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A new automated setup for stable isotope analysis of dissolved organic carbon

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Abstract

The measurement of stable isotope ratios on dissolved organic carbon (DOC) has long posed analytical problems and limited the use of this powerful tracer in biogeochemical studies in aquatic systems. Here, we provide a detailed description of a successful coupling of a custom-modified total organic carbon analyzer (Thermo HiPerTOC) to an isotope-ratio mass spectrometer (IRMS). The method is based on the wet oxidation of up to ~20 mL aqueous sample in a closed reactor, whereby complete oxidation is ensured by a combination of sodium persulfate addition, heating, and UV irradiation. The produced CO_2 is carried over a water trap and purification column in a stream of He and introduced into the IRMS via a conventional open-split interface, enabling both quantification and $\delta^{13}C$ analysis. Typical reproducibility of $\delta^{13}C$ analyses on DOC in this setup is in the order of 0.2‰ or better, comparable to that obtained in a more conventional elemental analyzer-IRMS setup. With appropriate blank correction procedures, accurate analyses can be obtained on concentrations as low as 0.5 mg DOC L^{-1}, representing the lower limit typically observed in marine systems. Typical overall system blank values for DOC analysis are in the order of 1 µg C. Analytical conditions (reaction time, reagent concentrations) were optimized for samples from brackish and marine environments, so that a single method can handle all types of environmental DOC samples. Although no certified DOC standards exist for $\delta^{13}C$, we analyzed the $\delta^{13}C$ values of a DOC "consensus reference material" from a deep-ocean environment (cfr. Hansell 2005) and found a $\delta^{13}C$ value of –19.5 ± 0.4‰ (n = 3), which is consistent with its oceanic origin.

Introduction

Importance of dissolved organic carbon in estuarine and marine biogeochemistry—Dissolved organic carbon (DOC) often constitutes the major form of organic carbon in aquatic systems, as most rivers and estuaries show DOC/POC (POC: particulate organic carbon) ratios in excess of 1 (e.g., Ludwig et al. 1996). Sources of DOC in aquatic systems (whether in freshwater, estuarine, or ocean environments) can be derived either from terrestrial or lateral inputs or from in situ production by benthic or pelagic primary producers. The DOC is considered a highly reactive organic carbon pool (despite the fact that not all components may be equally reactive) and can be mineralized by bacteria, oxidized by UV irradiation (photo-oxidation), exported, or converted to POC by flocculation.

In some estuaries, DOC appears to behave conservatively (Abril et al. 2002), but in other systems either internal production of DOC (e.g., Peterson et al. 1994; Raymond and Bauer 2001a; Abril et al. 2002; Otero et al. 2003) or net internal removal of DOC (e.g., the Scheldt estuary, Abril et al. 2002; Mekong estuary, own unpublished data) have been observed. However, a good fit of DOC concentrations with the pattern expected for conservative mixing does not necessarily imply that no significant processing of DOC takes place, since it only demonstrates that the *net effect* of production and consumption of DOC along the estuarine gradient is balanced. Peterson et al. (1994) were among the first to show the potential of using the stable isotope composition of DOC ($\delta^{13}C_{DOC}$) in studying estuarine carbon cycling. Stable isotope signatures of various potential carbon sources are often distinct and can thus be used as a tracer to distinguish or constrain carbon sources—for example, C3 and C4 plant material typically have distinct $\delta^{13}C$ signatures (approximately –27‰ and –13‰, respectively) owing to inherent differences in the isotope fractionation associated with their photosynthetic pathways. In some estuaries along the East and Gulf coasts of the United States, Peterson et al. (1994) found that DOC concentration profiles were consistent with conservative mixing, whereas

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the $\delta^{13}C_{DOC}$ data did not obey this pattern and indicated that significant processing of DOC took place in the estuary, with input and removal processes balancing each other out. Thus, whereas DOC concentration profiles provide information on the *net* dynamics of DOC, $\delta^{13}C_{DOC}$ mixing curves provide information on the *gross* DOC dynamics. In several other estuarine systems, DOC and $\delta^{13}C_{DOC}$ data have confirmed the existence of significant inputs of either estuarine phytoplankton-derived DOC or lateral inputs of DOC from tidal marshes. The few available studies that have combined stable isotope analyses on both POC and DOC in aquatic systems indicate that the relative contribution of various sources to both pools are not necessarily similar: Ziegler and Fogel (2003), for example, demonstrated that the contribution of various potential sources to the DOC pool was much more variable than in the case of POC in a freshwater tidal wetland. Similarly, Bianchi et al. (2004) and Bouillon et al. (in press) showed a consistently lower contribution of C4 material in the DOC pool (note: high-molecular-weight DOC pool in the case of Bianchi et al. 2004) than in the POC pool in 2 mixed C3-C4 river systems, the Mississippi (USA) and Tana (Kenya), respectively. Along the same lines, studies on the ^{14}C content of DOC and POC in rivers and estuaries suggest that the age and composition of both pools may differ substantially (e.g., Raymond and Bauer 2001a,b; Mayorga et al. 2005).

DOC is often the dominant organic carbon (OC) source transported by rivers (e.g., Ludwig et al. 1996), as well as the major form of OC exported from intertidal systems such as mangroves and salt marshes (e.g., Lee 1995). Because the sources of POC and DOC may be distinct in some cases (see above) and given that the majority of studies only provide data on the isotope composition of POC, it is clear that our current knowledge of the sources of organic carbon transported by river systems and the changes in its composition in the estuarine zone is limited and would benefit from more comparative data on the sources of both POC and DOC. Similarly, the number of studies that have used this tracer in marine systems is very limited, as the low concentrations of DOC and the high salt content pose additional analytical problems (Bauer 2002). The advent of continuous-flow isotope ratio mass spectrometry (CF-IRMS) has resulted in a huge increase in sample throughput, and stable carbon isotope analysis on solid samples is now a fairly routine procedure in many labs. In contrast, there is to our knowledge currently only 1 published report of a successful and automated coupling of a DOC analyzer to an IRMS (St-Jean 2003), but its suitability for samples from estuarine or marine environments was not examined or reported.

