mode, using a HP-5 or BPX-70 column (30 or 60 m, 0.32 mm ID) with a He flow rate of 2 ml/min. δ^{13}C data of PLFA are corrected for the addition of the methyl group by simple mass balance, and were calibrated by our own internal and external FAME standards. Reproducibility is estimated to be 0.6‰ or better. Elemental analyses (TOC, TN, as % of sediment dry weight) and bulk TOC δ^{13}C analyses were performed by elemental analyzer-IRMS. For a more elaborate description of sampling and analytical details,

BGD
2, 1617–1644, 2005

we refer to Boschker (2004) and Bouillon et al. (2004a).

3. Results and discussion

The two most important data types collect in our study are the stable carbon isotopic ratio of the sediment organic matter ($\delta^{13}C_{TOC}$) and of the bacterial PLFA ($\delta^{13}C_{i+a15:0}$). The relationship between these two data types and the way we analyze them is presented in Fig. 1. The TOC in sediments is generally a mixture of different source materials with different isotopic ratios and the sediment isotopic ratio ($\delta^{13}C_{TOC}$) is therefore indicative for the carbon sources that contribute to the TOC pool. Bacteria will probably only utilize a fraction of the TOC as their substrate as the various source materials are characterized by differences in degradability or accessibility. It is generally accepted that isotopic ratios of bacteria or heterotrophic organisms in general reflects their substrate (with no discernable fractionation, e.g. Fry and Sherr, 1984; Hullar et al., 1996). However, we analyzed bacterial PLFA as representatives of the bacterial biomass and there generally is an offset between the total biomass and PLFA (the latter being more ^{13}C-depleted) due to fractionation effects during fatty acid synthesis. This fractionation factor is however not well constrained, but appears fairly constant for diverse bacterial communities growing on complex substrates as found in sediments (Boschker et al., 1999). In order to relate the isotopic ratio of the bacterial PLFA to the substrates the bacteria were using, this offset must be known and relatively constant. In the discussion below, we will first discuss general trends in the isotopic composition of the TOC in our data set and then turn our attention to the bacterial biomarker data and their relationship with the TOC.

3.1. Sources of organic carbon in coastal sediments

Organic carbon in coastal sediments mostly consists of a mixture of different sources, including locally produced macrophyte material, microphytobenthos, and suspended

organic matter, with a variable composition, imported from outside the ecosystem boundaries – through sedimentation in the water column (subtidal seagrass beds) or during tidal inundation (mangroves and salt marshes). A combination of sediment organic carbon concentrations and $\delta^{13}C$ of sediment TOC can be used to document the variation in the relative importance of local versus allochthonous carbon sources (Middelburg et al., 1997). For mangrove and salt marsh systems, a good overall relationship between $\delta^{13}C_{TOC}$ and sediment TOC levels was found (Fig. 2a), with $\delta^{13}C_{TOC}$ values approaching those of the local macrophyte inputs in systems with high TOC of more than 10% (i.e. ~−26‰ in mangroves and ~−14‰ in *Spartina* marshes), whereas in low TOC settings (up to between 5 and 10% TOC), the $\delta^{13}C_{TOC}$ is much more variable and reflects a variable contribution by benthic microalgae, phytoplankton and detrital inputs. Such allochthonous inputs can have a wide range of $\delta^{13}C$ signatures, and depending on the ecosystem considered may consist of terrestrial C (either from C3 or C4-dominated catchments), marine or estuarine phytodetritus, microphytobenthos and seagrass-derived C. Based on silt content data that were available for a subset of the sediments (not shown), this range of low TOC sediments also presents a transition from predominantly sandy to silty sediments.

The patterns observed in Fig. 2a confirm those reported earlier for *Spartina* marshes (Middelburg et al., 1997) and mangroves (Bouillon et al., 2003a, 2004a), whereby it was proposed that variations in sediment TOC and $\delta^{13}C_{TOC}$ can in general be adequately described as resulting from simple admixture of local macrophyte C and tidal inputs of suspended matter. Sediments where local macrophyte inputs dominate are typically peaty (i.e. high %TOC), with $\delta^{13}C_{TOC}$ close to those of the macrophyte vegetation; whereas the more mineral-rich sediments result largely from sedimentation and trapping of suspended material and its associated organic matter – hence, such sediments are characterized by a lower TOC content and highly variable $\delta^{13}C_{TOC}$, often deviating significantly from the $\delta^{13}C$ signature of the dominant vegetation. Moreover, for salt marshes where data from both vegetated systems and adjacent mudflats or unvegetated patches are available, unvegetated areas typically have a lower TOC content and

show $\delta^{13}C$ signatures which deviate from those of the dominant vegetation, i.e. lower $\delta^{13}C$ for *Spartina* marshes, and higher $\delta^{13}C$ for C3 marshes (Fig. 2). The latter pattern indicates, as expected, that vegetated patches have an important but variable contribution from local macrophyte carbon, and that unvegetated sites are more dominated by tidally imported C and, possibly, microphytobenthos.

For seagrass and unvegetated systems, such clear relationships between %TOC and $\delta^{13}C_{TOC}$ are not observed (Fig. 2a), but it should be kept in mind that (i) the overall range of TOC is much smaller than that in marshes and mangroves, and (ii) the variability in macrophyte $\delta^{13}C$ values in seagrass systems is much larger, since the $\delta^{13}C$ of the seagrass biomass is in part determined by the $\delta^{13}C$ of the dissolved inorganic carbon pool (DIC) and the growth conditions, which can be highly variable in coastal settings (e.g. Hemminga and Mateo, 1996).

