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Tracing terrigenous dissolved organic matter and its photochemical decay in the ocean by using liquid chromatography/mass spectrometry

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Abstract

Reversed-phase liquid chromatography/mass spectrometry (LC/MS) is introduced as a new molecular fingerprinting technique for tracing terrigenous dissolved organic matter (DOM) and its photochemical decay in the ocean. DOM along a transect from the mangrove-fringed coast in Northern Brazil to the shelf edge was compared with mangrove-derived porewater DOM exposed to natural sunlight for 2–10 days in a photodegradation experiment. DOM was isolated from all samples via solid-phase extraction (C18) for LC/MS analysis. DOM in the estuary and ocean showed a bimodal mass distribution with two distinct maxima in the lower *m/z* range from 400 to 1000 Da (intensity-weighted average of 895 Da). Terrigenous porewater DOM from the mangroves was characterized by a broad molecular mass distribution over the detected range from 150 to 2000 Da (intensity-weighted average of 1130 Da). Polar compounds, i.e., those that eluted early in the reversed-phase chromatography, absorbed more UV light and had on average smaller molecular masses than the more apolar compounds.

After 10 days of irradiation (~70 kWh/m^2), mangrove DOM resembled open-ocean DOM in its mass distribution (intensity-weighted average of 885 Da). In addition, a large fraction of UV-absorbing compounds, which were present in mangrove samples but absent in offshore samples, were not detected after photodegradation. However, the bimodal mass distribution in ocean waters was not reproduced during photodegradation. This mass distribution is certainly a reflection of specific molecular properties of marine DOM which systematically differed from the mangrove samples. The molecular patterns of DOM in the ocean did not show significant contribution of terrigenous DOM, which is in contrast to previous stable carbon isotope analysis. If, however, photochemical modifications of the terrigenous component are considered as an additional mechanism besides simple mixing of two end members (marine and mangrove), the observed molecular patterns of oceanic DOM are consistent with the contribution of terrigenous DOM in these samples. In order to explore the molecular mass information, the mass spectra of the different samples were compared through multivariate statistics. Cluster analyses and multi-dimensional scaling (MDS) revealed significant differences between mangrove and oceanic DOM that successively disappeared in the course of the photodegradation experiment. With help of discriminant analyses, the similarity between photodegraded mangrove DOM and open-ocean DOM could be confirmed on an individual *m/z* level. Though conventional

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ion trap mass spectrometry (resolving power of ~1000) does not resolve the complexity of DOM at the level of single molecules, it does provide detailed molecular fingerprints. This molecular fingerprinting technique provides a means to trace DOM and modifications in its molecular structure in aquatic systems.

Keywords: Carbon cycle; Dissolved organic matter; Mangroves; Mass spectroscopy; Photochemistry; Molecular fingerprinting; Brazil; Amazonia

1. Introduction

Dissolved organic matter (DOM) contains more carbon than any other non-fossil organic carbon reservoir in the ocean (Hedges et al., 1997). Rivers and tidal zones combined deliver enough DOM to the ocean to account for its amount and turnover (Hedges et al., 1997). Lignin, an unambiguous molecular tracer for vascular plant organic matter, reveals the presence of terrigenous DOM in all deep ocean basins (Meyers-Schulte and Hedges, 1986; Hernes and Benner, 2006); however, its concentration does not correspond to the huge continental flux of DOM (Hedges et al., 1997; Hernes and Benner, 2006). Thus the turnover of terrigenous DOM in the ocean is evidently much faster then the turnover of the bulk of marine DOM, which has an average age of several thousand years (Williams and Druffel, 1987; Bauer et al., 1992).

Photolysis in the upper ocean may be an important removal mechanism for terrigenous DOM in the ocean (e.g. Vodacek et al., 1997; Miller and Zepp, 1995; Mopper et al., 1991). The high aromaticity and absorptive capacity of terrigenous DOM makes it particularly susceptible to photochemical reactions, and lignin is therefore rapidly lost during exposure to sunlight. The molecular signature of lignin in the deep ocean also shows signs of photochemical decay (Hernes and Benner, 2002). DOM consists of multiple pools with varying photoreactivity, and the complete photochemical mineralization of DOM into inorganic carbon in the ocean is probably slow. Therefore, the concentration of DOM in estuaries and on continental shelves often behaves conservatively on time-scales of up to several years (e.g. Mantoura and Woodward, 1983; Moran et al., 1991; Dittmar and Kattner, 2003a).

With the advent of soft ionization techniques (e.g., electrospray ionization [ESI]) that transfer entire organic molecules out of an aqueous solution into a mass spectrometer, new analytical tools for the molecular characterization of DOM became available. Conventional mass spectrometry (MS) provides molecular information in a far higher resolution than other bulk characterization techniques (such as fluorescence or absorbance spectroscopy). In ESI–MS, the molecular mass distribution of DOM over a wide range of several thousand Dalton is readily obtained in ~1 Da steps. ESI–MS thus provides an important structural overview of DOM and enables the comparison of different DOM samples based on detailed molecular information. Chromatographic separation prior to mass spectrometry (LC/MS) adds an additional dimension and further increases the amount of molecular information available. LC/MS provides a highly complex molecular fingerprint composed of several thousand data points per DOM sample. In combination with suitable statistical tools an LC/MS molecular fingerprint may enable us to trace DOM from the different sources as it cycles through marine systems.