Existing methodologies for DOC and $\delta^{13}C_{DOC}$ analysis—The analysis of DOC in natural water samples, in particular in the marine environment, has long been problematic. One of the important events in the evolution of DOC analysis that demonstrated the analytical uncertainties in the quantification of DOC were the reports in the 1970s that DOC concentrations measured with high temperature oxidation (HTO) were higher than those measured with the (at the time standard) wet oxidation method, and therefore that the stock of DOC in the ocean could be much higher than previously thought (e.g., Sharp 1997). Although there are now a variety of methods commercially available, DOC analysis still has many pitfalls during both sampling and sample storage (Kaplan 1994; Spyres et al. 2000), incomplete conversion to CO_2 depending on the methodology used, reagent volumes and concentrations (e.g., McKenna and Doering 1995), and various blank contributions (Sharp 1997). Moreover, as demonstrated by intercomparison studies, differences in sampling/analysis procedures can lead to significant variability in results obtained between various laboratories (Peltzer et al. 1996, Abril et al. 2002). Blanks may consist of both instrumental blanks (e.g., resulting from C adsorption on catalysts in HTO methods), reagent blanks (e.g., C present in acid or persulfate reagent), and blanks associated with the preparation of standard (calibration) solutions, e.g., the C present in the deionized water used to prepare standard series. In view of the reported differences between methodologies, there have been several efforts to conduct large-scale interlaboratory and method comparison studies (e.g., Hedges et al. 1993, Peltzer et al. 1996, Sharp et al. 2002). As an example of how systematic differences in results are common, Abril et al. (2002) give some intercalibration results on estuarine DOC samples measured in 4 different laboratories; although correlations between the results were often reasonable for pairwise comparisons (with 1 exception, R^2 values ranged between 0.62 and 0.93), there was typically a significant offset in the linear correlation and/or a slope deviating substantially from 1. Appropriate blank correction procedures have been proposed as one of the underlying problems (Sharp et al. 2002). Moreover, McKenna and Doering (1995) demonstrated that incomplete conversion occurred in marine waters when using persulfate oxidation if care was not taken to use higher amounts of persulfate than required for freshwater samples. This was thought to be due to interactions between the chloride ions and the oxidation reaction. Because there was no decrease in precision, use of less than asymptotic concentrations of persulfate go undetected, and this may in part explain some of the differences observed between HTO and wet oxidation techniques in intercomparison studies.

Although drying or freeze-drying of water samples and subsequent analysis by elemental analyzer–isotope ratio mass spectrometry (EA-IRMS) has been proposed as a reliable method for DOC analysis on freshwater samples (Ghandi et al. 2004), this approach is not appropriate for DOC from estuarine or marine environments, because such samples, once dried or freeze-dried, consist mainly of salts with only a minor amount of organic C. Some of the earlier methods overcame the problem of low concentrations and high salt content by ultrafiltration or cross-flow filtration (see Raymond and Bauer 2001b). However, because this typically results in the isolation of only a limited fraction of the total DOC (as low as 20% to

30% in marine DOC), the representativeness of such data remains to be confirmed. Methods where the entire DOC pool was analyzed for $\delta^{13}C$ have included sealed-tube combustion (Fry et al. 1993; Peterson et al. 1994, used e.g., in a modified form by Otero et al. 2003), UV irradiation, UV-persulfate oxidation, and high-temperature oxidation (see Raymond and Bauer 2001b). In each of these cases, analyses were performed "off-line" followed by cryopurification of the evolved CO_2 and subsequent analysis on a dual-inlet IRMS system. These methods are all time-consuming, however, and the absence of a high-throughput methodology for $\delta^{13}C_{DOC}$ analysis suitable for samples from a range of environments has no doubt hampered a more wide-scale application of stable isotope studies on DOC in estuaries and marine systems. Recently, however, an automated system for $\delta^{13}C_{DOC}$ analysis has been described, consisting of a commercially available TOC instrument that was adapted to work under continuous-flow conditions necessary for IRMS work (St-Jean 2003). We opted for a more versatile instrument (Thermo HiPerTOC), which has the option to run samples by either high-temperature combustion (HT), UV-assisted breakdown, and/or heated persulfate to convert DOC to CO_2. Given the small sample volumes that can be processed with the HT module, UV and/or persulfate methods are inherently more suitable for continuous-flow IRMS applications, but the high-temperature option gives the opportunity to compare DOC concentration measurements between different methods or perform a rapid screening of DOC concentrations prior to $\delta^{13}C$ measurements without loss of large amounts of sample, and the high-temperature oven can be used to house the reduction/scrubber interface (see system description). The Theus software allows users to fully customize volumes, reaction times, and valve operations, which was a prerequisite to optimize the analytical procedure for continuous-flow measurements of saline samples.

In this article, we describe the modifications and analytical conditions used to analyze DOC and $\delta^{13}C_{DOC}$ in aqueous samples ranging from freshwater to fully marine environments. Although the specific modifications needed to adapt currently available TOC analyzers for a successful coupling to an IRMS will to some extent be type- and manufacturer-specific, many of them will be similar to those we have made, and we therefore describe the rationale behind the modifications in our system in some detail.

Materials and procedures

Rationale for hardware layout modifications—The analytical setup consists of a Thermo HiPerTOC TIC/TOC analyzer (which includes a 55-position autosampler with movable arm) coupled to a Thermo Delta Plus XL IRMS via a Conflo III interface. Tests with the original HiPerTOC configuration and preloaded methods indicated that there were major incompatibilities that precluded a simple coupling to the IRMS, which we will list below. Although a description of the current configuration (Figure 1) may seem most relevant, we will first describe these restrictions and their solutions in some detail since many of them will also be encountered by those wishing to modify other TOC analyzers to couple to their IRMS system.

Leakages in the gas and liquid flow paths. Given that the TOC analyzer is designed to work at atmospheric pressure, typically with O_2 as the carrier gas, it was not unexpected that some problems might arise when operating the system on He at the pressure needed to force the carrier gas through the GC column at an acceptable flow rate (~1 bar overpressure). These leakages resulted not only in overconsumption of He, but also in an unstable gas flow, leakage of reagents from valves, and the introduction of atmospheric gases (N_2, O_2) which interfere with the measurements.

Large dead volumes. A number of the original system components had relatively large volumes, which resulted in extremely wide and tailing CO_2 peaks when working on the flow rates acceptable for continuous-flow IRMS work (~90 to 150 mL min^{-1}, vs. 300 mL min^{-1} when used as a standalone). To reduce this problem, the Peltier cooling element was redesigned (Figure 2) and all wide-diameter tubing in the gas flow path was replaced by 1/16-inch stainless steel tubing.

Gas-liquid separation. The original configuration included a gas-liquid separator, used to remove H_2O during analyses with the high-temperature module and as a drain for the Peltier element. Because this created a high dead volume that was not flushed properly, the modified Peltier element was placed directly above the exit of the UV reactor so that condensed water could flow back into the reactor either by gravity or during the reactor drainage (Figure 1). To eliminate H_2O in the carrier gas stream, an additional H_2O trap (either Nafion or magnesium perchlorate) needed to be installed in line after the Peltier element (Figure 1).

Reactor volume and design. The UV reactor in the original version could accommodate ~15 mL of sample liquid (including reagents), and consisted of an open quartz tube with the UV lamp centrally located, i.e., in direct contact with the sample liquid. Although this resulted in excellent sample conversion, the sample volumes were insufficient to properly analyze low DOC samples for $\delta^{13}C$, and the design suffered from an inherent blank problem caused by the need for a seal in direct contact with the UV lamp. A new and larger volume reactor designed by the manufacturer lifted both these restrictions: it now accommodates ~31 mL liquid (note that the total reactor volume is 77 mL; see Figure 2) and consists of a double-walled quartz tube with a central 1/4-inch entrance at the bottom and a 1/4-inch exit at the outer edge; the UV lamp is inserted in the open interior and is thus no longer in direct contact with the sample liquid or with seals. To be able to handle larger volumes of liquid, the sample loop was elongated (and now holds ~31 mL), and the original 10-mL syringe was replaced by a 25-mL gas-tight version (see Figure 1).

