Tracing terrestrial organic matter by δ^{34}S and δ^{13}C signatures in a subarctic estuary

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Abstract

   A key issue to understanding the transformations of terrestrial organic carbon in the ocean is to disentangle the latter from marine-produced organic matter. We applied a multiple stable isotope approach using δ^{34}S and δ^{13}C isotope signatures from estuarine dissolved organic matter (DOM), enabling us to constrain the contribution of terrestrial-derived DOM in an estuarine gradient of the northern Baltic Sea. The stable isotope signatures for dissolved organic sulfur (δ^{34}S$_{DOS}$) have twice the range between terrestrial and marine end members compared to the stable isotope signatures for dissolved organic carbon (δ^{13}C$_{DOC}$); hence, the share of terrestrial DOM in the total estuarine DOM can be calculated more precisely. DOM samples from the water column were collected using ultrafiltration on board the German RV *Maria S Merian* during a winter cruise, in the Bothnian Bay, Bothnian Sea, and Baltic proper. We calculated the terrestrial fraction of the estuarine DOC (DOC$_{ter}$) from both δ^{13}C$_{DOC}$ and δ^{34}S$_{DOS}$ signatures and applying fixed C : S ratios for riverine and marine end members to convert S isotope signatures into DOC concentrations. The δ^{34}S$_{DOS}$ signature of the riverine end member was +7.02‰, and the mean signatures from Bothnian Bay, Bothnian Sea, and Baltic proper were +10.27, +12.51, and +13.67‰, respectively, showing an increasing marine signal southwards (δ^{34}S$_{DOS}$ marine end member = 18.1‰). These signatures indicate that 87%, 75%, and 67%, respectively, of the water column DOC is of terrestrial origin (DOC$_{ter}$) in these basins. Comparing the fractions of DOC$_{ter}$ in each basin—that are still based on few winter values only—with the annual river input of DOC, it appears that the turnover time for DOC$_{ter}$ in the Gulf of Bothnia is much shorter than the hydraulic turnover time, suggesting that high-latitude estuaries might be efficient sinks for DOC$_{ter}$.

Alerts about carbon leakage from thawed permafrost in the taiga–tundra region in the Northern Hemisphere (Freeman et al. 2001; Tranvik and Jansson 2002) have set focus on the role of dissolved organic carbon (DOC) in the global carbon cycle (Hedges et al. 1997), because soil carbon in this region corresponds to about 70 times the annual CO_2 emission from anthropogenic sources (Gorham 1991). The Arctic Ocean, although it is the smallest ocean with respect to area and volume (holds only 1% of the global ocean water), receives about 10% of the global river discharge, draining the carbon-rich taiga and tundra regions. The rivers entering the Arctic Ocean have among the highest DOC concentrations in the world (Dittmar and Kattner 2003). As a possible response to global warming, the discharge of fresh water to the Arctic Ocean has already been altered (Peterson et al. 2002) and this makes the Arctic Ocean and its drainage area particularly interesting when studying climate change issues and in particular the fate of terrestrial-derived carbon in the oceans. Terrestrial DOC comprises only a small fraction (0.2–2.4%) of the total DOC in the global ocean, and has an oceanic residence time (20–132 yr) which is much shorter than that of the bulk marine dissolved organic matter (DOM) (Hedges et al. 1997; Opsahl and Benner 1997), i.e., terrestrial DOC will be removed on its way to the open ocean. Thus, the argument on conservative behavior of DOC in high-latitude seas as inferred from conservative mixing lines (Kattner et al. 1999; Dittmar and Kattner 2003) can be questioned.

   The general role of estuaries in retaining terrestrial-derived matter is well known, whereas for DOM the role of respiration and sedimentation as potential removal pro-

cesses are not yet clearly understood (Hedges and Keil 1999; Kattner et al. 1999; Raymond and Bauer 2001*a*). 90% of total marine organic matter burial is believed to occur in the estuaries and shelf areas (Berner 1989), but a precise estimate of how much of this buried matter originates from terrestrial matter and how much is derived from full marine- and estuarine-derived matter is still missing (Humborg 1997; Raymond and Bauer 2001*a*). Furthermore, respiration as a possible removal process is the least constrained rate in carbon mass balances for seawaters in general (del Giorgio and Duarte 2002). Thus, there are no general agreements on the C removal processes in the estuarine areas of high latitudes (Hedges et al. 1997; del Giorgio and Duarte 2002; Algesten et al. 2006)

Property salinity plots of total organic carbon (TOC) and of DOC often show conservative behavior in high-latitude estuaries (Wedborg et al. 1994; Kattner et al. 1999), whereas studies from the Baltic Sea have shown that humic substances as typically land-derived material showed a negative deviation from the conservative mixing line, indicating significant removal during estuarine mixing (Fonselius 1995). Removal processes could be masked by estuarine primary production in salinity–DOC plots (Raymond and Bauer 2001*a*), and the key issue to understanding the retention and the fate of terrestrial-derived matter in estuaries is to be able to determine the contribution of allochthonous (terrestrial) vs. autochthonous (marine and estuarine) organic carbon to total DOC (Hedges et al. 1997). End-member mixing analysis (EMMA) based on isotope signatures of DOM is often used to identify mixing proportions of different sources in a compiled sample. For the case of organic material in an estuary, following equation could be used:

$$\text{DOM}_e \times \text{I}_e = \text{DOM}_t \times \text{I}_t + \text{DOM}_m \times \text{I}_m + \text{DOM}_a \times \text{I}_a \qquad (1)$$

where e = estuary, t = terrestrial, m = marine, and a = DOM added in the estuary. DOM_x = concentration of DOM, and I_x is the isotope signature from that source. For additional information about abbreviations, *see* Table 1.