From the direct comparison of $\delta^{13}C_{TOC}$ with $\delta^{13}C_{plant}$ (Fig. 3a), it is clear that in most cases, local macrophyte production is not the dominant C input to the sediment TOC pool. If macrophyte material would dominate the TOC pool then most of the data would plot close to the 1:1 line in Fig. 3a, i.e. sediment $\delta^{13}C_{TOC}$ would reflect the signature of the macrophyte vegetation. The deviation from this expected pattern for all systems considered (i.e. more positive $\delta^{13}C$ in C3 marshes and mangroves, more negative $\delta^{13}C$ in C4 marshes, seagrasses and macroalgae) is consistent with extensive inputs from suspended organic C and/or microphytobenthos, and this pattern is similar to that recently reported based on a more comprehensive data compilation on seagrass sediment $\delta^{13}C$ data (Kennedy et al., 2004; Bouillon et al., 2004b). A second point worth noting in Fig. 2 is that settings in which local macrophyte inputs dominate the TOC pool (i.e. with high TOC content and $\delta^{13}C$ values close to those of the macrophyte vegetation) are overall quite scarce. Although it could be argued that the dataset here is too limited to generalize this conclusion, this pattern appears to be maintained if other datasets on TOC in coastal sediments are included (e.g. for mangrove systems, 65% of the data we have compiled show a TOC of less than 5%, and 82% of the data have less than 10% TOC, n=650). This implies that a substantial

BGD
2, 1617–1644, 2005

input of non-local organic carbon should be considered to be more rule than exception in coastal sediments (see also Duarte et al., 2004). The sediment organic matter in most systems therefore consists of a mixture of carbon sources potentially sustaining bacterial mineralization processes and growth.

3.2. Bacterial carbon sources in coastal sediments

Given the wide range of $\delta^{13}C_{TOC}$ and $\delta^{13}C_{plant}$ in coastal ecosystems, we used a compilation of concurrent $\delta^{13}C_{TOC}$ and $\delta^{13}C$ data of bacterial PLFA (i+a15:0, Fig. 2b) to determine the extent to which bacteria assimilate various available carbon sources. The coastal ecosystems covered here are typically very productive, and a substantial part of this production is by macrophytes (saltmarsh plants, mangroves and seagrasses). The compilation of data presented here, however, shows that this local macrophyte production is not the dominant carbon source in most systems, and thereby generalizes and confirms some of our earlier case studies (e.g. Boschker et al., 1999; Bouillon et al., 2004a). When compared to the stable isotope signatures of the dominant vegetation (Fig. 3b), the $\delta^{13}C_{i+a15:0}$ data clearly demonstrate that in C3-dominated systems (mangroves and C3 marshes), bacteria typically consume carbon sources more enriched in ^{13}C than local macrophytes; whereas in *Spartina* marshes, seagrass and macroalgae beds (where local macrophytes are characterized by heavy $\delta^{13}C$ signatures), the substrate used by bacteria is generally much more depleted in ^{13}C than the local macrophytes. The general trend is therefore that the bacterial PLFA show more average isotopic ratios whereas the local macrophytes are found at the extremes of the range. Given the evidence mentioned above that tidally imported carbon sources form a significant and often isotopically distinct (Fig. 2a) input in these systems, this provides good evidence that these imported C sources often are a major C source sustaining benthic mineralization.

Considering that a variety of C sources can be available in coastal sediments, and that such different sources may have a different lability or accessibility, the question

BGD
2, 1617–1644, 2005

arises if and to which extent bacteria make selective use of certain sources. In the simplest scenario whereby no selectivity would occur, we would expect to see an excellent relationship between $\delta^{13}C_{TOC}$ and $\delta^{13}C_{i+a15:0}$, with an offset caused by isotope fractionation during fatty acid biosynthesis (determined experimentally for i+a15:0 as −5.6±1.8‰ by Boschker et al., 1999). Although the entire dataset (Fig. 4) shows a reasonable positive relationship (R^2=0.58, slope close to unity) between $\delta^{13}C_{TOC}$ and $\delta^{13}C_{i+a15:0}$, the variability observed is much larger than would be expected given the analytical precision of both parameters (better than ±0.2‰ and ±0.6‰, respectively) and the variability in isotope fractionation between PLFA and carbon source in the experiments by Boschker et al. (1999) (−5.6±1.8‰) or estimated from our data set (−3.7±2.1‰, n=29, see further). For any given $\delta^{13}C_{TOC}$, the range in $\delta^{13}C_{i+a15:0}$ typically spans 10‰ or more, which indicates that bacteria in many cases do not assimilate carbon sources merely in proportion to their relative abundance in the sediment TOC pool. It is also apparent from Fig. 4 that the majority of the points are located above the expected line, which suggests that in a substantial number of sediments the bacteria preferentially utilize an isotopically enriched carbon source.

A further interesting pattern in bacterial selectivity can be discerned when plotting the difference between both bacterial PLFA and TOC (hereafter referred to as $\Delta\delta$, i.e. $\delta^{13}C_{i+a15:0}-\delta^{13}C_{TOC}$) as a function of the organic carbon content of the sediments considered (Fig. 5). As discussed above, if bacteria show no selectivity against the different carbon sources in the TOC, the $\Delta\delta$ values would be more or less constant and show a slightly negative offset due to isotopic fractionation in bacterial lipid synthesis. In order to evaluate possible trends in this dataset, we first performed a cumulative sum analysis based on the median value. This indicated that there were three distinct regions (%TOC<0.8, 0.8<%TOC<2.3, and %TOC>2.3), and $\Delta\delta$ values in these regions were found to differ significantly (Dunn's multiple comparison test, p<0.05).