Need for a constant flow. To ensure stable background values and to avoid intrusion of atmospheric air in the Conflo interface, a stable and uninterrupted carrier gas flow is required dur-

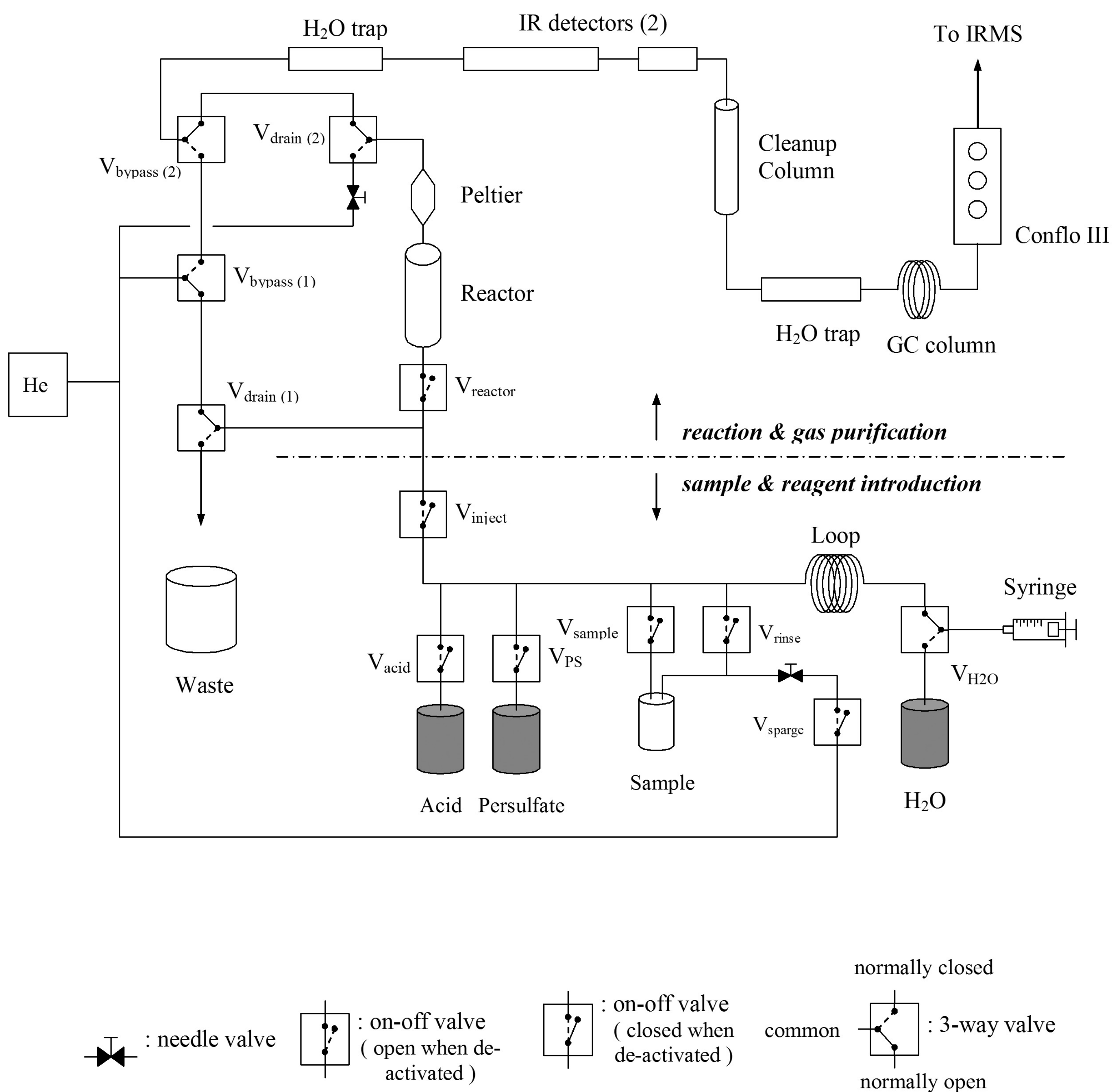

Fig. 1. General layout of the gas and liquid flow scheme of the modified system for automated analysis of $\delta^{13}C_{DOC}$.

ing the entire measurement procedure. Because it became evident that optimal sample conversion for samples containing salt was feasible only when closing the reactor for a certain amount of time, the valve layout had to be redesigned to allow a carrier gas bypass during closure of the UV reactor (see Figure 1).

Interfering gases and background problems. Further hardware modifications were needed to ensure that a clean carrier gas stream containing only CO_2 was carried to the IRMS. Because this requirement does not hold for the original configuration, where CO_2 concentrations are measured by IR-detection, there was a need to purify the gas stream of interfering gases and to separate the CO_2 peak from gases that could not be entirely removed (e.g., dissolved N_2) by a GC column. This was also noted by St-Jean (2003) and was confirmed by our own observations when comparing chromatograms with and without the clean-up column and GC column. During initial tests, we used the existing column configuration of our Thermo Flash EA 1112 elemental analyzer (i.e., oxidation

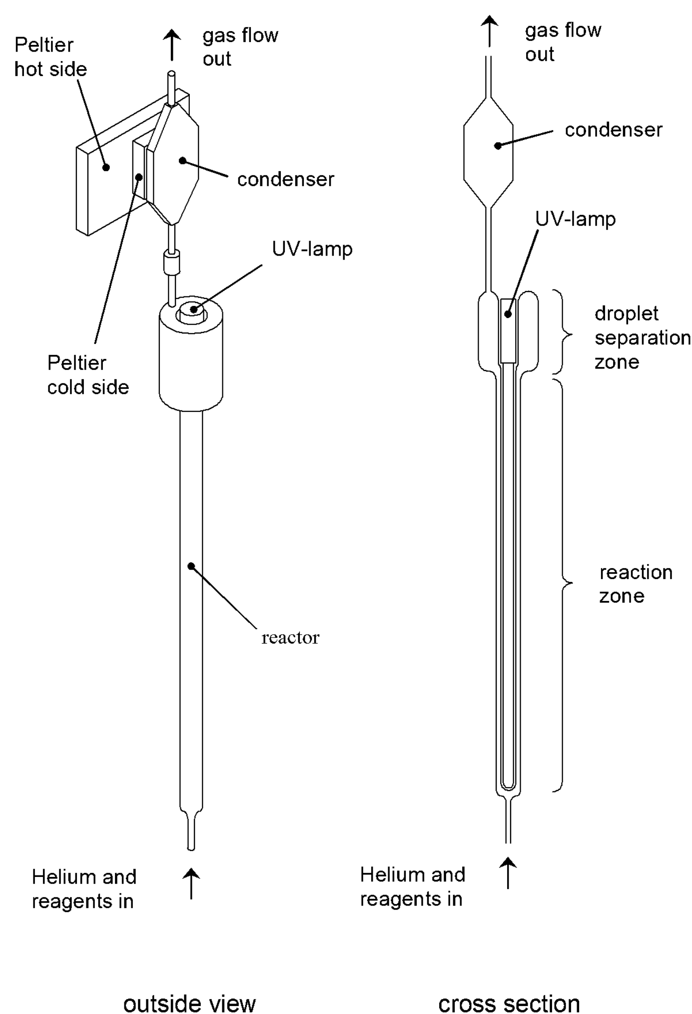

Fig. 2. Diagram showing the modified double-walled UV reactor and the modified Peltier element.