Whereas the isotopic signatures of the riverine and marine end members can be characterized rather easily, the isotopic signatures of DOM added by estuarine production are difficult to estimate, because estuarine production assimilates biogenic elements originating from both marine and riverine sources. In the case of dissolved inorganic carbon (DIC; mainly CO_3^{2-} and HCO_3^-), the concentration in marine waters is only about two times higher than the concentration of DIC in the rivers entering the estuary. Consequently, estuarine production will assimilate DIC from full marine sources (0 to +2‰), from riverine DIC (±0‰ to −15‰) as well as from respired terrestrial and marine organic material with δ^{13}C signatures of about −28‰ and −22‰, respectively (Chanton and Lewis 1999; Peterson and Fry 1987). Even though δ^{13}C is one of the most commonly used stable isotope signatures for tracing terrestrial matter (Raymond and Bauer 2001*b*), the terrestrial input to estuarine DIC implies a difficulty using δ^{13}C in these environments. In other words, primary production in the estuarine areas uses significant amounts of terrestrial DIC for photosynthesis, making it difficult to identify this autochthonous material and to distinguish from allochthonous (terrestrial) material, because the δ^{13}C signature from the estuarine production is a third end member in the EMMA, as described in Eq. 1.

A possible way to avoid a significant contribution of the estuarine end member to the total isotopic signatures of DOM in estuaries would be to choose a biogenic element for which concentrations in the marine realm are by orders of magnitude different from concentrations in the terrestrial realm. In this study, we aim to explore the use of δ^{34}S in dissolved organic sulfur (DOS) as a tracer for terrestrial-derived DOC in brackish environments and yet in a multiple stable isotope approach. For this purpose, δ^{34}S isotope signatures appear to have the following advantages: (1) even estuarine phytoplankton production will have a typical marine δ^{34}S signature, which will make it possible to distinguish the terrestrial matter from the marine and the estuarine produced organic matter; (2) the isotopic signatures of terrestrial- and marine-derived organic matter have a wider range than for δ^{13}C; and (3) assimilation and degradation of sulfur under oxic conditions fractionates the δ^{34}S signature no more than 1–2‰ (Peterson et al. 1985; Peterson and Fry 1987). Thus, δ^{34}S$_{DOS}$ signature appears to be a stable tracer in oligotrophic and well-oxygenated estuaries such as are often found in high latitudes.

The aim of this study was to test a multiple-isotope approach (δ^{13}C and δ^{34}S) addressing the relative amounts of terrestrial- vs. marine-derived organic matter in one of the best-monitored high-latitude estuaries, i.e., the Gulf of Bothnia. The Gulf is ice-covered during winter and the rivers draining into the Gulf have similar organic matter concentrations as the large Siberian and Canadian rivers (Smedberg et al. 2006). Thus the Gulf of Bothnia can be seen as a model system for the much larger Arctic estuaries, but with more constrained physical borders and better-known fluxes of water and elements in and out of the system (Wulff et al. 2001). The relative proportions of terrestrial- and marine-derived organic matter as inferred from the isotope signatures of DOM will finally be compared with the hydraulic turnover time for the Gulf of Bothnia.

Methods

Sampling and sampling sites—The northern part of the Baltic Sea is called the Gulf of Bothnia, and a sill of 45-m depth close to the island of Åland separates it from the Baltic proper. The area of the Gulf of Bothnia is 115,500 km^2 and the volume is 6369 km^3 (Bernes 1988).

The Gulf of Bothnia is divided into two major basins: the Bothnian Bay in the North and the Bothnian Sea in the south, which are separated by a sill of 25-m depth. The water is highly influenced by the rivers entering both basins; the salinity in the Bothnian Bay is 2.0–3.8, and in the Bothnian Sea 4.8–6.0 (Fonselius 1995).

Samples were taken during a winter cruise with the German RV *Maria S Merian* between 28 Feb 06 and 16 Mar 06. Therefore, all values obtained are winter values, which are assumed unaffected by seasonal fluctuations

Table 1.   All abbreviations used in Eqs. 1 and 3–7 in order of appearance.*

Abbreviation	Explanation	Used in Eq.
DOM	Dissolved organic material, i.e., organic carbon <0.7 μm. Consists of about 50% carbon (DOC) and 0.5% sulfur (DOS). H, O and N are other major elements in DOM.	1
DOC	Dissolved organic carbon.	3, 6, 7
DOS	Dissolved organic sulfur.	4, 5, 6
DOC_{ter}	Fraction of *DOC-TOT* in retentate that has a terrestrial origin.	3, 6, 7
$\delta^{13}S_{DOC\text{-}TOT}$	$\delta^{13}C$ signature measured in the retentate.	3
$\delta^{13}C_{DOCmar}$	$\delta^{13}C$ signature in marine end member, i.e., the phytoplankton production, $= -21$‰ to -22‰ (Peterson and Fry 1987).	3
$\delta^{13}C_{DOCter}$	$\delta^{13}C$ signature in terrestrial end member, mainly deriving from vascular plants, $= -28$‰ (Peterson and Fry 1987).	3
$Conc_{SO4}$	Concentration of SO_4^{2-} in the retentate (measured with IC).	4
$Conc_{S\text{-}TOT}$	Total S concentration in the retentate (measured with ICP-OES).	4
$Conc_{DOS}$	Concentration of DOS in the retentate, calculated as difference between $Conc_{S\text{-}TOT}$ and concentration of $Conc_{SO4}$.	4
$\delta^{34}S_{SO4}$	$\delta^{34}S$ measured in the precipitated $BaSO_4$.	4
$\delta^{34}S_{TOT}$	$\delta^{34}S$ measured in the freeze-dried concentrated DOM from retentate.	4
$\delta^{34}S_{DOS}$	$\delta^{34}S$ for the organic fraction of the $\delta^{34}S_{DOS}$ signature in the retentate.	4
$\delta^{34}S_{DOSter}$	The $\delta^{34}S_{DOS}$ of the terrestrial end member measured at the Kalix River mouth.	5
$\delta^{34}S_{DOSmar}$	The $\delta^{34}S_{DOS}$ of the marine end member, taken from literature values of Baltic plankton (Hartmann and Nielsen 1969).	5
DOC_{ter}	The fraction of *DOS-TOT* in retentate with a terrestrial origin.	5
$\delta^{34}S_{DOS\text{-}TOT}$	The calculated $\delta^{34}S_{DOS}$ signature from Eq. 4.	5
$C:S_{ter}$	The ratio (molar ratio) between carbon concentration and sulfur concentration in the terrestrial fraction of the sample, assumed to be 300 (Hessen and Tranvik 1998; Findlay and Sinsabaugh 2003).	6, 7
$C:S_{mar}$	The ratio between carbon concentration and sulfur concentration (molar ratio) in the marine fraction (derived from phytoplankton production) of the sample, assumed to be 100 (Ho et al. 2003; Twining et al. 2004).	6, 7
DOC_{mar}	The fraction of *DOC-TOT* that has a marine origin $(1 - DOC_{ter})$.	7