The objective of this study was to establish LC/MS in combination with statistical tools as a molecular fingerprinting technique for tracing terrigenous DOM and its photochemical decay in the ocean. The mangrove-fringed North Brazilian shelf served as a unique system for this case study. Mangroves play a major role in global biogeochemical cycles, linking land and ocean of the tropics in an exceptional way. Tidal flushing forces up to half of the mangrove litter production into the ocean (Robertson et al., 1992; Dittmar et al., 2001; Jennerjahn and Ittekkot, 2002). On a global scale, DOM export from mangroves is approximately 2.2×10^{12} mol C $year^{-1}$ which is similar to the annual Amazon River discharge (Dittmar et al., 2006). On the continental shelf southwest of the Amazon Estuary, the terrigenous component of DOM is mainly derived from mangrove organic matter. As DOM flushes from the mangrove-fringed coast to the outer shelf it undergoes extensive photochemical modifications. Up to half of the mangrove-derived DOM is mineralized and phenolic compounds are almost entirely lost (Dittmar et al., 2006). These modifications can be reproduced in experiments with DOM from mangrove porewater which is mainly derived from mangrove leaf litter (Dittmar and Lara, 2001) and has not yet undergone photolysis.

2. Materials and methods

2.1. Sampling and field experiments

The coast in North Brazil is almost entirely fringed by mangroves. This mangrove forest is one of the largest in the world and is well developed, with trees reaching heights of >20 m. Sampling was performed at ~1°S with the cutter *Tubarão II* during the dry season of 2001 at six stations along a transect from the mangroves to the outer shelf (Fig. 1). At the innermost station, the mouth of a mangrove-fringed estuary, samples were taken at low and high tide. Sampling was performed during daytime. The water column of the shallow shelf (water depths generally <30 m) was well-mixed by strong diurnal tidal currents and the action of waves. Therefore, sampling of water was restricted to the surface. Water-column stratification was checked with a CTD (electric conductivity, temperature, and depths) probe.

In Northern Brazil, the tidal flushing of sedimentary porewater is the main transport mechanism of mangrove-derived DOM from the forests into the tidal creeks and onto the continental shelf (Dittmar and Lara, 2001; Dittmar et al., 2001). Porewater, as a reference for mangrove-derived DOM, was sampled from a mangrove site at the Caeté Estuary, near the town of Bragança in Northern Brazil. Most of the area is flooded biweekly during spring tides, when the tidal range is 4–5 m. The sampling site is a mixed forest dominated by *Rhizophora mangle* and *Avicennia germinans* trees, and a low abundance of *Laguncularia racemosa*. Sampling was performed during the dry season in October 2001 at neap tide, so that the site was not flooded during the period of sampling. To obtain porewater, three holes of 1.5-m depth were carefully dug into the mangrove sediment. After the holes filled with porewater, the water was removed, and again the hole was allowed to refill with porewater. This was repeated two more times before sampling, to reduce effects of disturbance and contamination. The salinity of the porewater was 30.9 and the dissolved organic carbon (DOC) concentration 2.77 mM.

Immediately after sampling, all samples were filtered (0.2 μm) and aliquots were stored frozen in 20-mL pre-combusted glass ampoules. For the isolation of DOM, aliquots (varying volumes of 0.3–10 L depending on the DOC concentration) of all samples were acidified (pH=2) with 10 M HCl (p.a.) and pumped through solid-phase extraction cartridges (C18-SPE, Varian Bond Elut, 5 g). The cartridges were rinsed with methanol (HPLC grade) immediately