A first observation is that the $\Delta\delta$ data converge to a median value −3.8‰ (interquartile range: 1.5‰) for the data where %TOC exceeds 2.3% (see Fig. 5b, where average $\Delta\delta$ values are plotted for binned data). If we consider only the data with a TOC con-

BGD
2, 1617–1644, 2005

tent higher than 10%, and hence where macrophyte material is the (only) dominant C source available (Fig. 2a), the average $\Delta\delta$ values is -3.7 ± 2.1‰ (n=29), and our dataset therefore provides an empirical verification of the fractionation between substrate and i+a15:0 under field conditions. This relationship is very robust as it holds for both sediments from C3-dominated mangroves and C4, *Spartina* marshes, which have very different isotopic ratios in the sediment TOC. This value is also within the range reported by Boschker et al. (1999) and the expected value of −3‰ due to fatty acid synthesis in general (Hayes, 2001), and confirms that possible variations in the degree of isotope fractionation with environmental conditions (e.g. under anoxic or oxic conditions (Teece et al., 1999) are not likely to be a major limitation in our interpretations. Moreover, this field verification suggests that the large range in lipid fractionations found in experiments with single (simple) substrates and/or specific bacterial strains (e.g. Pelz et al., 1997; Abraham et al., 1998; Teece et al., 1999) likely can be ruled out in natural, highly diverse communities where bacteria process more complex natural organic substrates. However, we do find a considerable variation around the mean $\Delta\delta$ values (SD of binned ranges vary between 1.8 and 3.3‰, Fig. 5a), which could be due to both a selective use of certain organic matter sources and to some variation in fractionation in lipid synthesis between samples.

Secondly, in all sediments with a TOC content above 0.8%, the difference between δ^{13}C for TOC and the bacterial biomarkers is found to be also fairly constant; even though a Dunn's multiple comparison test indicates a slightly higher $\Delta\delta$ values where 0.8<%TOC<2.3 as compared to where %TOC>2.3, this difference is small (∼1‰). This uniformity in $\Delta\delta$ values is remarkable because it suggests that in this intermediate range (roughly between 1 and 10% TOC) the bacteria on average also utilized the TOC as found in the sediment with little preference between the different source materials. However, our $\delta^{13}C_{TOC}$ data (Fig. 2a) suggest that in this range the TOC is made of a mixture of source materials derived from various algal and macrophyte sources, which in general have a greatly different degradability. Material from algae is mostly much more available to bacteria and is degraded with a much higher rate

than macrophyte derived materials (Schoenberg et al., 1990; Hee et al., 2001). This difference in degradability is though to be the result of differences in biochemical composition between algal and macrophyte derived materials. These intrinsic differences in degradability appear, however, on average not to be expressed in the sediments that we studied, indicating that other mechanisms may determine the degradability of organic materials in these sediments. Most of the TOC found in sediments is sorbed to the mineral, clay phase of the sediment, and it has been shown that this greatly reduces the availability for bacterial degradation (Keil et al., 1994). A hypothesis to explain our results may be that this sorption determines the degradability of all source materials to a similar extent and that the availability of the organic matter is largely determined by the rate at which the sorbed substrates are released from the mineral phase.

Thirdly, below a TOC content of 0.8% there is a significant and large shift to more positive $\Delta\delta$ values (median $\Delta\delta$ −0.2‰). Under the assumption that isotope fractionation in lipid synthesis is similar as in the high TOC environments, this shift indicates that in these low TOC sediments, bacteria are preferentially utilizing an easily degradable, relatively ^{13}C-enriched carbon source with a limited abundance in the total TOC pool (Fig. 5). The data in this range of %TOC are mainly from C4 marshes and seagrass beds, which both have relatively enriched ratios in the local macrophyte material, and also from unvegetated sediments. At the vegetated sites, this may suggest that the enriched source material used by bacteria may be organic material, such as root exudates, released from seagrasses and C4 marsh plants (*Spartina* spp.), which are generally simple organic molecules that are readily available to bacteria. This effect is also clearly seen in the $\delta^{13}C_{i+a15:0}$ data from C4, *Spartina* marshes in Fig. 2b, which show substantial an increase in low TOC sediments after reaching minimum values at approximately 5% TOC. However, this explanation is not consistent with the pattern observed in the $\delta^{13}C_{i+a15:0}$ data presented in Fig. 3b. Especially for the unvegetated sediments where a role for root exudates or other readily available macrophyte materials is unlikely, the data are also consistent with an important role for microphytobenthos-derived carbon as a carbon source for bacteria, since this source has δ^{13}C signatures

BGD
2, 1617–1644, 2005

generally more positive than those of the sediment TOC pool (typically between −20 and −13‰, e.g. see France 1995). The reason why this effect is only expressed in low TOC sediments, is likely related to the lower availability of organic matter in these sediments. The role of microphytobenthos as a carbon substrate for bacteria has been suggested previously in several studies (Boschker et al., 1999; Bouillon et al., 2004a; Cook et al., 2004), and this view is consistent with results from ^{13}C-labeling experiments where a very rapid transfer of microphytobenthos C to bacteria was found (Middelburg et al., 2000; own unpublished data from mangroves in Kenya).