column at 1020°C with cobaltic oxide and chromium oxide; reduction column with reduced copper at 640°C; 3-meter Porapak Q 50-80 mesh column, 1/4 inch), but because the HiPerTOC has its own heating oven for the HTO model, we opted to combine both oxidation and reduction columns in a single quartz column heated to 680°C (Figure 1), filled with both reduced copper wire (to reduce possible N oxides and to eliminate dissolved O_2, which may both cause interferences in the IRMS measurements) and silvered cobaltic oxide (mainly intended to trap halogens). A 2-meter Porapak Q column (50-80 mesh, 1/4 inch, i.e., similar but slightly shorter than that used in the EA) was placed directly behind the cleanup column and (Mg perchlorate) water trap (Figure 1).

Software modifications, aside from configuring new sequences of valve operations and command timings, mainly consisted of including a command that sends a signal to the IRMS PC during each HiPerTOC analysis. The sequences of samples in both IRMS and HiPerTOC software need to be identical, and each IRMS run waits for the signal sent out during the HiPerTOC method. Because the overall runtime of the HiPerTOC method is longer than that of the IRMS method, the two instruments can run unattended without timing problems.

Current system layout—The general layout of the modified setup is illustrated in Figure 1 and basically consists of 2 major modules: (i) the reaction and gas purification system (upper part of Figure 1), and (ii) the sample and reagent introduction system (lower part of Figure 1). Gas and liquid flows are directed by a series of twelve 3-way valves (see legend of Figure 1), whereby the gas flow rate of the carrier stream is regulated by a fixed pressure on the main He line; the gas flows to sparge the sample vials and to drain the reactor are regulated by needle valves (Figure 1).

In normal mode (i.e., all valves are deactivated), the carrier gas is directed through the UV reactor and passes through the entire gas purification and detection system. The latter consists of 2 built-in IR detectors (with 6-inch and 15-inch sample cells), H_2O traps, the abovementioned cleanup column at 680°C with reduced Cu wire and cobalt/cobaltic oxide, and a GC column, before transferring the gas stream to the IRMS via a Conflo interface.

Sample and reagent can be taken up by operating the relevant valves (V_{acid}, V_{PS}, V_{sample}, and V_{H2O}; see Figure 1) and are taken up in a sample loop by movement of a motor-driven 25-mL glass syringe. To introduce the liquid phase in the reactor, the V_{inject} valve is activated, and the syringe injects the total volume taken up into the UV reactor.

Methods and analytical conditions—Our optimized method for DOC and $\delta^{13}C_{DOC}$ analysis consists of the following sequence of events:

(1) While the carrier gas flows through the UV reactor (all valves above the dotted line in Figure 1 are deactivated), reagents and sample are taken up in the following order: (i) a certain volume of deionized water (DI) and H_3PO_4, (ii) a variable volume of sample, and (iii) a second volume of DI. Typical volumes and reagent concentrations can be found in Table 1. The DI water volumes are needed to buffer for possible dilution effects during the transit time in the system tubing and to make sure that the entire volume of sample and reagent can be fully introduced into the reactor, i.e., the DI water volume needs to be in excess of the tubing volume leading to the reactor. The entire volume of sample and reagents is then injected into the UV reactor by dispensing the syringe while the V_{inject} valve is activated.

Table 1. Typical reagent volumes and concentrations used in the determination of DOC and $\delta^{13}C_{DOC}$ using the modified HiPer-TOC-IRMS setup.

Reagent	Concentration	Typical volume
Deionized H_2O (first intake)	—	0.5 mL
'Front buffer' H_3PO_4	1 M	1 mL
Sample	Variable	Variable (0-23 mL)
Deionized H_2O (second intake)	—	1 mL
Deionized H_2O (third intake)	—	0.5 mL
Na persulfate	1.5 M	2.5 mL
Deionized H_2O (fourth intake)	—	0.5 mL

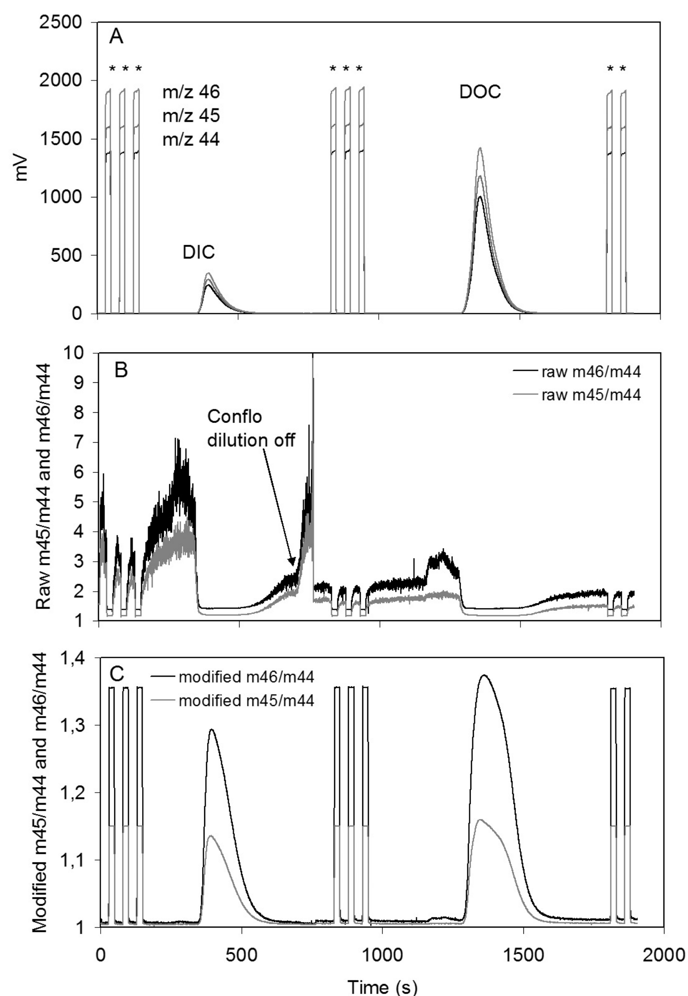

Fig. 3. Typical IRMS response of an estuarine water sample using the HiPerTOC-IRMS method described in the text. (A) The raw m/z traces for the 3 masses (upper trace: m/z46, middle trace: m/z 45, lower trace: m/z 44); (B) raw traces of the ratio of m/z 45 to m/z 44 and of m/z46 to m/z44; and (C) modified ratios as they are shown by default in the Isodat software, i.e., adding 100 mV to the raw m/z values for each mass before conversion into their ratios. Peaks marked with an asterisk in the upper panel indicate the CO_2 reference gas peaks supplied by the Conflo interface; the arrow in panel B indicates the point where the He dilution is turned off in the Conflo interface after the DIC peak.