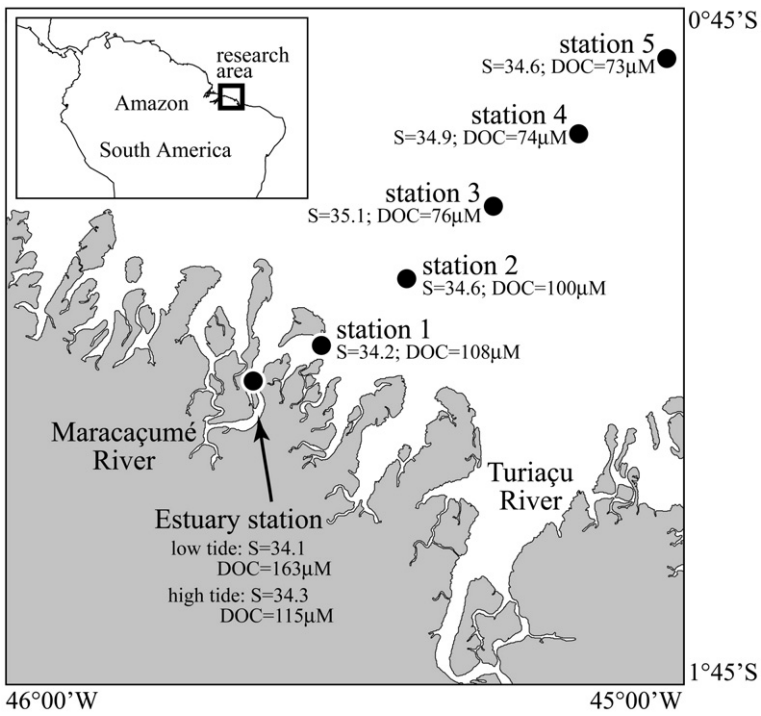

Fig. 1. The location of the sampling stations in North Brazil. Salinity and DOC concentrations for each station.

before use. Immediately after adsorption, remaining salts were washed off the cartridges with 0.01 M HCl, and DOM was eluted onboard with 30 mL methanol into pre-combusted glass ampoules. The eluted samples were freeze-dried and stored at −18 °C in the dark. The solid-phase extraction was checked with artificial seawater blanks (DOC < 0.5 μM). Freeze-dried blank extracts did not contain significantly higher amounts of DOC than seawater blanks. To minimize possible artifacts introduced by selective isolation of specific compounds classes, the same DOM extraction procedure was applied to all samples. The extraction efficiency for DOC was relatively uniform among samples, and 39 ± 3% for all samples considered in this study. Systematic differences in extraction efficiencies between inshore samples (estuary and station 1: 43 ± 1%) and offshore samples (stations 2–5: 36 ± 1%) were small, though significant. DOC was determined by high-temperature catalytic oxidation with a modified MQ Scientific TOC analyzer (Peterson et al., 2003).

Photodegradation processes were simulated experimentally. Sterile-filtered (0.2 μm pore size) mangrove-DOM was exposed on site to natural sunlight for up to 14 days. Dark controls and the production of bio-labile DOM during photodegradation and its presence after the experiment (Dittmar et al., 2006) indicates that re-growth of microorganisms in the sterile-filtered samples were probably not significant. The pre-combusted thin-walled borosilicate glass ampoules (Wheaton, 100 mL) used for this experiment were tested for their UV absorption over 190–400 nm in a spectrophotometer. They absorbed less UV-light than conventional quartz bottles employed in most photodegradation experiments. Constant water-cooling kept the temperature at ambient levels (26–30 °C). A headspace of air eliminated possible oxygen limitation. Oxygen concentrations (WTW electrode) and UV-absorption spectra (190–300 nm) were measured every second day on a subset of ampoules. For sampling of DOM, triplicates (three ampoules) were removed every second day, and kept frozen (−18 °C) until further analyses. Photodegraded DOM (0.3 L, pooled sample from the three replicas) was concentrated via C18-SPE as described above with an extraction efficiency similar to the offshore samples.

During the experiment, DOC decreased asymptotically to ~65% of its initial concentration. For more details on the degradation experiment and for additional data collected on the samples (inorganic nutrients, stable carbon isotopes, physicochemical parameters) refer to Dittmar et al. (2006).

2.2. Liquid chromatography/mass spectrometry (LC/MS)

For LC/MS analysis, aliquots of the freeze-dried C18 extractable DOM samples (~1 mg) were re-dissolved in water (0.5 mL). Samples were analyzed in triplicate on an ion trap LC/MS (Thermo Finnigan, LCQ). 15 μL of marine sample (stations 1–5) and 10 μL of mangrove and photodegradation samples (estuary and porewater) were injected directly onto a C18 reversed-phase aqueous column (Alltech Alltima C18, 5 μm, 150 × 2.1 mm). The difference in injection volume compensated for differences in the carbon concentration between samples so that similar amounts of DOC were loaded onto the column. Liquid chromatography used a gradient of water and methanol, both modified with 0.05% formic acid, at 30 °C. The flow rate was kept constant at 250 μL min^{-1}. The gradient started at 80% water and decreased linearly to 50% water at 6 min and 1% water at 15 min. Elution continued at 1% water until 23 min, then the column was slowly equilibrated to initial conditions. Absorption spectra were obtained between 200 and 600 nm by a diode array detector (DAD) at 1-nm resolution. Flow was directed from the DAD into the electrospray source of the MS without splitting. Electrospray ionization (ESI) has been shown to be a suitable ionization technique for humic substances (Stenson et al., 2002, 2003) and coastal dissolved organic matter (Koch et al., 2005, Seitzinger et al., 2005). While ESI is still selective and does not transform all dissolved organic compounds into gas-phase ions, it is particularly suitable for acquiring detailed molecular information on DOM composition. The electrospray source was operated in positive ion mode at a capillary voltage of 4.8 kV, N_2 gas flow at 70 units with auxiliary gas at 10 units and a capillary temperature of 335 °C, similar to previous LC/MS work on DOM (e.g. Seitzinger et al., 2005). Mass spectra were collected in a mass to charge ratio (m/z) range of 150–2000 m/z. All electrospray and trap conditions were determined by tuning the instrument to the specific flow conditions and sample types. Tuning was conducted on both mangrove and marine end member samples that were infused into the solvent stream for a total flow into the ESI chamber of 250 μL min^{-1}. This ensured a steady electrospray plume, good sample protonation and a high MS signal for all sample types. For all analyses, the mass spectrometer was operated at one unit mass resolution and calibrated using a standard solution prescribed by Thermo Finnigan (caffeine, MRFA and Ultramark). All solvents used were of environmental analytical grade (Burdick and Jackson).