The data from the C3 marshes in Schiermonnikoog (the Netherlands), however, do not follow the pattern as discussed above, as they show a higher $\Delta\delta$ values compared the other data above a TOC of 1% (Fig. 5, although a cumulative sum analysis including the Schiermonnikoog data gave the same pattern as without). Since they are also distinct from the other C3 marsh data, we consider this to be a site-specific case for which we have no conclusive explanation. Root exudation can not be an explanation as for C4 marshes and seagrass beds, because the local C3 macrophyte material has a depleted signature. However, this marsh is situated next to the very extensive mud-flats of the Wadden Sea that are prone to wind induced erosion due to their long wind fetch. It has been shown that the seston in the tide water of the Wadden Sea contains high amounts of benthic diatoms eroded from the mud-flat surface, and benthic diatoms even dominate the plankton during high winds (de Jonge and van Beusekom, 1995). As sedimentation of suspended materials on salt marshes mainly occurs during high wind conditions, our data may indicate that the bacteria in the sediment of the Schiermonnikoog marsh thrive to a large extent on imported microphytobenthos material that was produced on the nearby mud-flats. Another exception is the study by Cook et al. (2004) on a pristine intertidal mud-flat in Tasmania (data not shown in Fig. 5). Here the TOC was dominated by terrestrial C3 material, but the bacteria were probably mainly growing on material produced by benthic microalgae leading to $\Delta\delta$ ratios between 2 to 7‰.

BGD
2, 1617–1644, 2005

4. Conclusions

4.1. Representativeness of the current data set

Despite the relatively large number of data compiled in this study (~340, from a variety of coastal systems), the question should be raised whether this dataset is sufficiently representative. For mangrove systems, we feel the data are likely to cover most types of settings, since they span the full range of %TOC and $\delta^{13}C_{TOC}$ encountered in the literature (e.g. see compilation in Bouillon et al., 2003a), but there are some less frequently encountered cases for which no $\delta^{13}C_{i+a15:0}$ are available. One such example is the situation described by Wooller et al. (2003), where high %TOC (29–36%) coincide with high $\delta^{13}C_{TOC}$ (−24.6 to −20.2‰) due to particularly large inputs of seagrass material. Similarly, considering the range of %TOC and $\delta^{13}C_{TOC}$ covered by our data (Fig. 2a), we can argue that *Spartina* marshes and seagrass systems are likely to be covered in a representative way. Data on C3 marshes are more scarce, however, and as discussed above, may be somewhat biased since the majority of data come from a single site with a possibly exceptionally high contribution of resuspended benthic diatoms in the tidal inputs.

Finally, we must stress that there is an almost complete lack of data on macroalgae-based systems, despite the fact that of all the vegetated coastal systems, they globally cover the largest surface area and their integrated benthic mineralization rate (247 Tmol C yt^{-1}) is larger than that of the other systems combined (208 Tmol C y^{-1}, Middelburg et al., 2004).

4.2. Distinction between local macrophyte production and terrestrial carbon

Terrestrial organic carbon sources transported to the coastal zone generally fall in two categories, C3 and C4-plant derived matter, each with a distinct and non-overlapping $\delta^{13}C$ range (typically ~−27 and −13‰, respectively). Since most of the data presented here come from regions where C3 vegetation dominates the catchment areas,

we must keep in mind that terrestrial C and some local producers (C3 marshes and mangroves) are isotopically indistinguishable, and hence, that part of the C we ascribe to local macrophytes might in fact be terrestrial C (as part of the suspended matter pool deposited during inundation). One particular case, however, are the data from the Tana delta (northern Kenya), where a significant part of the catchment area is dominated by C4 grasslands, and where riverine suspended matter is comprised by ~50% C4-derived C (S. Bouillon, unpublished data). In this particular case, the contribution by C4-derived C is also reflected in the $\delta^{13}C_{TOC}$ data of the mangrove sediments (~−21‰) and in the $\delta^{13}C_{i+a15:0}$ data (~−25.5‰), which could indicate that this terrestrial carbon is a significant C source for sedimentary bacteria. It is not implausible that such a pattern is more widespread, but if the terrestrial C pool is derived from C3 vegetation, this would likely go undetected with the techniques used here except for seagrass systems and C4-marshes.

4.3. Conclusions and implications for carbon dynamics in the coastal zone

Our study clearly illustrates that mineralization in coastal sediments is often not fuelled by local macrophyte production and that in certain systems, bacteria may be selectively degrading more labile carbon sources such as microphytobenthos and carbon imported or settled from the water column. This may have implications for budgeting studies, since community respiration rates (where no source characterization is done) may overestimate the role of mineralization in the C budget of a particular ecosystems' production (e.g. see Bouillon et al., 2004b). Moreover, if such additional sources are preferentially mineralized, this implies that the fraction of carbon available for further export or long-term burial will differ in source, age, and composition to the total C pool available. Furthermore, these results may also have some implications for our understanding of N cycling in coastal systems, since the assimilated algae-derived material typically has a much higher N content than organic matter derived from vascular plants.

In summary, our meta-analysis on bacterial carbon sources in near-coastal sediments demonstrates that:

1. $\delta^{13}C$ of bacterial PLFA show a large variability over the entire range of $\delta^{13}C_{TOC}$ data, indicating that in most settings, sedimentary bacteria may depend on C from various origins,

2. systems where local macrophyte production is the major supplier of C for in situ decomposition are generally limited to organic carbon-rich sites (TOC>10%), which are likely to make up only a small part of the global areas of salt marsh and mangrove systems. In this respect, there appears to be a major difference in functioning between "open" and "closed" systems – the former with more pronounced exchange and subsidy of organic matter with adjacent systems.

3. in the majority of systems with ~1 to 10% TOC, bacterial PLFA $\delta^{13}C$ data indicate that non-macrophyte sources such as microphytobenthic production or imported carbon sources become important substrates but there is on average no apparent preferential use of the different source materials.

4. Only for sediments with less than ~1% TOC, bacteria clearly make preferential use of an isotopically heavy carbon source. These sediments were mostly from C4 *Spartina* marshes, seagrass beds and unvegetated sites suggesting that this heavy carbon source may be either root exudates from macrophytes or material produced by benthic diatoms. A similar effect was however also found for a C3-marsh where the macrophyte material is relatively depleted, suggesting that import or local production of microphytobenthos is a likely explanation.