(2) At this stage, the CO_2 resulting from acidification of the sample dissolved inorganic carbon pool is bubbled out of the reactor by the carrier gas stream. Complete removal of DIC usually takes less than 6 min. During the purging of the DIC, the gas stream that is ultimately transferred to the IRMS sources is diluted with He in the Conflo interface, since for most surface water samples, the DIC content is much larger than the DOC content.

(3) After the DIC has been sparged out of the reactor, a volume of persulfate reagent (Table 1), buffered with DI, is injected into the reactor after which the latter is closed ($V_{reactor}$ activated), and at the same time, the carrier gas flow is redirected via the bypass (activation of the 2 V_{bypass} valves). The UV lamp is turned on, and the reaction is allowed to take place for 10 min. Due to the (continuous) heating applied to the reactor, the liquid reaches a temperature of 85 to 95 °C. While this is an excessive reaction time for easily degradable compounds and many natural freshwater samples, tests with humic acid and salt solutions (~ seawater salinity) indicated that in some cases, such reaction times were needed to ensure complete conversion and absence of peak tailing.

(4) After the DOC conversion, the carrier gas flow is again directed through the reactor to bubble out the resulting CO_2. The latter is carried through the purification and detection system for quantification and $\delta^{13}C_{DOC}$ analysis.

(5) When all the CO_2 has been purged from the reactor (usually within 4 to 5 min, but the procedure is set for 6 min), the remaining liquid in the reactor is drained by reversing the gas flow through the reactor (V_{bypass} valves and V_{drain} are actuated).

Quantification of the CO_2 peaks can be performed by either the Theus software based on the response of the IR detectors or the area of the CO_2 peak on the IRMS. In all our experiments, we used the latter data because the IRMS response gave more stable background levels. Note that during this method, the sample DIC is also directed to the IRMS, in principle allowing for a quantification and $\delta^{13}C$ analysis of DIC. However, when only DIC or $\delta^{13}C_{DIC}$ analysis would be desired, a shorter method can be used whereby steps (3) and (4) are omitted. Similarly, when only DOC and $\delta^{13}C_{DOC}$ needs to be performed, the method can be significantly shortened by acidifying the sample vials in the autosampler tray and purging out the resulting CO_2 before picking up the sample in the analyzer. However, we opted to remove the DIC internally, because this provides a visual check on the removal of DIC, and because adding a significant amount of (dilute) acid to the sample vial causes a dilution of the sample which can only be quantified and corrected for if sample vials are completely filled and known to have a constant volume—both of which are not always the case (see also Fukushima et al. 1996).

A typical IRMS response of the above-described method is illustrated in Figure 3. The upper panel shows the raw traces for the 3 m/z (44, 45, and 46) and visualizes the overall peak shape. Reference injections of CO_2 from a tank are made via the Conflo interface at the start of each analysis, between the DIC and DOC peaks, and at the end of each analysis. The lower 2 panels illustrate the ratios of the m/z traces (45/44 and 46/44), which is useful to trace impurities in the gas stream. Note that the default settings in the IRMS software modify these ratios by adding 100 mV to each of the traces before converting them into ratios (Figure 3C), whereas the raw ratios (Figure 3B) show more variability, since slight changes in the background levels (e.g., when turning the He dilution off in the Conflo interface, as indicated by the arrow in panel B) can have a large impact on the mass ratios.

Reference samples for system calibration and verification—To verify the analytical performance of the methodology, we performed a series of experiments using different compounds and different concentration ranges. Before these experiments, we evaluated the

Table 2. Results of the elemental analyzer–IRMS analyses on materials used to prepare standard solutions for DOC and $\delta^{13}C_{DOC}$.

Compound	%C (average ± 1 SD)	$\delta^{13}C$ (average ± 1 SD)	n
Acetanilide	71.09 %*	–30.05 ± 0.11‰	6
IAEA-CH-6	42.7 ± 0.7%	–10.4‰*	6
IAEA-C6	43.1 ± 0.9%	–10.40 ± 0.10‰	6
Humic acid	51.2 ± 1.2%	–26.97 ± 0.10‰	7

*Reference values, based on which the results for other compounds were calibrated. The %C was calibrated based on acetanilide (Merck, 71.09% C); $\delta^{13}C$ values were calibrated versus IAEA-CH-6 (–10.4‰).

reagent volumes and reaction time by analyzing series of both sucrose and humic acid solutions prepared in both DI water and NaCl-amended DI (salinity equivalent to normal seawater).

Repeatability of the DOC and $\delta^{13}C_{DOC}$ was tested with a series of analyses of a standard solution of sucrose (IAEA-C6) at a given concentration. Similarly, the consistency of data when varying concentrations and volumes are used (resulting in a different signal magnitude on the IRMS, and a variable contribution by the overall system blank) was tested by analyzing a dilution series of both sucrose (IAEA-C6) and humic acid (Aldrich, cat. no. H 1675-2) in concentrations ranging from 0.5 to 25 mg L^{-1} C. The C content and $\delta^{13}C$ composition of both compounds was verified or measured separately by running 6 weighted samples of each on an EA-IRMS (Thermo Flash EA 1112 and Delta Plus XL), and calibrated versus IAEA-CH-6 ($\delta^{13}C$ = –10.4‰) and acetanilide (71.09%C). Results of the EA-IRMS analyses are given in Table 2.

Finally, because no certified reference material for DOC or $\delta^{13}C_{DOC}$ exists, we analyzed a commonly used intercomparison material ("consensus reference material," CRM, see Hansell 2005). This CRM is deep-ocean water, collected at 2600 m depth from the Sargasso Sea, and is freely distributed to labs analyzing DOC in an effort to make DOC results from different methods and laboratories more directly comparable, by stimulating researchers to run this standard in their analysis sequence and report on the resulting data. The commonly accepted concentration for these deep-ocean DOC samples is 44 to 46 μM DOC (W. Chen, University of Miami, personal communication).

