**Efficient removal of recalcitrant deep-ocean dissolved organic matter during hydrothermal circulation**

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**Oceanic dissolved organic carbon is an important carbon pool, similar in magnitude to atmospheric CO2, but the fate of its oldest components is not well understood1,2. Hot hydrothermal circulation may facilitate the degradation of otherwise un-reactive dissolved organic matter, playing an important role in the long-term global carbon cycle. The oldest, most recalcitrant forms of dissolved organic carbon, which constitutes the majority of oceanic DOC, can be recovered by solid-phase extraction. Here we present measurements of solid-phase extractable dissolved organic carbon from samples collected between 2009 and 2013 at seven vent sites in the Atlantic, Pacific, and Southern Oceans, along with magnesium concentrations, a conservative tracer of water circulation through hydrothermal systems. We find that magnesium and solid-phase extractable dissolved organic carbon concentrations are correlated, suggesting that solid-phase extractable dissolved organic carbon is almost entirely lost from solution via mineralisation or deposition during circulation through hydrothermal vents with fluid temperatures 212-401 °C. In laboratory experiments, where we heated samples to 380 °C for four days, we found a similar removal efficiency. We conclude that thermal degradation alone can account for the loss of solid-phase extractable dissolved organic carbon in natural hydrothermal systems, and that its maximum lifetime is constrained by the timescale hydrothermal cycling, at about 40 million years3.**

The reservoir of oceanic dissolved organic carbon (DOC) is quantitatively similar to that of atmospheric CO2 (660 vs. 850 Pg C). Oceanic DOC is composed of a myriad of structurally distinct molecules at extremely low concentrations1 making up a mixture totalling 35-48 µM C throughout the deep ocean2. The accumulation of recalcitrant DOC in the deep ocean plays an important role in the long-term storage of carbon, and the biotic and abiotic mediation of carbon transfer between inorganic and organic pools in ocean water forms a critical control on atmospheric and marine concentrations of CO2 and O2, and marine nutrient fluxes2,4. Recalcitrant DOC in the deep ocean basins has a very low reactivity, with an apparent turnover time of 6,000-30,000 years, and therefore appears to survive several global thermohaline cycles1,2,5,6. Most (>95%) of deep-sea DOC remains uncharacterized on an individual molecular level1, but an inherent molecular feature of oceanic DOC is that a major and reproducible fraction of about 60-65% is extractable on styrene-divinyl benzene resin, operationally defined as solid-phase extractable DOC (SPE-DOC)6. Oceanic SPE-DOC is similar in apparent age and distribution to bulk DOC6 , meaning that it is formed in the surface ocean after photosynthetic reduction of CO2 and further microbial processing of this reduced carbon, and is removed over millennia. However, there is limited evidence for loss mechanisms that operate on such timescales.

During various stages of hydrothermal circulation, deep ocean water is subjected to high temperatures (>300 °C), low pH and increased and diversified microbial activity7–9, and hydrothermal circulation has a profound effect on organic and inorganic forms of carbon in seawater7,8. Under simulated hydrothermal conditions in the laboratory (i.e. >200 °C, >200 bar) organic molecules can be removed through gasification to CO2, H2, and CH410. During gasification, water can act as acidic reagent and solvent10, allowing hydrolysis and functional group cleavage, among other reactions11. The high ionic strength of seawater and the provision of mineral catalysts in the oceanic lithosphere likely promote these abiotic reactions10,11. An important outstanding uncertainty is whether the mixture of recalcitrant dissolved organic molecules found in deep-ocean water is removed during high-temperature hydrothermal circulation.