Acknowledgements. Funding for this work was provided by the Fonds voor Wetenschappelijk Onderzoek (FWO-Vlaanderen, contract G.0118.02), EC-INCO project contract ERB IC18-CT98-0295, the NSF Microbial Observatories Project "Salt Marsh Microbes and Microbial Processes", the NIOZ-NIOO cooperation project and the NWO-VIDI grant of HTSB. SB is a postdoctoral fellow with the FWO-Vlaanderen. We are grateful to the following people for help during sample collection, fieldwork logistics, and analyses: I. De Mesel, A. Borges, J. Kairo, J. Bosire, A. Rao, F. Dahdouh-Guebas, V. Sharma, A. Wielemaker, J. de Brouwer, V. Créach, M. Steegstra, B. Schaub, S. Meger, H. van Wijnen, J. Bakker, M. Holmer, C. Hopkinson and

J. Hobbie. B. Jones (Texas A&M University) kindly provided his raw data on sediment, macrophyte, and PLFA δ^{13}C. A. Borges, F. Dehairs, D. Gillikin, P. Mangion, J. Middelburg, and N. Koedam provided useful comments on an earlier draft of this manuscript. This is NIOO-KNAW publication no. XXXX.

References

Barrón, C., Marba, N., Terrados, J., Kennedy, H., and Duarte C. M.: Community metabolism and carbon budget along a gradient of seagrass (*Cymodocea nodosa*) colonization, Limnol. Oceanogr. 49, 1642–1651, 2004.

Abraham, W. R., Hesse, C., and Pelz, O.: Ratios of carbon isotopes in microbial lipids as an indicator of substrate usage, Appl. Environ. Microbiol. 64, 4202–4209, 1998.

Borges, A. V.: Do we have enough pieces of the jigsaw to integrate CO_2 fluxes in the coastal ocean ?, Estuaries 28, 3–27, 2005.

Boschker, H. T. S., de Brouwer, J. F. C., and Cappenberg, T. E.: The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments : stable carbon isotope analysis of microbial biomarkers, Limnol. Oceanogr. 44, 309–319, 1999.

Boschker, H. T. S., Wielemaker, A., Schaub, B. E. M., and Holmer, M.: Limited coupling of macrophyte production and bacterial carbon cycling in the sediments of *Zostera* spp. meadows, Mar. Ecol. Prog. Ser. 203, 181–189, 2000.

Boschker, H. T. S. and Middelburg, J. J.: Stable isotopes and biomarkers in microbial ecology, FEMS Microbiol. Ecol. 40, 85–95, 2002.

Boschker, H. T. S.: Linking microbial community structure and functioning: stable isotope (^{13}C) labeling in combination with PLFA analysis, pp. 1673–1688, in: Molecular Microbial Ecology Manual II, edited by: Kowalchuk, G. A., de Bruijn, F. J., Head, I. M., Akkermans, A. D., and van Elsas, J. D., Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004.

Boschker, H. T. S., Kromkamp, J., and Middelburg, J. J.: Biomarker and carbon isotopic constraints on bacterial and algal community structure and functioning in the highly polluted Scheldt estuary, Limnol. Oceanogr., 50, 70–80, 2005.

Bouillon, S., Dahdouh-Guebas, F., Rao, A. V. V. S., Koedam, N., and Dehairs, F.: Sources of organic carbon in mangrove sediments : variability and some possible implications for ecosystem functioning, Hydrobiologia, 495, 33–39, 2003a.

Bouillon, S., Frankignoulle, M., Dehairs, F., Velimirov, B., Eiler, A., Etcheber, H., Abril, G., and Borges, A. V.: Inorganic and organic carbon biogeochemistry in the Gautami Godavari estuary (Andhra Pradesh, India) during pre-monsoon : the local impact of extensive mangrove forests, Glob. Biogeochem. Cycles, 17, 1114, doi:10.1029/2002GB002026, 2003b.

Bouillon, S., Moens, T., Koedam, N., Dahdouh-Guebas, F., Baeyens, W., and Dehairs, F.: Variability in the origin of carbon substrates for bacterial communities in mangrove sediments, FEMS Microbiol. Ecol. 49, 171–179, 2004a.

Bouillon, S., Moens, T., and Dehairs, F.: Carbon sources sustaining benthic mineralization in mangrove and adjacent seagrass sediments (Gazi bay, Kenya), Biogeosciences 1, 71–78, 2004b.

Cifuentes, L. A. and Salata, G. G.: Significance of carbon isotope discrimination between bulk carbon and extracted phospholipid fatty acids in selected terrestrial and marine environments, Org. Geochem., 32, 613–621, 2001.

Cook, P. L. M., Revill, A. R., Clementson, L. A., and Volkman, J. K.: Carbon and nitrogen cycling on intertidal mudflats of a temperate Australian estuary, III. Sources of organic matter, Mar. Ecol. Prog. Ser., 280, 55–72, 2004.

Duarte, C. M., Middelburg, J. J., and Caraco, N.: Major role of marine vegetation on the oceanic carbon cycle, Biogeosciences, 2, 1–8, 2004,
SRef-ID: 1726-4189/bg/2005-2-1.

France, R. L.: C-13 Enrichment in benthic compared to planktonic algae – foodweb implications, Mar. Ecol. Prog. Ser., 124, 307–312, 1995.

Freeman, K. H., Hayes, J. M., Trendel, J. M., and Albrecht, P.: Evidence from carbon isotope measurements for diverse origins of sedimentary hydrocarbons, Nature, 343, 254–256, 1990.