* *DOC-TOT*, $\delta^{13}C$ signature found in the retentate; IC, ion chromatography; ICP-OES, inductively coupled plasma–optical emission spectrometry; *DOS-TOT*, $\delta^{34}S$ signature found in the retentate.

caused by primary production. All stations sampled for this study are shown in Fig. 1.

On board the research vessel, samples for ultrafiltration were taken with a pump connected to a conductivity, temperature, and depth (CTD) probe (Seabird®). This made it possible to get the necessary water for the analyses at any desired depth. The ultrafiltration samples, between 50 and >100 liters each, were taken in surface water at 5-m depth. At each station, TOC samples from the entire water column were also taken from a Rosette sampler connected to a CTD. The samples were not filtered (and are therefore referred to as TOC, not DOC) and analyzed directly onboard. An ultrafiltration sample from the Kalix River mouth (Kamlunge) was taken during spring flood at 19 Jun 06 as an end-member sample and prefiltered and ultra-filtered in the same way as the Baltic samples.

Preparation of the samples—The major challenge in using $\delta^{34}S_{DOS}$ lies in its relatively low concentration, i.e., the SO_4^{2-} concentration in marine waters dominates the $\delta^{34}S$ signature, and the signal of $\delta^{34}S_{DOS}$ is not detectable as such in bulk measurements of marine waters (the percentage sulfur in DOM is only around 0.5% [C.-M. Mörth unpubl.]). With DOC concentrations around 350 μmol L^{-1} and SO_4^{2-} concentrations of about 2.8 mmol L^{-1} in the Gulf of Bothnia, the SO_4^{2-} concentrations were about 1600 times higher than the DOS

concentrations; in the river water the ratio of SO_4^{2-} to DOS was roughly only 2.5 because of the much lower SO_4^{2-} concentration.

Ideally, to measure both $\delta^{34}S_{DOS}$ and $\delta^{34}S_{SO4}$, the DOS concentrations and SO_4^{2-} ions should be in the same concentration range. This was achieved by diluting the samples with MilliQ water to decrease the SO_4^{2-} concentration, followed by a concentration of DOS by means of ultrafiltration.

The concentration of DOM was performed at the ship using ultrafiltration with a >1-kDa filter cutoff, according to the method described in Larsson et al. (2002). Prefiltration to distinguish between particulate organic matter and DOM was made by pumping the sample through a GF/F filter (pore size range 0.5–0.7 μm, preheated to 500°C for 4 h, Whatman® GF/F) before starting the ultrafiltration. Prior to the sampling cruise, filters made by regenerated cellulose had been tested, and proved not to become negatively charged and consequently to repel and concentrate SO_4^{2-} ions. The samples were concentrated to 0.5 liters and frozen on the ship. The filters were washed between the samples with 5 liters of NaOH (pH 10–11), 5 liters of MilliQ water, 5 liters of HCl (pH 2–3), and 5 liters of MilliQ water. All fractions (before prefiltration, after prefiltration, retentate >1 kDa and permeate <1 kDa) were measured for organic carbon for calculations of recovery. Organic carbon concentrations for both
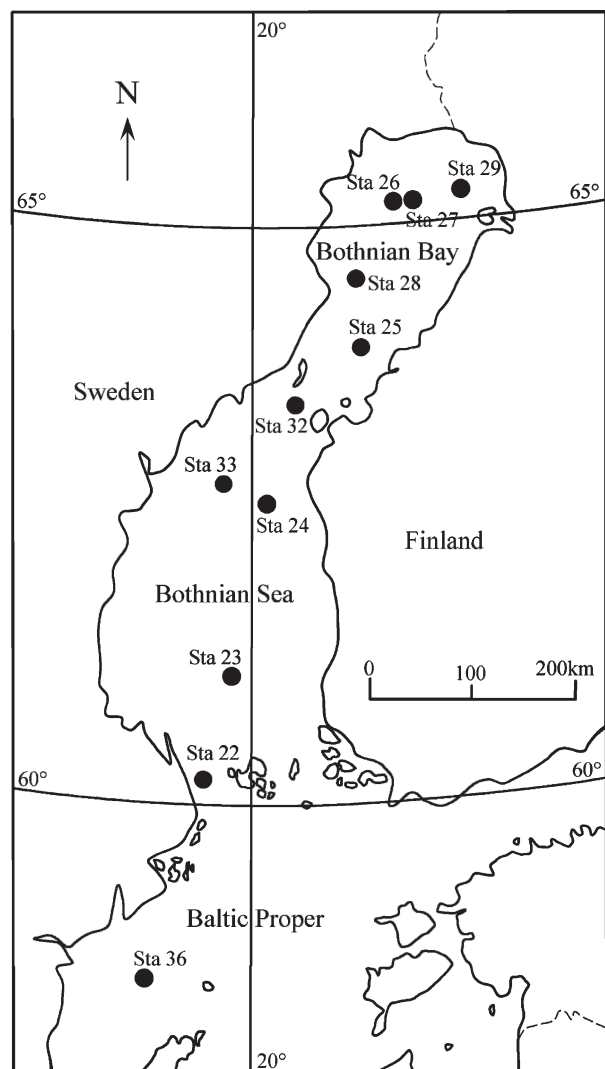
Fig. 1. Investigation area and sampling stations of the RV *Maria S. Merian* cruise, Feb 06–Mar 06.

ultrafiltration samples and the samples from the water column were determined on board by means of catalytic carbon combustion (Shimadsu TOC-VCPH). For $\delta^{13}C_{DOC}$ analyses, subsamples were taken from each retentate sample before further dilution.