2.3. Multivariate statistics

To explore differences between the samples, the mass spectra were analyzed with multivariate statistics. The chromatograms were divided into 2-min intervals between a retention time of 6 to 16 min and the average background subtracted mass spectra (*m*/*z* distribution) of these intervals were used for hierarchical agglomerative cluster analysis, multi-dimensional scaling (MDS) and discriminant analysis.

Cluster analysis and MDS are based on the Bray–Curtis similarity matrix in which all parameters (masses) of all samples are compared. For both statistical techniques normal distribution is not required. The Bray–Curtis coefficient (S; Bray and Curtis, 1957) was computed from standardized and untransformed data sets (Primer 5.0) and represents the extent of similarity of samples referring to their mass spectra. S increases with the degree of similarity.

From the similarity analysis a group average cluster analysis is computed and results are displayed in a dendrogram. The branching in the dendrogram is dependent on the similarity of the samples. A coefficient of S=100 would represent samples with identical relative intensities for all peaks in the mass spectra.

MDS is a statistical technique for representing multi-dimensional systems (e.g. mass intensities in a mass spectrum) on a visual low dimensional grid (2D or 3D). The similarity of samples decreases with the distance in the MDS plot. The stress value gives an indication for the quality of the MDS analysis. For 2-dimensional ordinations a stress value of less than 0.05 gives an excellent representation of the analyzed data. Stress values of greater than 0.3 indicate that the points are too close to being arbitrarily placed in a two dimensional space (Clarke and Warwick, 1994).

Different from MDS and cluster analyses, discriminant analysis, in this case a two-stage principal component analysis (Hoogerbrugge et al., 1983), allows the identification of individual variables (*m*/*z* values) that contribute most to the differences observed between the samples. In this technique, principal component analysis is first done on the entire data set after z-scoring and variance-scaling. Then the first quarter of the resulting principal components is chosen, and the scores from replicate samples are averaged. These averaged scores are used for a second principal component analysis (the discriminant analysis) in order to maximize variance among samples and to minimize the variance among replicates. The results from the discriminant analysis are in the form of scores, which reflect relationships among the samples, and loadings, which show relationships among the individual variables (*m*/*z* values) contributing to the relationship observed between the samples. For ease in viewing, the loadings-plots have been rescaled by multiplying each *m*/*z* value by its data-set standard deviation, and are thus reconstructed difference spectra rather than classical loadings plots. In this LC/MS study, discriminant analysis was applied to time-averaged mass spectra, with each 2-min time slice across the chromatogram treated as a separate data set.

3. Results and discussion

3.1. Reversed-phase chromatography of DOM

Dissolved organic carbon (DOC) concentrations on the shelf ranged from 73 to 108 μM, decreasing from near- to offshore. Higher values of 115 and 163 μM, were reached in the mangrove-fringed estuary, showing a tidal signature with a maximum at low tide (Fig. 1). Reversed-phase chromatography of DOM yielded one unresolved broad peak as identified by total ion current (TIC, Fig. 2), with no resolution of individual compounds. This unresolved peak is consistent with the complex and polydisperse nature of natural organic matter and the strong interactions that occur between the individual compounds during chromatography. The peak maximum shifted toward longer retention times along the transect offshore. At the outermost station, the peak maximum eluted 10% (or 1 min) later than at low tide in the estuary. Stable carbon isotope ratios ($\delta^{13}C$ values) increased from −25.7‰ in the mangrove-fringed estuary to −23.8‰ offshore (Dittmar et al., 2006), indicating a decrease of mangrove-derived DOM along the transect across the shelf. For comparison, $\delta^{13}C$ of the mangrove DOM end member was −26.6‰ (before photodegradation) and $\delta^{13}C$ of the marine end member was −23.7‰ (Dittmar et al., 2006). Based on a two-source mixing model, the terrigenous proportion of DOM can be estimated to be ~40% in the estuary and at stations 1 and 2, and it decreased at the offshore stations from ~40% (station 2) to values <5% (station 5). The retention time of the peak maximum of the total ion current (TIC) inversely correlated with $\delta^{13}C$ (linear, $r=-0.94$, $n=7$, $p<0.001$). The shift toward longer retention times offshore and the negative correlation with $\delta^{13}C$ is consistent with an overall lower molecular polarity of marine DOM compared to mangrove-derived DOM. This finding matches earlier observations of a significant amphiphilic fraction in marine DOM which is virtually absent in terrigenous DOM (Dittmar and Kattner, 2003b).