Fry, B. and Sherr, E. B.: $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems, Contrib. Mar. Sci., 27, 13–47, 1984.

Gattuso, J. P., Frankignoulle, M., and Wollast, R.: Carbon and carbonate metabolism in coastal aquatic ecosystems, Annu. Rev. Ecol. Syst., 29, 405–434, 1998.

Harvey, H. R., Ederington, M. C., and McManus, G. B.: Lipid composition of the marine ciliates *Pleuronema* sp. and *Fabrea salina*: shifts in response to diet, J. Eukar. Microbiol, 44, 189–193, 1997.

Hayes, J. M.: Fractionation of carbon and hydrogen isotopes in biosynthetic processes, in: Organic geochemistry of contemporaneous and ancient sediments, edited by: Valley, J. S.

and Cole, D. R., Mineralogical Society of America, 225–277, http://www.blackwell-synergy.com/na102/home/ACS/publisher/synergy/journals/entities/2013.gif, 2001.
Hee, C. A., Pease, T. K., Alperin, M. J., and Martens, C. S.: Dissolved organic carbon production and consumption in anoxic marine sediments: A pulsed-tracer experiment, Limnol. Oceanogr., 46, 1908–1920, 2001.
Hemminga, M. A. and Mateo, M. A.: Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies, Mar. Ecol. Prog. Ser., 140, 285–298, 1996.
Holmer, M., Andersen, F. O., Nielsen, S. L., and Boschker, H. T. S.: The importance of mineralization based on sulfate reduction for nutrient regeneration in tropical seagrass sediments, Aquat. Bot., 71, 1–17, 2001.
Holmer, M., Duarte, C. M., Boschker, H. T. S., and Barrón, C.: Carbon cycling and bacterial carbon sources in pristine and impacted Mediterranean seagrass sediments, Aquat. Microb. Ecol., 36, 227–237, 2004.
Hullar, M. A. J., Fry, B., Peterson, B. J., and Wright, R. T.: Microbial utilization of estuarine dissolved organic carbon: A stable isotope tracer approach tested by mass balance, Appl. Environ. Microbiol., 62, 2489–2493, 1996.
Jones, W. B., Cifuentes, L. A., and Kaldy, J. E.: Stable carbon isotope evidence for coupling between sedimentary bacteria and seagrasses in a sub-tropical lagoon, Mar. Ecol. Prog. Ser., 255, 15–25, 2003.
Keil, R. G., Monlucon, C. B., Prahl, G. F., and Hedges, J. I.: Sorptive preservation of labile organic matter in marine sediments, Nature, 370, 549–551, 1994.
Kennedy, H., Gacia, E., Kennedy, D. P., Papadimitriou, S., and Duarte, C. M.: Organic carbon sources to SE Asian coastal sediments, Estuar. Coast. Shelf. Sci., 60, 59–68, 2004.
McAllister, S. L., Bauer, J. E., Cherrier, J. E., and Ducklow, H. W.: Assessing sources and ages of organic matter supporting river and estuarine bacterial production: a multiple-isotope ($\Delta^{14}C$, $\delta^{13}C$, and $\delta^{15}N$) approach, Limnol. Oceanogr., 49, 1687–1702, 2004.
Middelburg, J. J.: A simple rate model for organic-matter decomposition in marine- sediments, Geochim. Cosmochim. Acta, 53, 1577–1581, 1989.
Middelburg, J. J., Barranguet, C., Boschker, H. T. S., Herman, P. M. J., Moens, T., and Heip, C. H. R.: The fate of intertidal microphytobenthos carbon: an in situ ^{13}C labeling study, Limnol. Oceanogr., 45, 1224–1234, 2000.
Middelburg, J. J., Duarte, C. M., and Gattuso, J. P.: Respiration in coastal benthic communities, in: Respiration in aquatic ecosystems, edited by: del Giorgio, P. A. and Williams, le B., P. J.,

Oxford University Press, 2004.
Middelburg, J. J., Nieuwenhuize, J., Lubberts, R. K., and van de Plassche, O.: Organic carbon isotope systematics of coastal marshes, Estuar. Coast. Shelf Sci., 45, 681–687, 1997.
Pelz, O., Hesse, C., Tesar, M., Coffin, R. B., and Abraham, W. R.: Development of methods to measure carbon isotope ratios of bacterial biomarkers in the environment, Isotopes Environ. Health Stud., 33, 131–144, 1997.
Raymond, P. A. and Bauer, J. E.: DOC cycling in a temperate estuary: a mass balance approach using natural ^{14}C and ^{13}C isotopes, Limnol. Oceanogr., 46, 655–667, 2001.
Schoenberg, S. A., Benner, R., Armstrong, A., Sobecky, P., and Hodson, R. E.: Effects of acid stress on aerobic decomposition of algal and aquatic macrophyte detritus – direct comparison in a radiocarbon assay, Appl. Environm. Microbiol., 56, 237–244, 1990.
Teece, M. A., Fogel, M. L., Dollhopf, M. E., and Nealson, K. H.: Isotopic fractionation associated with biosynthesis of fatty acids by a marine bacterium under oxic and anoxic conditions, Org. Geochem., 30, 1571–1579, 1999.
Volkman, J. K., Jeffrey, S. W., Nichols, P. D., Rogers, G. I., and Garland, C. D.: Fatty acids and lipid compositions of 10 species of microalgae used in mariculture, J. Exp. Mar. Biol. Ecol., 128, 219–240, 1989.
Wooller, M., Smallwood, B., Jacobson, M., and Fogel, M.: Carbon and nitrogen stable isotopic variation in *Laguncularia racemosa* from Florida and Belize: implications for trophic level studies, Hydrobiologia, 499, 13–23, 2003.