Assessment and discussion

System layout and method development—Preliminary trials indicated that adequate conversion of sample DOC was not feasible without closing off the reactor for a certain amount of time. Bubbling out the produced CO_2 during the reaction itself was acceptable for simple compounds (e.g., sucrose dissolved in DI water) but for more complex compounds (such as humic acid), saline solutions, or natural water samples, this procedure resulted in low signals and long peak tailing due to long reaction times. Hence, the valve layout was redesigned to be able to close the reactor after sample and reagent injection, with the possibility of bypassing the carrier gas flow (Figure 1) with an equivalent flow rate. A reaction time of 10 min was found to be sufficient based on series of sucrose and humic acid samples prepared in both DI and NaCl-amended DI, with a final salinity equivalent to seawater. To minimize possible blank effects when using different methods, we used a single method for all further measurements.

The choice of reagent volumes was similarly based on trial experiments with varying amounts of reagents. Sufficient buffering DI water is needed to ensure that no sample dilution or diffusion takes place during the uptake of the sample and its transfer to the reactor. Standard solutions were analyzed with varying amounts of persulfate: below a certain level, signals decreased in intensity and showed clear signs of peak tailing (corresponding to further oxidation after opening of the reactor), but peak shape and height stabilized with higher persulfate volume. It is worth noting that the molar persulfate/chloride ratio used in our system is significantly lower that that proposed by McKenna and Doering (1995), with a minimal ratio of ~0.35 in our system for fully marine samples, versus 8.75 in McKenna and Doering 1995. However, it should be stressed that our method also used UV irradiation in combination with persulfate and heating, which forms a likely explanation for this difference, and our ratio is still higher than that used in other wet oxidation methods (see McKenna and Doering 1995 for references).

Blank correction procedures—Blanks and blank correction procedures are an important aspect to consider in DOC analysis and have been proposed to be the cause for some of the inconsistencies in results from intercomparison studies and differences in results obtained from different methodologies for DOC analysis (Sharp 1997). With the UV/persulfate methodology, 2 types of blanks should be considered: (i) a "system blank," which we define here as DOC resulting from the addition of deionized water, acid, and persulfate reagent, and DOC introduced by the instrument itself, e.g., by leaching due to contact of the sample with tubing, valves etc., and (ii) blanks associated with the preparation of standards, which is equivalent to the DOC still present in the deionized (DI) water used to prepare standard solutions (hereafter "DI blank"). The first type of blank is in theory constant as long as the same analytical conditions (e.g., volume of reagents) are used for all samples and standards and should be used to correct all data from unknown samples. The second type of blank is superimposed on the first and that should be taken into account when treating the results for the standard series, i.e., to calculate the conversion factor between the quantity of DOC and the instrument response. The overall blank correction procedure used here thus consists of the following:

1. Establishing the system blank. The system blank can be determined by running "dummy" samples whereby no sample/standard material is taken up; the resulting DOC peak is then equivalent to that from the DOC still present in the different reagents and possible DOC leached from system components during the analysis. The area of the CO_2 peak on the IRMS is used to quan-

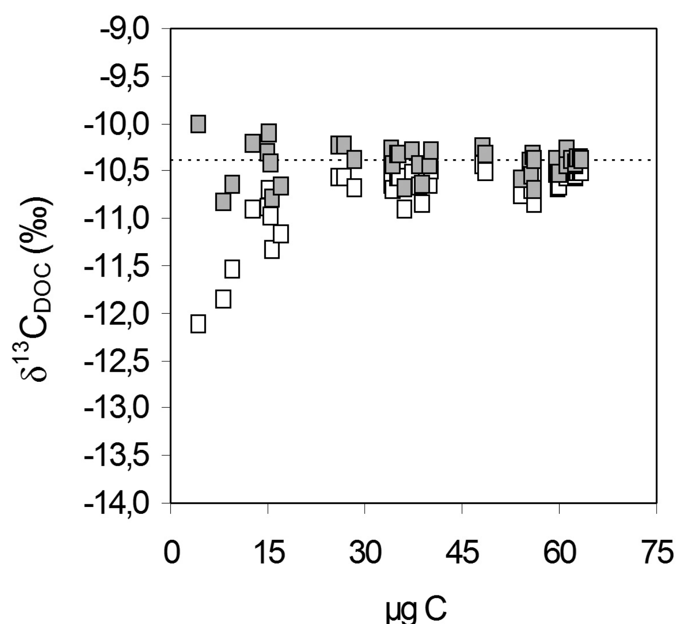

Fig. 4. Typical response of the IRMS to different concentrations or volumes of standard solutions (□, IAEA-C6 sucrose). Blank-corrected values are shown by ■ (see text for details).

tify the system blank. An independent approach to determine or verify the system blank is to use the intercept of the regression line that relates the quantity of standard DOC to the IRMS response, after correcting for the DI blank (see further).

2. Establishing the DI blank. To determine the DI blank, a series of deionized water samples can be analyzed with varying volumes (but similar amounts of reagents). In case the deionized water contains a measurable and significant blank, a relationship between the DI volume and the overall DOC peak should be found, and the intercept can be used to determine the DI blank. In our experiments, however, where DI water was UV-treated before preparation of standards, we found no increase of the DOC blank with increasing volumes of DI water. Therefore, this blank was not taken into account in our data processing. However, prior tests using DI water without prior UV treatment had indicated that the DI blank was measurable.

To determine the DOC concentration in an unknown sample, the regression equation between the amount of DOC in each standard (equaling the standard DOC concentration, multiplied by its volume) and the IRMS response (peak area in mVs) is used. Typical blank values obtained were in the order of 0.7 to 1.5 µg C for DOC analysis.

Although quantification of the DOC blanks is relatively straightforward, it is also crucial to establish the $\delta^{13}C$ value of the system blank, because correction for blanks is based on the following equation, which is a reformulation of a simple mass balance:

$$\delta^{13}C_{sample} = (\delta^{13}C_{measured} * A_{measured} - \delta^{13}C_{blank} * A_{blank}) / (A_{measured} - A_{blank}) \quad [1]$$

where $\delta^{13}C_{sample}$ is the blank-corrected $\delta^{13}C$ value of the sample; $\delta^{13}C_{measured}$ is the measured $\delta^{13}C$ value of the sample, which includes the system blank; $\delta^{13}C_{blank}$ is the (unknown) value of the system blank; A_{sample} is the blank-corrected IRMS response (area of the CO_2 peak) of the sample; $A_{measured}$ and A_{blank} are the IRMS response of the sample (including blank) and of the blank, whereby $A_{measured} = A_{blank} + A_{sample}$.