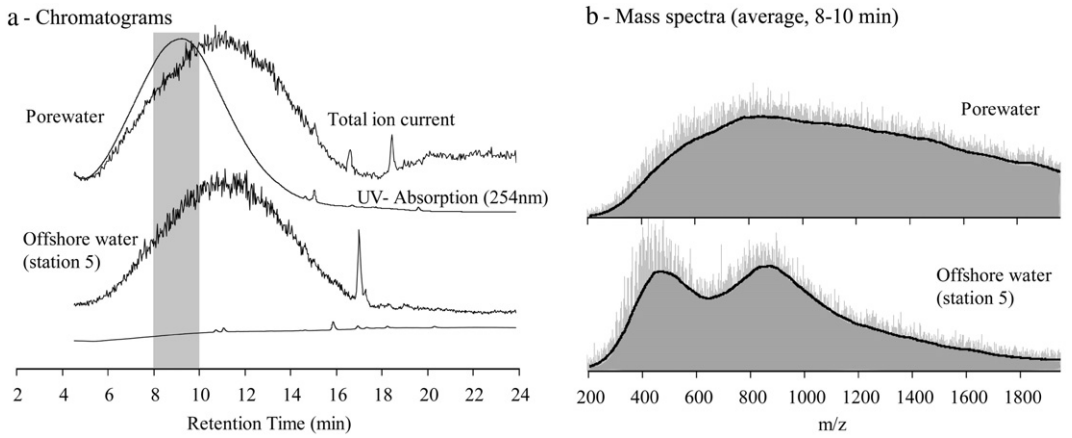

Fig. 2. LC/MS of DOM extracted from mangrove porewater and from offshore water (station 5). (a) Reversed-phase chromatography with detection of total ion current (TIC, mass spectrometer) and UV-light absorption (254 nm) (b) time-averaged mass spectra of the 8–10 min chromatography interval, including moving averages (black line) of the signal intensities of the mass spectra.

In the porewater sample, the peak maximum with UV-absorbance detection (254 nm) eluted 15% (or 1.5 min) earlier than with TIC detection (Fig. 2). UV absorption at 254 nm is a rough proxy for the aromaticity of natural organic matter (e.g. Dittmar and Kattner, 2003b; Weishaar et al., 2003). The shift in retention time between the two detectors is an indication of a higher polarity, and probably higher functionality, of aromatic compounds compared to the aliphatic, and more hydrophobic, fraction of DOM.

The absorptivity at 254 nm was much higher in the porewater compared to the water column samples (Fig. 2a) and decreased sharply in the offshore direction along the transect (data not shown). In order to compare the UV absorptivity between samples, we normalized the absorption at the chromatographic peak maximum to the TIC signal intensity, as an approximation for DOM concentration. The normalized absorption decreased from 100% in the estuary to 66% at station 1 to 22% at station 2 and to almost undetectable levels further offshore. Contrary to absorption, TIC signal intensity did not fluctuate significantly between samples, because similar amounts of DOC were always used for LC/MS. The normalized UV absorption (254 nm) inversely correlated with $\delta^{13}C$ (linear, $r=-0.96$, $n=7$, $p<0.001$) which supports the finding that terrigenous DOM is usually characterized by a far higher aromaticity than marine DOM (e.g. Dittmar and Kattner, 2003b).

These major cross-shelf molecular changes, i.e. a shift toward more hydrophobic and less aromatic properties, were also observed during the photodegradation experiment of mangrove-derived DOM. The chromatographic peak maximum shifted by 15% (1.5 min) toward longer retention times, and the UV absorptivity decreased to 15% of the initial value after 10 days of irradiation with natural sunlight (~70 kWh/m^2). Proton nuclear magnetic resonance spectroscopy (^1H NMR) showed the same trend (Dittmar et al., 2006). As the photodegradation experiment progressed, aromatic compounds were almost completely lost and functionalized aliphatics (mainly carboxylic acids) decreased significantly. NMR chemical changes from near- to offshore were consistent with the experimentally mediated alterations. The slight shifts in solid-phase extraction efficiencies from in- to offshore (from 43±1% to 36±1%) and during photodegradation are probably also a reflection of systematic compositional changes, and consistent with the LC/MS results.

3.2. Mass spectrometry

The mass spectra reflect the molecular complexity of DOM throughout the chromatographic analysis. All nominal masses were present in almost the entire mass window recorded (150–2000 *m/z*; Fig. 2). Within the complex mass spectra, some regular patterns appeared, e.g., signal intensities for odd nominal masses were higher than for even nominal masses. Because of the positive ionization mode the mass of one proton has to be subtracted to obtain actual masses (or in case of sodium adducts one sodium ion; Koch et al., 2005). The dominance of nitrogen-free molecules in DOM can thus explain the observed odd versus even signature (Koch et al., 2007). Sinuous patterns also appeared in the mass spectra with a wavelength of 14 *m/z*; these patterns may be attributed to homologous series of compounds whose molecular formulae differ in the number of CH_2-units present (Koch et al., 2005). In the course of

chromatography the molecular size slightly changed. The intensity-weighted average at the beginning of the unresolved peak (6–8 min) was 907±11 Da (average of all water column samples) and increased steadily throughout the chromatographic analysis to 957±11 Da (at 14–16 min). Varying sample amounts (2–20 μL injection volume) resulted in proportional changes of TIC, but did not cause any significant changes in molecular mass distribution.