Table 1. Overview of study site characteristics and data sources.

Site	Latitude, Longitude	Number of samples per ecosystem type					Data source
		C4 marsh	C3 marsh	Mangrove	Seagrass	Unvegetated and macroalgae	
Schiermonnikoog (the Netherlands)	53°30' N 6°10' E		35			6	1
St Annaland (the Netherlands)	51°36' N 4°08' E	2			3	3	1, 2
Kattedijke (the Netherlands)	51°38' N 3°56' E	3				2	3
Waarde marsh (the Netherlands)	51.24°' N 4°07' E	17	9			13	1, 3
Valkenisse (the Netherlands)	51°23' N 4°08' E	2				2	1
Ritthem (the Netherlands)	51°27' N 3°40' E	13				8	1
Mont St. Michel Bay (France)	48°36' N 1°48' E	3					1
Plum Island Sound (MA, USA)	42°45' N 70°50' W	13				10	1
Great Marshes (MA, USA)	41°43' N 70°21' W	3				2	3
Canary Creek (DW, USA)	38°47' N 75°09' W	2				2	1
North River (NC, USA)	34°45' N 76°35' W	2	2		2	1	4
North Inlet (SC, USA)	33°20' N 79°10' W	2				2	1
Chunnambar (India)	11°53' N 79°48' E			3			5
Pambala (Sri Lanka)	7°35' N 79°47'E			24			5
Pichavaram (India)	11°27' N 79°17' E			8			5
Gazi Bay (Kenya)	4°22' S 39°30' E			41	12		6
Tana delta (Kenya)	2°30'S, 40°30'E			15			1
Nyborg Fjord (Denmark)	55°17' N 10°49' E				6	6	2
Arcachon Bay (France)	44°40' N 1°10' E				9	9	2
Mallorca, various sites (Spain)	39°09'N 2°56'E				10	14	7
Laguna Madre (TX, USA)	26°09' N 97°12' W				8	4	8
Ban Pak Klok (Thailand)	8°03' N 98°25' E				6		1, 9

Data sources: 1: this study, 2: Boschker et al. (2000), 3: Boschker et al. (1999), 4: Cifuentes and Salata (2001), 5: Bouillon et al. (2004a), 6: Bouillon et al. (2004b), 7: Holmer et al. (2004), 8: Jones et al. (2003), 9: Holmer et al. (2001).

BGD

2, 1617–1644, 2005

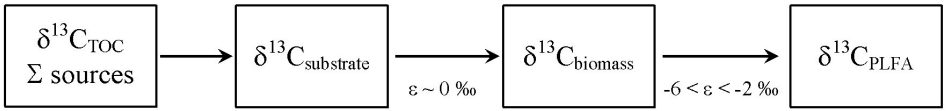

Fig. 1. Analytical scheme used for the stable isotope data collected in this study.

BGD
2, 1617–1644, 2005

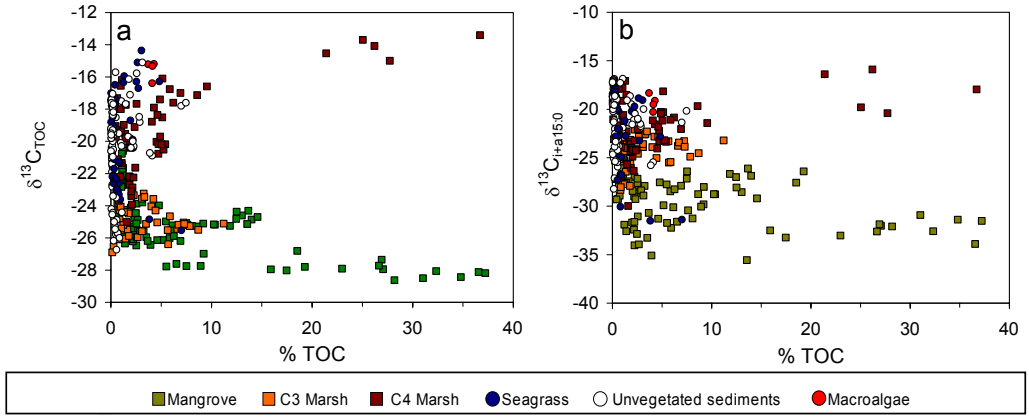

Fig. 2. Compilation of δ^{13}C signatures of **(a)** bulk sediments organic carbon and **(b)** δ^{13}C of bacterial PLFA as a function of sedimentary organic carbon content in different types of coastal ecosystems. Note the different scales on the Y-axes.