Reported DOC concentrations in hydrothermal vent fluids are highly variable (15-539 µM) compared to background deep ocean DOC (35-48 µM)7,12,13. Hydrothermal processes involved in the supply and consumption of DOC include thermal degradation, alteration and mobilization of carbon-rich sediments14–16, microbial reduction of dissolved inorganic carbon (DIC)8, excretion of DOC by macrofauna, microbial fixing of CH4 and H217 and possibly abiotic synthesis18–20. These processes can lead to increased concentrations of small organic compounds such as formate, acetate, methanol, methanethiol and amino acids7,11,13,15,16,20–23, and mixtures of larger hydrocarbon chains (petroleum)14,15. The rock substratum, extent of sedimentation and geochemical constraints on the fluid composition (e.g. pH, H2) may all play an important role in the dissolved carbon system. Here we aimed to investigate the fate of DOC in a geochemically diverse range of hydrothermal systems in order to assess the importance of high temperature hydrothermal circulation in the oceanic cycling of DOC.

We quantified two operationally defined fractions of DOC in focussed and diffuse vent fluids from sites in the Atlantic, Pacific and Southern Oceans (Figure 1, Table 1). These were ‘bulk DOC’, the fraction that is stable in solution after purging with inert gas at pH 2; and solid-phase extractable DOC ‘SPE-DOC’, that is sufficiently hydrophobic at pH 2 to adsorb onto styrene-divinyl benzene (PPL, Varian) and is non-volatile after heating at 50 °C overnight (see supplementary methods for detailed explanation of methods and data treatment). We use the conservative tracer magnesium to determine the percentage of hydrothermal fluid in a sample, as this element is almost completely removed from solution during high temperature seawater-rock interaction3.

Table 1: Vent site characteristics

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Site | Host rock | Chimney type | Region | pH (25°C, lowest measured) | Temperature (°C, highest measured) |
| Menez Gwen | Basalt | White smoker | Atlantic | 4.7 | 280 |
| Endeavour | Basalt | Black smoker | Pacific | 3.9 | 351 |
| Axial Volcano | Basalt | Black smoker | Pacific | 3.6 | 330 |
| Beebe (AKA Piccard) | Basalt | Black smoker | Caribbean | 2.9 | 401 |
| E2 | Basalt | Black smoker | Southern | 3.0 | 351 |
| Logatchev | Serpentinite | Black smoker | Atlantic | 3.9 | 350 |
| Von Damm | Gabbro | Talc chimneys | Caribbean | 6.0 | 215 |

SPE-DOC concentrations co-varied with magnesium at all sites, suggesting simple conservative mixing between a globally consistent hydrothermal end-member (1.6 ± 1.2 µM) and seawater (25 ± 1.2 µM; 99% confidence level; Figure 2). Assuming that seawater in the hydrothermal recharge zone is similar in composition to the ambient seawater at the vent sites sampled, this represents a globally consistent removal of 93.5 ± 4.7% of the deep-ocean SPE-DOC (Figure 2). Local variability in SPE-DOC concentrations in diffuse venting areas (<10% hydrothermal end-member) was far higher than the bulk differences in SPE-DOC concentrations between the ocean basins.

To test the effect of heat and pressure alone on SPE-DOC concentrations in seawater, we extracted DOC from North Equatorial Pacific Intermediate Water, re-dissolved the SPE-DOC in artificial seawater to ~2 mM SPE-DOC, and thermally degraded the 2 mM SPE-DOC sample in a modified Dickson-type hydrothermal apparatus in two experiments (300 °C, 400 bar, 2 days heating and 380 °C, 400 bar, 4 days heating; see supplementary methods). The concentration of SPE-DOC decreased rapidly over the first hour of heating from room temperature and then stabilised with 15.0% remaining (300 °C) and 7.1% remaining (380 °C) after 2-4 days (Figure 2 and supplementary information), a result which is remarkably consistent with the amount of degradation calculated for natural systems (93.5 ± 4.7%). Under natural conditions, fluids are likely to reside in the hydrothermal reaction zone for several years24 at temperatures >415 °C25. This result provides convincing evidence that thermal degradation alone can decrease the accumulated SPE-DOC in deep-ocean waters to near-zero concentrations.