In order to further decrease the SO_4^{2-} concentration in the retentate that still showed SO_4^{2-} concentrations between 2.8 and 5.6 mmol L^{-1} after the first ultrafiltration (similar to the original sampled water) and DOS concentrations around 0.01 mmol L^{-1}, the samples were diluted in MilliQ water (50–140 liters, depending on original salinity) and ultrafiltered again to a volume of 0.5 L. After this second ultrafiltration, similar SO_4^{2-} and DOS concentrations could be achieved, ranging between 6 and 70 μmol L^{-1} SO_4^{2-} and between 6 and 20 μmol L^{-1} DOS (Table 2).

Recovery—Recovery for the ultrafiltration of the Bothnian Bay and Bothnian Sea samples was low; though in normal range for oceanic samples between 10% and 17%.

For the river sample, it was satisfying, with 43% of total DOC (Benner et al. 1992). Ultrafiltration isolates DOM based on size rather than chemical properties, compared to, e.g., macroreticular XAD resin chromatography. The cross-flow rate (retentate flow : permeate flow) was kept well over 50; under that condition, Pellicon® 2 filters achieve a good recovery of the colloid fraction >1 kDa (Larsson et al. 2002).

Analyses—The SO_4^{2-} concentration ($Conc_{SO4}$) in each retentate was measured with ion chromatography (IC; Dionex DX-300 system equipped with an AS14 column using electrical suppression, Dionex) and the total S concentration ($Conc_{S\text{-}TOT}$) was measured with inductively coupled plasma–optical emission spectrometry (ICP-OES) analysis. The analyses were performed at the same day and with the same calibration solutions for both measurements, in order to minimize the errors. Based on standard measurements, the errors for IC measurements are better than 1% and for ICP-OES better than 2%. The DOS concentrations ($Conc_{DOS}$) were calculated as the difference between $Conc_{S\text{-}TOT}$ and $Conc_{SO4}$ (Table 2).

After measurements of the S concentrations in the retentate, the samples were freeze-dried and homogenized. SO_4^{2-} ions were precipitated from the permeate using $BaCl_2$, forming $BaSO_4$, in order to measure the inorganic $\delta^{34}S$ in each sample. The $\delta^{34}S$ signature of the total DOM solution ($\delta^{34}S_{TOT}$ of the retentate) was determined directly by measuring the freeze-dried organic matter of the retentate. After measuring the two isotopic S signatures of the inorganic and total S fractions ($\delta^{34}S_{SO4}$ and $\delta^{34}S_{TOT}$) of the retentate, we were able to calculate the remaining isotopic signature of the dissolved organic fraction ($\delta^{34}S_{DOS}$; Eq. 4).

Analyses of isotopic composition were executed in a Finnigan mass spectrometer (a Finnigan Delta+ coupled to an elemental analyzer–isotope ratio mass spectrometry [IRMS] combination, Carlo Erba NC2500, using continuous flow). The freeze-dried retentate (about 1.5–2 mg) and $BaSO_4$ (about 300 mg) were mixed with V_2O_5 (approx. 2 mg for the freeze-dried retentate and 1 : 1 by weight for $BaSO_4$, normally about 300 μg) and placed in tin capsules. The reference gas was measured before every sample with a precision better than ±0.2‰. Results are given as per-mil deviation from a standard (Pee Dee Belemnite [PDB] for C and a Cañon Diablo Troilite for S) and denoted δ, defined by Eq. 2, where R is the ratio of heavy to light isotopes (e.g., $^{13}C : ^{12}C$ or $^{34}S : ^{32}S$):

$$\delta^{13}C \text{ or } \delta^{34}S \text{ (‰)} = (R_{sample}/R_{standard} - 1) \times 1000 \quad (2)$$

Calculations—In this multiple-isotope approach we calculated the terrestrial part (fraction) of the DOC based on $\delta^{13}C$ and $\delta^{34}S$ signatures found in the retentate (*DOC-TOT* and *DOS-TOT*). Whereas the $\delta^{13}C$ signature could be used directly to calculate the terrestrial fraction of the estuarine DOC, we had first to calculate the $\delta^{34}S_{DOS}$ signatures in the retentate from the measured $\delta^{34}S_{SO4}$ and $\delta^{34}S_{TOT}$ signatures and second to apply a C : S ratio to convert S isotope signatures into DOC concentrations. All

Table 2. Measured and derived (calculated) variables used for the determination of DOC_{ter} and DOC_{mar} along the estuarine gradient in the northern Baltic Sea. Abbreviations used are the same as in Table 1; SO_4^{2-} (%) and DOC (%) give the relative contribution of both forms in the retentate; C:S ratios are the measured ratios in the retentates.

Basin	Station	Conc_{SO4} (μmol L^{-1})	Conc_{DOS} (μmol L^{-1})	SO_4^{2-} (%)	DOS (%)	$\delta^{34}S_{TOT}$ (‰)	$\delta^{34}S_{SO4}$ (‰)	C:S	$\delta^{34}S_{DOS}$ (‰)	DOS_{ter} (%)	DOC_{ter} (%)	DOC_{mar} (%)
				Measured values					Calculated values			
Kalix River mouth		22.4	12.6	63.9	36.1	6.33	5.94	324	7.02	100	100	0
Bothnian Bay	25	35.2	12.9	73.3	26.7	18.23	20.08	219	13.15	44.6	70.7	29.3
	26	11.4	12.9	46.9	53.1	14.38	20.13	226	9.30	79.4	92.0	8.0
	27	6.1	8.8	41.0	59.0	13.74	20.07	266	9.35	79.0	91.9	8.1
	28	6.8	11.7	36.9	63.1	13.39	20.18	248	9.42	78.3	91.6	8.4
	29	10.5	18.9	35.6	64.4	13.72	20.15	236	10.15	71.7	88.4	11.6
Bothnian Sea	23	32.9	7.5	81.4	18.6	18.75	20.31	229	11.90	55.9	79.2	20.8
	24	67.7	8.8	88.6	11.4	19.54	20.37	234	13.12	44.9	71.0	29.0
Baltic proper	36	9.0	5.7	61.2	38.8	17.78	20.38	282	13.67	39.9	66.6	33.4

abbreviations used in Eqs. 1 and 3–7 are explained in Table 1 in order of appearance.