The samples along the transect showed very similar mass distribution patterns, with a pronounced bimodal mass distribution in all samples (Fig. 2b). Two distinct maxima were evident in all estuarine and shelf samples at ~450 Da and ~850 Da. Systematic changes along the transect were mainly observed in the high-molecular mass range (1000–2000 Da) where signal intensities decreased significantly from near- to offshore. The intensity-weighted average of the detected masses (150–2000 Da) continuously decreased along the transect from 935 Da in the estuary to 895 Da at the outermost station (station 5).

Different from the water column samples, terrigenous DOM from the mangrove porewater was characterized by a broad molecular mass distribution over 200–2000 Da, with a maximum at ~800 Da (Fig. 2b). These characteristics changed during the photodegradation experiment, where molecules in the high-molecular mass range were lost, and the peak maximum was shifted from 800 to 700 Da (Fig. 3a). Smaller molecules in the mass range of 250 to 700 Da became relatively enriched, either because they were produced during photodegradation or because of the preferential loss of large molecules. The intensity-weighted mass average continuously decreased during the experiment from 1131 Da in the original porewater sample to 1105 Da, 994 Da and 885 Da after 2, 4 and 10 days of photodegradation, respectively. This trend in intensity-weighted mass averages as a function of photochemically mediated degradation is similar to that observed in our transect from nearshore to offshore. After 10 days of irradiation, the overall molecular mass distribution in mangrove porewater resembled the mass distribution in open ocean waters (Fig. 3b), and the characteristic terrestrial higher molecular mass components were removed.

However, the bimodal mass distribution in ocean waters was not reproduced during photodegradation. This mass distribution is certainly a reflection of specific molecular properties of marine DOM which systematically differed from the mangrove samples and may thus contribute to molecular fingerprinting. Stable carbon isotope analyses (Dittmar et al., 2006) indicate

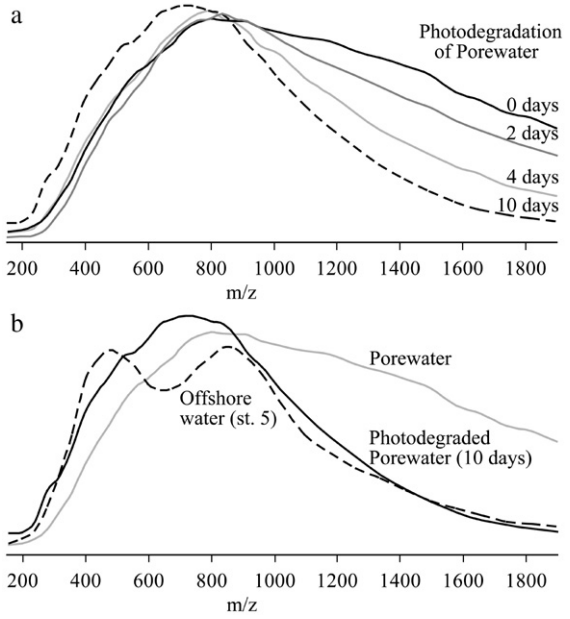

Fig. 3. Average mass spectra of DOM for the 8–10 min chromatography time interval: (a) DOM from mangrove porewater after 0, 2, 4, and 10 days of photodegradation with natural sunlight. (b) Mangrove-derived DOM before and after photodegradation compared to open-ocean DOM. For clarity only moving averages of the signal intensities are shown.

a significant mangrove-derived DOM component (~40% of DOC) in the estuary and at stations 1 and 2. However, the molecular patterns of DOM at these stations do not match this observation. Most notably, the abundant high-molecular weight fraction and the aromatic components observed in mangrove porewater were low at all stations and virtually absent offshore (stations 2–5). However, if photochemical modifications of the terrigenous component are considered as an additional mechanism besides simple mixing of two end members, the observed molecular pattern of oceanic DOM can be reconciled with the stable carbon isotope analyses indicating a significant contribution of terrigenous DOM.

3.3. Multivariate statistics

To explore the molecular mass information in its full complexity with regard to the various DOM characteristics, the mass spectra of the different samples were compared through multivariate statistics. The reversed-phase chromatography prior to mass spectrometry significantly enhanced this attempt. As a first step, the broad unresolved peak in the chromatography was divided into 5 intervals, each of 2 min, and an average mass spectrum was obtained over each interval. The

multivariate statistical analyses (cluster and MDS analyses) also indicated that differences between the samples were most pronounced during the 8–10 min and 10–12 min intervals. The differences between the individual mass spectra were less significant during the other time intervals within the chromatographic analysis, including the averages of the entire peak (6–16 min). Most aromatic compounds, which were far more abundant in mangrove DOM (porewater) compared to open-ocean DOM, eluted between 8 and 10 min as shown by the absorption maximum at 254 nm. The following discussion therefore focuses on the average mass spectra recorded during the 8–10 min chromatography interval of the different samples.