Unlike SPE-DOC, bulk DOC did not co-vary with magnesium at all sites and did not decrease to values <10% of seawater (i.e. <4 µM). The lowest concentrations of bulk DOC that we measured were between 7-30 µM at unsedimented basalt hosted sites (Menez Gwen, Axial Volcano, Endeavour, E2), similar to previously reported values7. Bulk DOC concentrations were 68-402 µM and 41-346 µM at hydrogen-rich vent sites21,26 from the Logatchev and Beebe vents, respectively and up to 762 µM at the hydrogen-rich and sediment covered Von Damm vent field21 (see supplementary information for all values). In hydrogen-rich fluids (i.e. H2>10 mM), CO2 can be reduced to formate during mixing of fluids with seawater, and the bulk DOC concentrations measured at the Von Damm vent field correspond closely to formate measured at the same site20, and abiotic production of formate during vent fluid mixing with seawater20,27 leads to higher bulk DOC in chimneys with increased sub-surface mixing (Figure SI1-2). Some formate (~5 µM) is also formed at the Beebe vent site20, and mobilization of previously buried organic matter has been identified as an important process which may further help to explain the raised DOC concentrations at this site21. Diffusive areas of venting at all sites hosted high bulk DOC concentrations (Figure 3 & Figure SI1), which may be related to microbial activity7,28, leaching from sediments16 or secretion by macrofauna. We also cannot rule out the possibility of some random DOC contamination related to the sampling techniques and ROV operations29 (SI Sheet 2), although our measurements are generally consistent with previous reports from various hydrothermal settings7,12,13 and recently published compound-specific concentrations from hydrogen rich fluids20,21.

The bulk DOC and SPE-DOC concentrations were thus decoupled in the hydrothermal environments, with variable amounts of additional non-extractable DOC present in many of the samples. The two processes - SPE-DOC loss and non-extractable DOC production - were probably largely unrelated, as the dominant DOC production results from the reduction of inorganic carbon20 and leaching of previously buried sedimentary particulate organic carbon15,16,21. Using laboratory-prepared solutions, we determined that organic acids (formic acid, acetic acid), amino acids (aspartic acid, glutamic acid) and methanol are all completely non-extractable by SPE using PPL, but fully detected as DOC by our analytical method (Figure SI3). This likely results from their having insufficient hydrophobic groups to be retained on the sorbent6 (see supplementary methods and supplementary information). These compounds all occur in hydrothermal environments13,20–23,29, where they can be formed sub-surface or in hydrothermal plumes both abiotically and biotically. They are also generally biologically labile and may be rapidly consumed by vent microbes28. Their rapid and patchy production and consumption make these compounds good candidates to explain the high and variable concentrations of bulk DOC observed in many vent environments.

Our data, along with other recent findings, suggest that hydrothermal fluids can provide an energy source for heterotrophic as well as autotrophic organisms and also valuable precursor compounds for basic biochemistry13,18,22,28. The production of non-extractable DOC was greatest at sites with hydrogen-rich fluids, where mixing of high temperature fluid with seawater leads to the production of formate from CO2 and H227. At the Von Damm Vent Field in the Caribbean Sea, of four chimneys with differing temperatures due to varied sub-surface mixing, the chimneys with the most sub-surface mixing had the highest bulk DOC concentrations, corresponding closely to similar observations of formate concentrations20. SPE-DOC concentrations were unaffected by this process (Figure SI3). Further re-working of formate and other labile non-extractable DOC by sedimentary and hydrothermal plume microbes may create important hotspots for carbon cycling in the otherwise relatively inactive deep-ocean environment. It is worth noting that a minor, possibly insignificant, fraction of SPE-DOC survived hydrothermal treatment both in nature and in our experiment. It is difficult to speculate about the nature and fate of this material, but future studies focusing on its molecular properties29 and availability to heterotrophs may shed more light on this. High temperature hydrothermal vents are unlikely to be a major source of highly recalcitrant dissolved condensed aromatic compounds to the deep ocean, as had been previously hypothesised30, because even if the 1.6 µM SPE-DOC that remains in hydrothermal fluids consisted entirely of condensed aromatic compounds, the timescale of this production would be insufficient to support the concentration of these compounds in the ocean30.