$$DOC_{ter} =$$
$$\left(\delta^{13}C_{DOC\text{-}TOT} - \delta^{13}C_{DOCmar}\right) / \left(\delta^{13}C_{DOCter} - \delta^{13}C_{DOCmar}\right) \quad (3)$$

$\delta^{34}S_{DOS}$ was calculated by using the isotope measurements for $\delta^{34}S_{SO4}$ and $\delta^{34}S_{TOT}$, and the concentration of SO_4^{2-} and total S:

$$\mathrm{Conc}_{SO4} \times \delta^{34}S_{SO4} + \mathrm{Conc}_{DOS} \times \delta^{34}S_{DOS} =$$
$$\mathrm{Conc}_{S\text{-}TOT} \times \delta^{34}S_{TOT} \quad (4)$$

The terrestrial vs. marine fractions of DOS were calculated by EMMA using the terrestrial end member measured at the Kalix River mouth ($\delta^{34}S_{DOSter}$) and the marine end member reported in the literature (Hartmann and Nielsen 1969) of Baltic plankton ($\delta^{34}S_{DOSmar}$ = 18.1‰) measured in the western Baltic. Unfortunately we did not get any of our own samples of plankton, because the cruise was performed during winter. $^{34}S_{DOSmar}$ data are few but all lay in the range between 18‰ and 20‰ (Peterson et al. 1985; Chanton and Lewis 1999).

$$DOS_{ter} =$$
$$\left(\delta^{34}S_{DOS\text{-}TOT} - \delta^{34}S_{DOSmar}\right) / \left(\delta^{34}S_{DOSter} - \delta^{34}S_{DOSmar}\right) \quad (5)$$

After determining the C:S ratio in respective end members (C:S_{ter} from Hessen and Tranvik 1998 and Findlay and Sinsabaugh 2003; C:S_{mar} from Ho et al. 2003 and Twining et al. 2004), the following equation for converting the fraction of terrestrial DOS to the fraction of terrestrial DOC could be used:

$$DOC_{ter} = (C:S_{ter}/C:S_{mar}) \times$$
$$DOS_{ter}/(1 - DOS_{ter} \times (1 - (C:S_{ter}/C:S_{mar}))) \quad (6)$$

In order to test our assumptions of the organic C:S ratios that are critical for the overall results on the terrestrial fractions of the estuarine DOC, we calculated the expected ratio in each sample with a variant of an EMMA (Eq. 7) and compared that with the measured ratios in each samples. If these values do correspond reasonably, the assumed ratios in both end members can be regarded as plausible.

$$DOC_{ter} \times C:S_{ter} + DOC_{mar} \times C:S_{mar} = C:S_{sample} \quad (7)$$

Sensitivity analysis using Monte Carlo simulation—Monte Carlo simulations were made to calculate the error propagation from errors in the analysis of sulfur concentrations and isotope values when calculating the terrestrial contribution of DOM. The errors in calculated DOS_{ter} to *DOS-TOT* according to these simulations ranged from 3% to 17%, with an average error of 3.5%. In the simulations, isotope signatures were assumed to have an error of ±0.2‰ and concentrations ±2% (deduced from the analytical precision in the measurements); number of simulations were n = 100,000 and normal distributed errors were also assumed. The high error in one of the estimates was caused by the high SO_4^{2-} concentration in that retentate. We recommend that the contribution of DOS and SO_4^{2-} to the total S concentration should be rather equal.

Testing the $\delta^{34}S$ method—To test whether ultrafiltration does not fractionate $\delta^{34}S_{DOS}$ from DOM, we mixed organic material made of 10-yr-old leach water from spruce needles with a typical terrestrial $\delta^{34}S_{DOS}$ signature of 4.23‰ with an SO_4^{2-} standard ($CaSO_4 \times 2H_2O$) with a $\delta^{34}S_{SO4}$ signature of 24.98‰. These were diluted in MilliQ water and adjusted to DOC and SO_4^{2-} concentrations similar to those in the Bothnian Bay (335 μmol C L^{-1} and 2.8 mmol S_{SO4} L^{-1}). After the first concentration the solution was diluted again with MilliQ water in order to decrease the SO_4^{2-} concentrations. The $\delta^{34}S$ for the retentate >1 kDa, containing both SO_4^{2-} and DOS, was determined to be 19.56‰ and the contribution of the $\delta^{34}S_{DOS}$ vs. the $\delta^{34}S_{SO4}$ to the total $\delta^{34}S$ signature were calculated by using Eq. 4. We calculated $\delta^{34}S_{DOS}$ to 3.97‰, only 0.2‰ from the measured original signature.

Results

Salinity and oxic conditions—The salinity of the sampled water ranged from 3.0 (Sta. 26) to 11.0 below the halocline at the Landsort Deep (Sta. 36). All stations were ice-covered, except for the Landsort Deep and the Åland Deep (Sta. 22). The water column of two northern Baltic basins was oxic throughout. The sub-halocline water in the Landsort Deep was, however, anoxic, and the Åland Sea may experience oxygen reduced intrusions in the immediate bottom layer. In the surface mixed layer of both regions, where the sampling was performed, no indication of adjacent sulfate reduction could be observed. This was seen in the high oxygen saturation values of all transect stations and was supported by the fact that ammonia was always at the detection limit. In the cases where ammonia could be detected at all in the water column, it had, with the exception of the Landsort Deep bottom water, a mean $NO_3^{2-} : NH_4^+$ molar ratio of 226, documenting a highly oxidized state of the surface water. Thus, the $\delta^{34}S_{DOS}$ signatures could be regarded as not influenced by anoxic processes in the Gulf of Bothnia.