Cluster and MDS analyses confirmed that offshore DOM (stations 2–5) was different from inshore DOM (estuary and station 1) (Figs. 4 and 5), and that these differences were not as pronounced as the differences between porewater and water-column DOM. After 2 days of photodegradation, the mass spectrum of the porewater sample had not significantly changed. After 4 days, however, the mass spectrum was already more similar to the water column samples than to the original porewater (Fig. 4). Further differences between porewater and water column samples successively disappeared, and after 10 days (~70 kWh/m^2 of irradiation), the mass spectra and hence the molecular patterns of

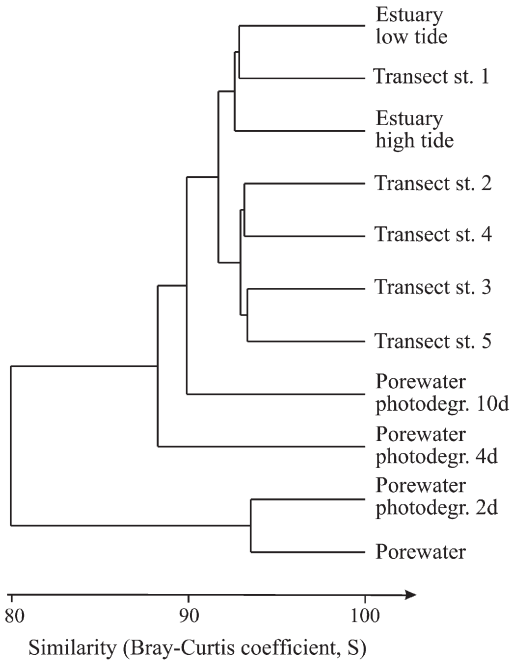

Fig. 4. Cluster analyses of mass spectra from different DOM samples: Photodegraded mangrove DOM shows a higher similarity to open-ocean DOM than to mangrove DOM before photodegradation.

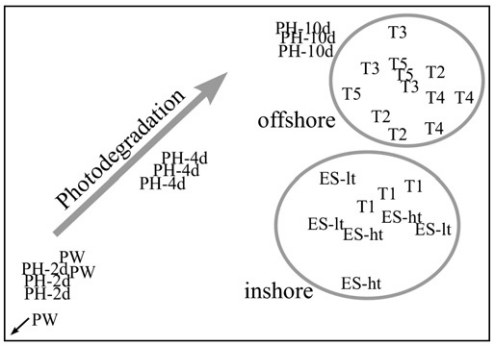

Fig. 5. Multi-dimensional scaling (MDS) analysis of mass spectra from different DOM samples: During photodegradation, the mass spectra of mangrove-derived DOM changed systematically, and after 10 days they were more similar to open-ocean DOM than to the mangrove DOM before photodegradation. The stress value for the MDS analysis was 0.12, i.e. the identified differences between the samples are significant.

mangrove-derived DOM resembled that of open-ocean DOM to a sufficient degree to account for the presence of mangrove-derived DOM in the open ocean.

In order to identify individual *m*/*z* values that contribute most to the observed differences in the mass spectra of porewater and open-ocean DOM, discriminant analyses were performed. Data from the photodegradation experiments were not included to ensure independence for later comparison. Discriminant analysis significantly differentiated between mangrove and marine samples. A reconstructed difference mass spectrum (Fig. 6), where the loadings are multiplied by the standard deviations for each *m*/*z* value prior to plotting, allows the mass spectra to look more familiar. The lower the value on the ordinate (negative range) for a given *m*/*z* value, the more significant was this *m*/*z* for mangrove-derived DOM, and, vice versa, the more positive the values on the ordinate, the more significant was the given *m*/*z* value for DOM from the offshore stations. Specific ions (*m*/*z*) that were enriched in either open-ocean (525, 295) or mangrove (295, 1136) samples were identified. The mass spectrometer intensities of these *m*/*z* values showing strongest enrichment towards open-ocean or mangrove end members (circled in Fig. 6) were plotted as a ratio of 525/295 and 848/1136 (marine/mangrove) against distance offshore (Fig. 7). Significant correlations were found between distance offshore and these intensity ratios. In both examples in Fig. 7, the ratios of mangrove-derived (porewater) and estuarine DOM were virtually identical. However, after 10 days of photodegradation, the mass ratios of porewater DOM were undistinguishable from that of open-ocean DOM. With the help of discriminant analyses, the results from the cluster and