The problem of establishing the blank $\delta^{13}C$ values is due to the fact that signals are too low for accurate and direct measurement, and hence, the $\delta^{13}C$ blank value must be determined indirectly. One of the approaches consists of measuring the δ values for varying quantities/concentrations of reference material and determining the blank $\delta^{13}C$ from the extrapolation of the relationship between $\delta^{13}C$ and 1/area, as has also been proposed for EA-IRMS work on small quantities of N_2 (Avak and Fry 1999). For $\delta^{13}C_{DOC}$ work, however, this did not seem to be the most appropriate method, since no certified $\delta^{13}C_{DOC}$ reference material exists. Our solution therefore consisted of determining the $\delta^{13}C$ value of the blank that resulted in the *least variation* on the corrected $\delta^{13}C$ values of standards prepared from IAEA-C6 sucrose. Briefly, this approach first calculates the corrected $\delta^{13}C$ values (i.e., $\delta^{13}C_{sample}$ in equation [1]) for a series of sucrose samples and the average and standard deviation on these data, using a predefined $\delta^{13}C$ value for the system blank. By using an iterative nonlinear optimization procedure (as used in Microsoft Excel Solver), the $\delta^{13}C_{blank}$ value is recalculated to result in a minimal standard deviation on the corrected $\delta^{13}C$ values for the standard series. This procedure typically resulted in $\delta^{13}C_{blank}$ values between –12 and –20‰ for DOC, and were similar to values obtained when an analogous procedure was followed whereby the criterion for $\delta^{13}C_{blank}$ optimization was to result in an average corrected $\delta^{13}C$ value for the sucrose series to approach the value obtained for this material by EA-IRMS (Table 2), which indicates the validity of the approach used. An example of raw data and blank-corrected data for a range of different concentrations of IAEA-C6 is shown in Figure 4, which illustrates the effect of the blank correction procedure.

Analytical performance—Series of standard solutions showed an excellent linear response in terms of CO_2 peak area on the IRMS (Figure 5), with an intercept that falls close to the area determined for blanks. Whereas the HiPerTOC also gives the opportunity to derive quantitative information based on the response of the IR detectors, we here only present the data based on the CO_2 peak as detected by the IRMS, since the latter provides a more stable baseline which allows for more consistent peak integration. Typical reproducibility of DOC determinations, expressed in terms of the coefficient of variation, is in the order of 0.5% to 2.5% for sucrose and humic acid solutions with concentrations ranging between 0.5 and 25 mg L^{-1} C, and better than 5% for natural DOC from estuarine waters, based a series of replicates performed on DOC samples from coastal environments.

Tests with sucrose and humic acid solutions in both DI water and DI water with NaCl (to a final concentration equivalent to seawater salinity) indicated that after blank correction, no significant differences could be observed in the area

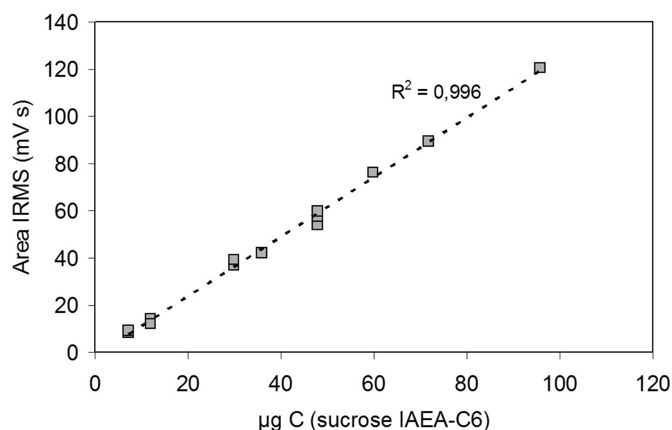

Fig. 5. Typical response of the IRMS for the quantification of DOC calibration standards (in this case, sucrose IAEA-C6).

of the CO_2 peaks or in the average $\delta^{13}C_{DOC}$ between saline and nonsaline samples (data not shown), indicating that our method is acceptable for both quantitative and stable isotope measurements under a range of salinity conditions.

We evaluated the performance of our setup in terms of $\delta^{13}C_{DOC}$ by comparing results of standard solutions of sucrose and humic acid with $\delta^{13}C$ data on these compounds as measured by traditional EA-IRMS (Table 3). It should be noted here, that since there is no referenced standard for $\delta^{13}C_{DOC}$ measurements, our raw (uncorrected) $\delta^{13}C_{DOC}$ data are obtained by setting an appropriate $\delta^{13}C$ value for the reference gas CO_2, which was calibrated both in a dual-inlet IRMS (Finnigan Delta Plus XL) with CO_2 generated from an internal carbonate standard and on the EA-IRMS based on results for IAEA-CH-6 sucrose. For sucrose, our results show that the HiPerTOC-IRMS setup performs well in terms of both accuracy (i.e., results of the HiPerTOC-IRMS and EA-IRMS are similar and not significantly different) and precision, with a standard deviation in the order of 0.07‰ to 0.18‰ (Table 3), which is comparable to typically cited external precision in EA-IRMS systems. For humic acid, the precision is slightly less than for sucrose but still within typical EA-IRMS limits, with standard deviations in our experiments between 0.16‰ and 0.23‰. However, for humic acid we found a small but significant offset between $\delta^{13}C$ values measured by EA-IRMS and those measured with the HiPerTOC-IRMS system, the latter giving $\delta^{13}C$ values ~0.7‰ less negative. Here, it should be noted that humic acid is considered one of the most problematic compounds for DOC analysis with wet oxidation methods. Comparison of CO_2 peak areas for sucrose and humic acid solutions indicated that our system yielded a recovery of 90% to 95% for humic acid, which is excellent compared to other wet oxidation methods, but apparently this may still cause a certain degree of fractionation. However, given that humic acids are usually not a dominant fraction of natural surface water DOC, and taking into account the typical reproducibility of the analysis (in the order of 0.2‰), we considered our analytical

Table 3. Results of tests on the consistency of DOC and $\delta^{13}C_{DOC}$ analysis on sucrose solutions (IAEA-C6) and humic acid solutions: a fixed concentration (25 mg L^{-1} C) and volume (5 mL); results of tests with varying concentrations (0.5 to 10 mg L^{-1} C) and volumes (2.5 to 20 mL), and overall results.

I. Fixed concentration and volume (~25 mg L^{-1} C; 5 mL)	
Average $\delta^{13}C$ ± 1 SD (*n*)	Reference value (see Table 2)
Sucrose: –10.3 ± 0.07‰ (11)	–10.4 ± 0.1‰
Humic acid: –26.3 ± 0.16‰ (6)	–27.0 ± 0.1‰
II. Variable concentration and volume (0.5-10 mg L^{-1} C; 2-20 mL)	
Sucrose: –10.3 ± 0.18‰ (12)	–10.4 ± 0.1‰
Humic acid: –26.2 ± 0.23‰ (10)	–27.0 ± 0.1 ‰
III. Combined results of I and II	
Sucrose: –10.3 ± 0.14 ‰ (23)	–10.4 ± 0.1‰
Humic acid: –26.3 ± 0.20‰ (16)	–27.0 ± 0.1‰

The reference value for the IAEA-C6 and humic acid materials were determined relative to the certified IAEA-CH-6 sucrose standard (see Table 2 and text), whereas the results for the DOC samples prepared from them are only calibrated relative to the reference CO_2 gas used, and given the absence of a certified $\delta^{13}C_{DOC}$ standard cannot be directly compared.

conditions sufficient for natural surface waters. For both sucrose and humic acid, it can also be noted that the reproducibility of $\delta^{13}C_{DOC}$ (as expressed by the standard deviation, Table 3) is better for solutions with a fixed concentration and sample volume, when the resulting response on the IRMS is constant. Replicates of 10 natural water samples similarly showed excellent reproducibility in terms of $\delta^{13}C_{DOC}$, with differences between duplicates being always less than 0.5‰.