TOC vs. salinity—TOC concentrations measured in water column samples showed decreasing values towards higher salinities, although TOC concentrations scattered significantly in the various basins (Fig. 2), and thus only 25% of the variability in TOC concentrations could be related to salinity. Highest mean concentrations (334 μmol L^{-1}) were found in the Bothnian Bay, followed by the Bothnian Sea (316 μmol L^{-1}) and the Baltic proper (302 μmol L^{-1}). Note that the TOC samples from the Baltic proper were taken at only one station, explaining the more homogenous patterns in comparison with the former two basins, where many more stations were sampled (Fig. 1). The difference between TOC and DOC concentrations, measured after filtering the water through 0.7-μm
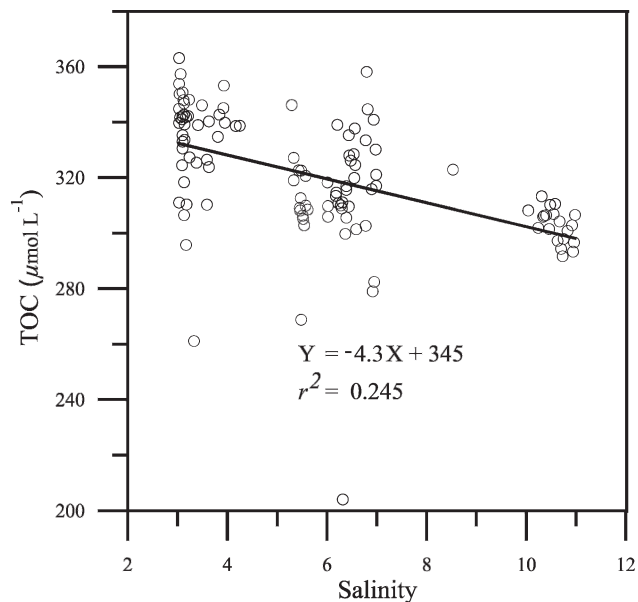

Fig. 2. TOC concentrations vs. salinity in the Bothnian Bay, Bothnian Sea, and Baltic proper in Mar 06.

GF/F filters, were small, often smaller than the analytical errors of the TOC measurements. Therefore, the TOC concentrations were also used as proxies for DOC concentrations in the mass balance calculation.

$\delta^{13}C$ signatures and C:N ratios of estuarine DOM—$\delta^{13}C_{DOC}$ showed the lightest signatures in the Bothnian Bay, with a mean of –26.7‰. The mean $\delta^{13}C_{DOC}$ signature in the Bothnian Sea, –25.4‰, was similar to the single sample from the Baltic proper of –25.5‰. Our end-member sample, the Kalix River mouth, showed a typical terrestrial signature of –27.8‰. The C:N ratios were highest in the Bothnian Bay (maximum 18.0) and lowest in the Baltic proper (10.0). The Kalix River mouth had a ratio of 13.6 (Table 3).

$\delta^{34}S$ signatures and C:S ratios of estuarine DOM—The terrestrial end member, measured at the Kalix River mouth, had a DOS isotope ($\delta^{34}S_{DOS}$) signature of +7.02‰. The mean signature for $\delta^{34}S_{DOS}$ in the Bothnian Bay was +10.27‰, in the Bothnian Sea +12.51‰, and in the Baltic proper +13.67‰, showing an increasing marine signal southwards. The C:S ratios of DOM ranged from 324 in the river mouth to a mean of 231 in the Bothnian Sea. The measured and the calculated (Eq. 7) C:S ratios for each sample were rather similar through all samples (except for Sta. 36, the Landsort Deep) and differed on average by 13.5% (Fig. 3).

Terrestrial vs. marine DOC in the Bothnian Bay and Bothnian Sea—The estuarine DOC in the Bothnian Bay, Bothnian Sea, and Baltic proper consisted of 87%, 75%, and 67%, respectively, terrestrial-derived matter as calculated from $\delta^{34}S_{DOS}$ signatures (Eqs. 5, 6). Calculated from the $\delta^{13}C_{DOC}$ signatures (Eq. 3), the terrestrial contribution to the DOC stock in the various basins amounted to 85% in

Table 3. $\delta^{13}C_{DOC}$ isotope signatures, C:N molar ratio, and the calculated (Eq. 3) relative contribution of DOC_{ter} and DOC_{mar} along the estuarine gradient in the northern Baltic Sea (*see* text for more details). Abbreviations used are the same as in Table 1.

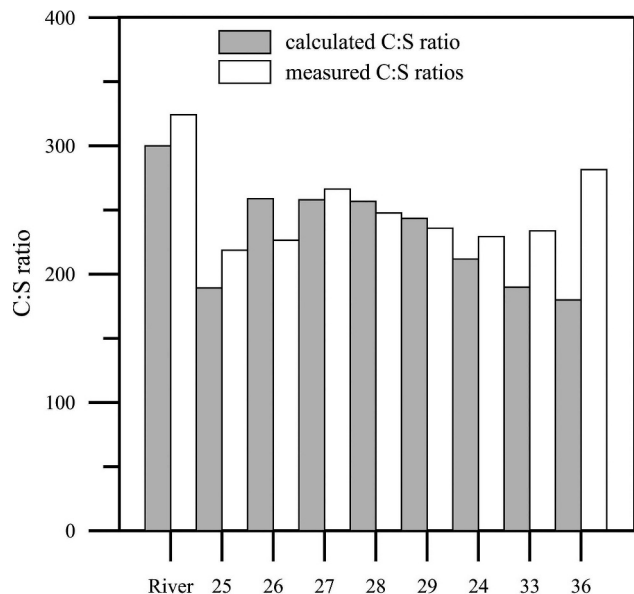| Basin	Station	$\delta^{13}C_{DOC}$ (‰)	C:N	DOC_{ter} (%)	DOC_{mar} (%)
Kalix River mouth		−27.75	13.6	100	0
Bothnian Bay	25	−27.09	16.6	90.3	9.73
	26	−26.88	17.9	87.1	12.9
	27	−26.40	18.0	80.0	20.0
	28	−26.71	15.4	84.5	15.5
	29	−26.54	16.9	82.1	17.9
Bothnian Sea	23	−25.42	15.8	65.5	34.5
	24	−25.51	16.4	66.8	33.2
Baltic proper	36	−25.49	10.0	66.4	33.6

Fig. 3. Calculated vs. measured C:S molar ratios for DOM in all samples. The ratios were calculated according to Eq. 7 from the assumed C:S ratios in the two end members. The mean difference between the measured and the calculated ratios was 13.5%.

the Bothnian Bay and to 66% both in the Baltic proper and in the Bothnian Sea.