BGD
2, 1617–1644, 2005

Bacterial carbon sources in coastal sediments

S. Bouillon and
H. T. S. Boschker

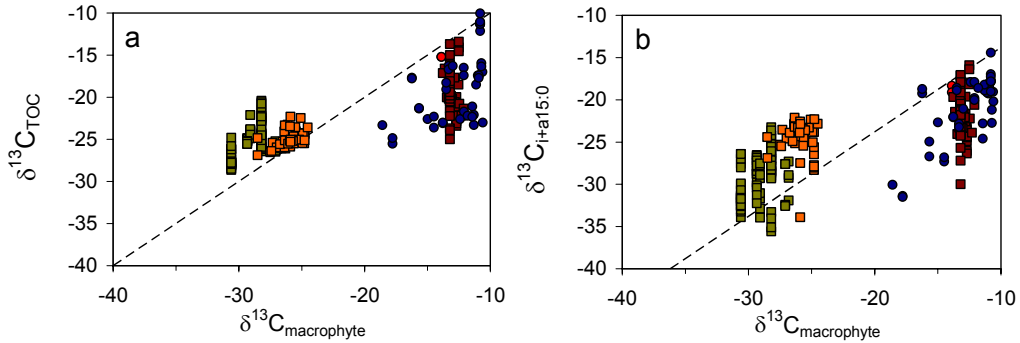

Fig. 3. Plot of **(a)** δ^{13}C of bulk sediment TOC and **(b)** δ^{13}C of bacterial PLFA *i*+*a*15:0 versus δ^{13}C of the dominant macrophyte vegetation for all vegetated coastal ecosystems considered. Note that for C3 marshes, C4 marshes, and mangroves for which no direct measurements of plant δ^{13}C were available, an average value of the data from other sites was assigned (i.e. −28.2‰ for mangroves, −25.9‰ for C3 marshes, and −13.2‰ for C4 marshes. The isoline in (a) is a 1:1 line, in (b) the isoline represents a shift of −3.7‰ to correct for fractionation between *i*+*a*15:0 and the substrate (see text for rationale). Symbols as in Fig. 2.

BGD
2, 1617–1644, 2005

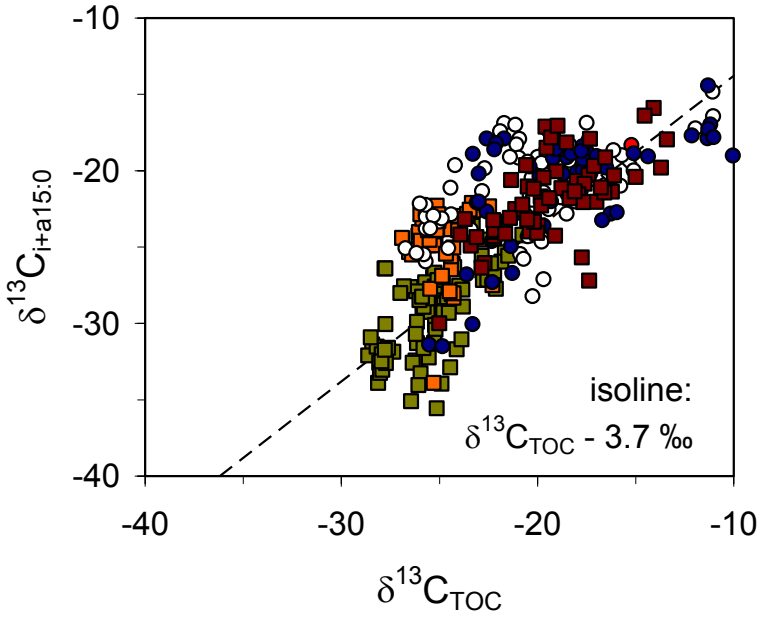

Fig. 4. Plot of δ^{13}C of bacterial PLFA ($i+a$15:0) versus δ^{13}C of the total sediment organic carbon pool (TOC), for various types of coastal ecosystems. The dotted line represents the expected $\delta^{13}C_{i+a15:0}$ when bulk TOC would be the main substrate (see text for details). Symbols as in Fig. 2.

BGD
2, 1617–1644, 2005

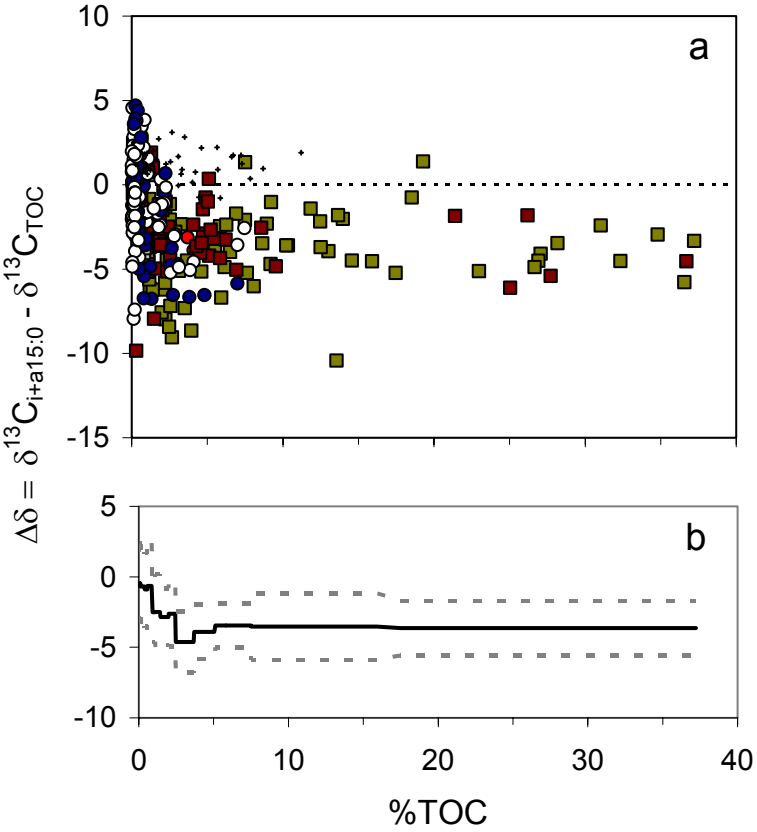

Fig. 5. **(a)** Plot of $\Delta\delta$ (defined as $\delta^{13}C_{i+a15:0} - \delta^{13}C_{TOC}$) as a function of the organic carbon content of coastal sediments. Note that the data for vegetated C3 marshes from one of the sites (Schiermonnikoog) fall outside the general pattern and are therefore plotted separately with small +. Symbols further as in Fig. 2. **(b)** shows the binned averages ±1 s.d., whereby each %TOC interval represents 20 datapoints.

BGD
2, 1617–1644, 2005