Considering the typical system blank (~ 1 µg C) and the sample volume that can be accommodated (up to ~20 mL, excluding reagents), our system is sufficiently sensitive to analyze $\delta^{13}C_{DOC}$ at the lower range of concentrations encountered in marine systems: deep ocean water with DOC concentrations of ~0.5 mg L^{-1} C thus yield about 10 µg C, allowing for adequate correction of the resulting blank (~9% contribution to the total signal).

Since the setup of our DOC-IRMS configuration, we have used this procedure to measure DOC and $\delta^{13}C_{DOC}$ in surface waters from a number of (sub)tropical coastal ecosystems, including the Tana estuary and delta (northern Kenya), two mangrove ecosystems along the Tanzanian coast, the Mekong estuary (Vietnam), and a series of tidal mangrove creeks in the Ca Mau province (Vietnam). Although an in-depth discussion of these data are not within the scope of this methodological paper, a comparison of these $\delta^{13}C_{DOC}$ data with concurrently collected $\delta^{13}C_{POC}$ data illustrates how both organic carbon pools may, in some cases, differ substantially in their origin (see Figure 6, where literature data from a number of other aquatic environments are shown as a comparison). In the Tana estuary and delta, for example, where the catchment area contains large areas of savannah (i.e., C4) grasslands, we

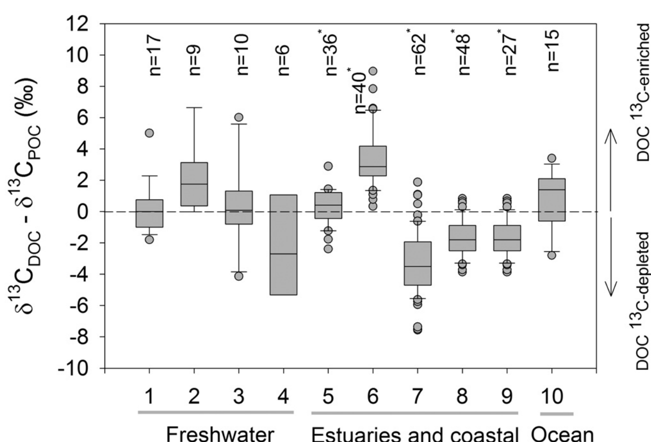

Fig. 6. Box-plot representation of differences in $\delta^{13}C_{DOC}$ and $\delta^{13}C_{POC}$ values for a range of different aquatic environments (indicative, rather than exhaustive). An asterisk indicates that the data were gathered with the described HiPerTOC-IRMS setup. Freshwater ecosystems: 1: Amazon basin (Mayorga et al. 2005); 2: Moore Creek, Arkansas, USA (Ziegler and Brisco 2004); 3: Huey Hallow, Arkansas, USA (Ziegler and Brisco 2004); 4: Santa Clara River, Louisiana, USA (Masiello and Druffel 2001). Estuaries and coastal ecosystems: 5: Mtoni mangrove creek, Tanzania (own unpublished data); 6: Ras Dege mangrove creeks, Tanzania (S. Bouillon, unpublished data); 7: Tana estuary and delta, Kenya (Bouillon et al., in press); 8: Mekong estuary, Vietnam (S. Bouillon and A.V. Borges, unpublished data); 9: Ca Mau mangrove creeks, Vietnam (S. Bouillon and A.V. Borges, unpublished data). Oceanic environment: 10: Middle Atlantic Bight (Bauer et al. 2001). Note that for the data from Mayorga et al. (2005), the $\delta^{13}C_{POC}$ data refer to the "fine" particulate organic carbon fraction, since this was found to be the dominant POC pool.

found a consistently lower contribution of C4-derived C to the DOC pool compared with POC (Bouillon et al., in press). Other systems, in contrast, show a distinctly more ^{13}C-enriched DOC pool compared with POC, as in the Ras Dege mangrove creeks (Tanzania) where the POC pool is to a larger extent of local mangrove origin (i.e., low $\delta^{13}C$ and high POC/PN ratios), whereas the DOC pool showed a high contribution of ^{13}C-enriched, marine and/or seagrass-derived material. Given the current scarcity of $\delta^{13}C_{DOC}$ data in studies on the biogeochemistry of organic matter in various aquatic ecosystems, these marked potential differences in relative contribution of various sources to DOC and POC have been mostly overlooked in past studies and stress the need to incorporate this tracer more widely in future work on aquatic carbon cycling and source characterization.

Comments and recommendations

Although we have demonstrated that our system is functional and shows good analytical performance, a number of further improvements of the system are foreseen to increase the system performance and, in particular, to decrease the system maintenance. First, efforts are being made to provide a more efficient and durable system for the purification of the gas stream, since the current cleanup column in some cases needs to be replaced after 30 to 50 samples, in particular during the analysis of saline samples, which indicates that the halogen loading is in part responsible. Furthermore, the built-in 3-way valves do not show a good long-term performance under the pressure conditions used, which results in occasional leaks and intrusion of atmospheric N_2 and O_2. The former may interfere with the IRMS measurements, whereas the oxygen inputs also contribute to the relatively short longevity of the cleanup/reduction column. A new type of valve is currently being installed that should eliminate these problems, and this would open up new possibilities for the analysis of dissolved gases or headspace analyses. Because the HiPerTOC is equipped with a built-in autosampler and a double needle, the valve layout would make it feasible to use the system to create a He headspace in the sample vials and sample the headspace, or alternatively, take up sample liquid from gastight vials (replacing the volume with He) and bubble out the dissolved gases in the UV reactor, which should enable automated analysis of, e.g., $\delta^{18}O$ of dissolved O_2 or $\delta^{13}C$ of dissolved inorganic carbon. To enable such dissolved gas or headspace analyses, improvements are needed at the level of the autosampler and/or sampling needle because, with the current double-walled needle system, the autosampler does not provide sufficient force to pierce commonly used septa. Finally, because $\delta^{13}C_{DOC}$ analyses are likely to become a more widespread analytical tool in the near future, it is imperative that common procedures for data calibration and standardization are developed. Given the current absence of a true certified reference solution, "consensus reference materials" (CRM) such as the one currently in use for DOC concentrations (Hansell 2005) could provide an appropriate way to standardize data and compare results of different methodologies and analytical setups, and we therefore aim at doing more extensive stable isotope measurements on this CRM.

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