The comparison of $\delta^{34}S_{DOS}$ and $\delta^{13}C_{DOC}$ signatures (Fig. 4) of the measured estuarine DOM gave valuable information on the covariation of these two isotopic signatures along this estuarine gradient. The regression model shows a significant relationship between these two isotopic signatures, with an R^2 value of 0.51 (0.90 if excluding the outlier in the Bothnian Bay). Moreover, the regression line indicates the isotopic signatures of the marine end member independently from our assumed literature data (Hartmann and Nielsen 1969). For the marine $\delta^{13}C_{DOC}$ end-member signature of −22‰, Fig. 4 shows a $\delta^{34}S_{DOS}$ signature of +20‰. The terrestrial $\delta^{13}C_{DOC}$ signature of −28‰ corresponds to a $\delta^{34}S_{DOS}$ signature of +8.0. The end members for $\delta^{34}S_{SO4}$ are also given in Fig. 4 (Rees et al. 1978; Ingri et al. 1997), also showing good correspondence with the regression line between our samples indicating, small or insignificant fractionation of $\delta^{34}S$ during the assimilation and degradation processes in the water column.

Discussion

To our knowledge, $\delta^{34}S$ signatures in DOM along an estuarine gradient have been measured for the first time as reported in this study. $\delta^{34}S_{DOS}$ can be used in the future for a more accurate and precise method to trace organic material in estuarine environments. Compared to estuarine studies that are based on $\delta^{13}C$ only (Raymond and Bauer 2001*a,b*), a multiple stable isotope approach that also uses $\delta^{34}S$ gives a more complete description of the DOM sources in estuaries. Unraveling the sources of estuarine DOM is crucial for carbon budgets modeling the terrestrial DOC in the ocean.
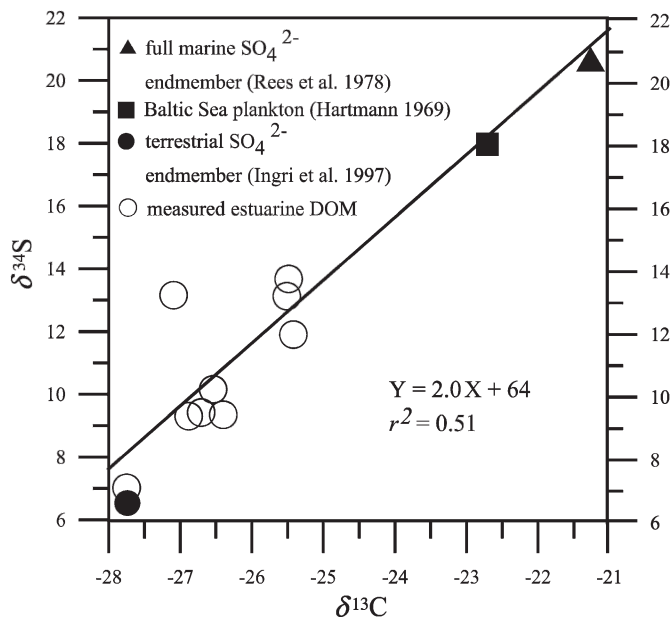

Fig. 4. The correlation between $\delta^{34}S_{DOS}$ and $\delta^{13}C_{DOC}$ in the ultrafiltered retentates. Literature values of marine sulfate end-member signature (+21‰; Rees et al. 1978), Baltic Sea plankton (+18.1‰; Hartmann and Nielsen 1969), and the terrestrial sulfate end-member sample in the Kalix River mouth (+6.0‰; Ingri et al. 1997) are marked as filled symbols in the figure, but are not included in the regression analysis.

Many other studies point to a conservative behavior of DOC in high-latitude estuaries and oceans (Kattner et al. 1999: Amon and Benner 2003), whereas Algesten et al. (2006) suggest a pronounced nonconservative behavior of DOC in the Gulf of Bothnia and a predominant role of respiration as likewise suggested for boreal lakes (Algesten et al. 2004). Thus, it is evident that these conflicting views on the fate of DOM in estuaries and shelf areas urge the need for increasing the precision in the determination of terrestrial vs. marine DOM sources. Our study showed that using $\delta^{34}S_{DOS}$ could be a valuable contribution to a multiple stable isotope approach to trace terrestrial DOM.

The use of $\delta^{34}S$ for tracing terrestrial DOM in estuaries— The advantage of using $\delta^{34}S_{DOS}$ in estuarine studies is foremost because of the fact that the estuarine DOS production is not influenced by terrestrial sources in the same way as DOC (Peterson et al. 1985; Chanton and Lewis 1999). The terrestrial-produced DOS could, though, be delineated with higher accuracy from the marine DOS by an EMMA as described in Eqs. 4–6. Phytoplankton as the marine end member for $\delta^{13}C_{DOC}$ in the open Baltic Sea water column is known to use riverine DIC for production and, thus, areas more influenced by riverine DIC show lighter $\delta^{13}C$ values in phytoplankton. Rolff and Elmgren (2000) measured the $\delta^{13}C$ signature of phytoplankton in the Bothnian Bay between –25‰ and –27‰, in the Bothnian Sea between –22‰ and –24‰, and in the Baltic proper between –19‰ and –22‰ (Rolff and Elmgren 2000). The $\delta^{13}C$ signature of phytoplankton is also known to vary

seasonally because of varying riverine DIC discharges (Chanton and Lewis 1999; Rolff 2000).