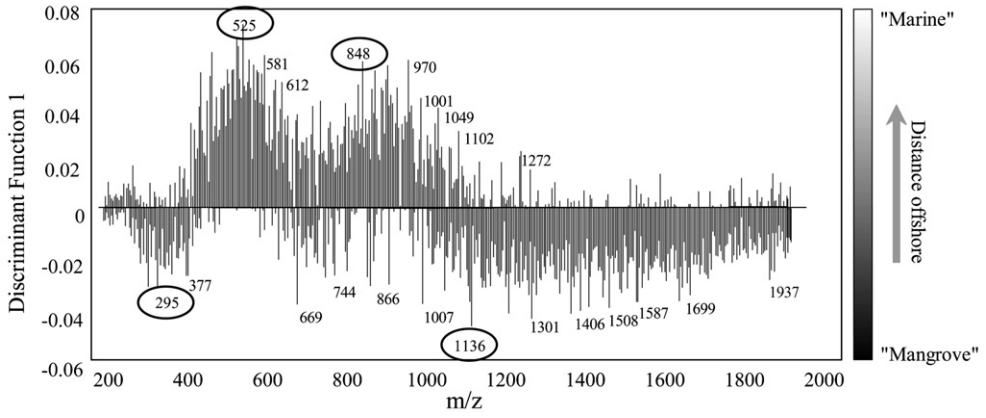

Fig. 6. Difference spectra constructed from the factor loadings for discriminant function 1. This discriminant analysis was performed on mass spectra from DOM samples taken along the estuary-to-offshore transect. Note that *m*/*z* values with negative loadings are most significant for nearshore DOM ("mangrove"), positive values are most significant for open-ocean DOM ("marine"). Discriminant function 1 is responsible for 10% of the variance in the data set and the ratio of variance between samples relative to variance between replicate mass spectral analyses ($n=3$) is 45.5.

MDS molecular fingerprinting approaches could thus be confirmed on an individual *m*/*z* level.

4. Conclusions

Molecular source tracers for DOM in the ocean are very limited. Mass spectrometry of a distinct DOM fraction, separated through solid-phase extraction and reversed-phase chromatography, produced detailed molecular fingerprints. These complex fingerprints, which consisted of 1800 individual mass spectrometry data points, were explored through various multivariate statistical approaches. MDS analysis distinguished between DOM from different sources, and the MDS plot offered a comprehensible approach to data representation for a multi-dimensional matrix. In addition, extensive molecular modifications of DOM that occurred during transport on the shelf might be related to a specific process, e.g. photochemically mediated degradation. The molecular pattern of DOM on the North Brazilian shelf cannot be explained simply by mixing terrigenous and marine sources. If photochemically mediated modifications of the terrigenous component are considered, the observed molecular patterns of DOM on the shelf are consistent with the observed contribution of terrigenous DOM in these samples, based upon stable carbon isotope values (Dittmar et al., 2006).

The statistical methods presented here are useful tools for the comparison of mass spectra of highly complex natural organic matter samples in general. Ion trap LC/MS has become more widely available to the aquatic sciences community in the past few years. While this technique does not resolve the complexity of DOM on the individual molecular level, it does provide

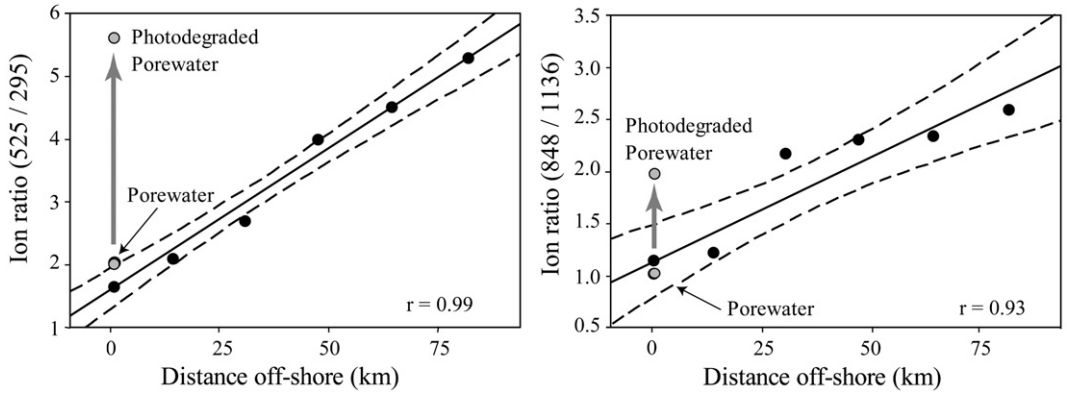

Fig. 7. Signal intensities of ions showing strong enrichment toward marine or mangrove end-members (circled in Fig. 6) were plotted as a ratio (marine/mangrove) against distance off-shore. The dashed lines show the 95% confidence interval. Ratios for a mangrove-derived DOM sample before and after exposure to sunlight are also plotted.

detailed molecular fingerprints. Routine application is possible and allows fast tracing of DOM as it travels through different aquatic systems and undergoes extensive modifications in its molecular structure.

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