The relationship between $\delta^{13}C_{DOC}$ and $\delta^{34}S_{DOS}$ —The good correlation between the measured $\delta^{13}C_{DOC}$ and $\delta^{34}S_{DOS}$ in the Gulf of Bothnia (Fig. 4), as well as the independent correspondence between the $\delta^{34}S_{DOS}$ values inferred from the regression line and the observed end-member signatures for Baltic Sea plankton (Hartmann and Nielsen 1969) and terrestrial and marine SO_4^{2-} (Rees et al. 1978; Ingri et al. 1997), confirms that $\delta^{34}S_{DOS}$ is a stable and accurate isotope signature to use in oxic aquatic environments. The range of the $\delta^{34}S_{DOS}$ found in the Gulf of Bothnia, in this study measured to 6.6‰ within a salinity range of 11, is two to three times higher compared to the measured $\delta^{13}C_{DOC}$ signature range (2.5‰), as also indicated by the regression equation ($y = 2.0\,x + 64$). The mean $\delta^{13}C_{DOC}$ signature of the Bothnian Sea differs only by 0.1‰ from the Baltic proper sample. Considering the error of up to 0.2‰ in the mass spectrometer analysis, a statistically significant difference between the two basins could not be detected using $\delta^{13}C$ signatures. Thus, the small range of $\delta^{13}C_{DOC}$ between the two sources (Fig. 4) restrains a better estimate of the terrestrial vs. marine part of DOM in coastal seas based on $\delta^{13}C$ signatures (Hedges et al. 1997).

C : S end-member ratios as critical for future mass balance applications—The application of DOS as a proxy for DOC requires known C : S ratios in terrestrial vs. marine DOM. Because we had only one terrestrial end-member sample and no measurements of marine phytoplankton, we had to rely on literature values for these ratios. Nevertheless, the expected C : S ratios (calculated from the assumed ratios in both end members) in each sample corresponded well with the measured ratios (Fig. 3). This indicates that the assumed end-member ratios are reasonable. A sensitivity analysis of the impact of the C : S ratios showed that a change of the ratio from C : S_{ter}/ C : S_{mar} = 3–4 (in Eq. 6) changes the mean relative contribution of DOC_{ter} in the Bothnian Bay from 87% to 90% and a change to 2 gives DOC_{ter} of 82%. The impact of the C : S ratios in the end members is highest when the relative contribution of DOS_{ter} is close to 50%. However, more data of C : S ratios as well as $\delta^{34}S$ signatures from terrestrial and marine sources are urgently needed.

The origin and fate of DOM in the Gulf of Bothnia as inferred from first DOC_{ter} data—The results of $\delta^{34}S_{DOS}$ signatures that were translated into terrestrial and marine DOC concentrations in the Gulf of Bothnia showed that DOC was dominated by terrestrial material, both in the Bothnian Bay (87%) and the Bothnian Sea (75%); in the Baltic proper, 67% came from terrestrial sources. The DOC_{ter} fraction if calculated from the $\delta^{13}C_{DOC}$ signatures also pointed to 60–90% DOC_{ter} in each basin; however, the values from the Bothnian Sea and the Baltic proper are so close to each other that the estuarine gradient shown by the calculations from $\delta^{34}S_{DOS}$ could not be seen. These estimates are corroborated by Skoog et al. (1996), who

estimated that 50–80% of the humic substances in the Gulf of Bothnia were of terrestrial origin.

As an example of how the refined estimates of DOC_{ter} and DOC_{mar} derived from our multiple-isotope approach can be used we show here a simple Land-Ocean Interactions in Coastal Zone (LOICZ) mass balance calculation (Gordon et al. 1996). The river discharge and salinity stocks were taken from Wulff et al. (2001) to calculate exchange fluxes of water and salt between the Bothnian Bay, Bothnian Sea, and Baltic proper in steady state but omitting their transition zones. The annual riverine inflows to the Bothnian Bay and Bothnian Sea are 110 km³ and 220 km³, respectively. This resulted in a volumetric exchange of 170 and 280 km³ yr⁻¹ for inflow and outflow to the Bothnian Bay and 770 and 990 km³ yr⁻¹ for the corresponding exchange for the Bothnian Sea to the Baltic proper, i.e., slightly lower exchanges than those observed by Wulff et al. (2001). We have also assumed that the hydrological conditions have been rather stable during the last decades. The DOC_{ter} stocks could be estimated based on the measured total TOC concentrations (Fig. 2) and our fractions of DOC_{ter} estimated from $\delta^{34}S_{DOS}$ signatures in each basin. The mean DOC_{ter} concentrations became 283, 241, and 225 μmol L⁻¹ for the Bothnian Bay, Bothnian Sea, and Baltic proper, respectively (i.e., a stock of 5.0 × 10⁹ and 13.0 × 10⁹ kg for the Bothnian Bay and Bothnian Sea, respectively). With DOC_{ter} riverine, atmospheric loads, and point sources of 790 × 10⁶, 40 × 10⁶, and 50 × 10⁶ kg DOC yr⁻¹ for the Bothnian Bay, and 600 ×10⁶, 70 ×10⁶, and 50 ×10⁶ kg DOC yr⁻¹ for the Bothnian Sea (Algesten et al. 2006), and a net export to the Baltic proper of 790 ×10⁶ kg DOC_{ter} yr⁻¹ based on the exchange fluxes of water with the Baltic proper, the sinks reached 400 ×10⁶ and 450 ×10⁶ kg C yr⁻¹ for the Bothnian Bay and Bothnian Sea, respectively. Hence, 53% of the total DOC_{ter} load was retained in the Gulf of Bothnia. The residence time, as calculated by DOC_{ter} loads and inflows, is 3.7 and 3.5 years for the Bothnian Bay and Bothnian Sea, respectively. The corresponding residence time for water becomes 5.3 and 3.9 years, i.e., longer than the DOC_{ter} residence time, indicating a nonconservative behavior of DOC_{ter} in the Gulf of Bothnia, at least in the Bothnian Bay. These first calculations based on few winter values suggest that the Gulf of Bothnia is an efficient trap for terrestrial dissolved organic carbon.

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