Even though bulk DOC concentrations were highly variable, the extractable fraction of DOC, representative of refractory deep ocean DOC, was efficiently removed during hydrothermal circulation across all the systems studied. While other processes (sedimentary, microbial) may contribute to DOC alteration or removal in lower temperature hydrothermal systems7,8,12, thermal degradation alone was sufficient to reproduce the SPE-DOC concentration loss found at high temperatures in the environment. Ocean water passes through high temperature hydrothermal systems with a recycling time of ~40 Ma3, due to a hydrothermal water flux of 3±1.5 x 1013 kg yr-1 and a global ocean volume of 1.33 x 1021 kg. Assuming a global average SPE-DOC concentration of 25±1.2 µM, and an average loss of 94% SPE-DOC during hydrothermal cycling, hydrothermal vents remove 8.5±4.2 x 109 g SPE-DOC from the ocean each year. If the thermal stability of marine SPE-DOC is representative of all refractory marine DOC (average refractory DOC concentration 40±2 µM2), the hydrothermal sink of refractory DOC would be 1.4±0.7 x 1010 g C yr-1. These should be considered as minimum estimates, because they only consider losses in axial reaction zones at >380 °C (94% removal), and no losses at lower temperatures in ridge flanks3,7,8. Hydrothermal removal rates are of minor relevance to the majority of deep-ocean DOC (4.3 x 1013 kg produced per year2). However, for the most refractory molecules, which are not removed by consumption, particle sorption or photo-degradation in surface waters2,5,6, this hydrothermal sink limits accumulation over geological time. Efficient hydrothermal removal therefore constrains the maximum possible lifetime for refractory DOC in the ocean.

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*Acknowledgements* We thank the captains, crews and ROV pilots from the numerous cruises that allowed us to gather this dataset. We also thank Matthias Friebe and Joaquín Pampín Baro for help with DOC measurements. J. A. Hawkes was funded by the Alexander von Humboldt Foundation. A. Stubbins was funded by a fellowship from the Hanse Institute for Advanced Studies (HWK, Delmenhorst, Germany) and NSF grant 1234704. This publication is partially funded by the Joint Institute for the Study of the Atmosphere and Ocean (JISAO) under NOAA Cooperative Agreement NA10OAR4320148, Contribution No. 1600, PMEL Contribution number xxxx. E. P. Achterberg acknowldges funding from the UK National Environmental Research Council (NE/G015732/1).

*Author Contributions* The study was designed by J.A.H., P.E.R., A.S., C.H. W.B. & T.D. Sampling and data analysis was conducted by J.A.H., P.E.R., A.S., C.H., D.A.B., A.K. & V.C., Cruise and project planning was conducted by D.A.B., D.P.C., A.K. & T.D. and all authors contributed to the writing of the manuscript.

*Competing financial interests* The authors declare no competing financial interests.

*Figure Captions*

Figure 1: **Study site map** showing sites sampled for this study

Figure 2: **Natural and experimental removal of SPE-DOC under hydrothermal conditions**

A: Environmental samples (A = Axial Volcano, B = Beebe Vents, E = Endeavour, e = E2, M = Menez Gwen, L = Logatchev, V = Von Damm). The extrapolated value in pure hydrothermal fluid is 1.6 ± 1.2 µM (93.5 ± 4.7% degradation). 99% confidence intervals of the regression line are shown as a shaded grey area, analytical error bars are indicated (see supplementary methods).

B: Hydrothermal degradation of an extract of North Equatorial Pacific Intermediate Water in the laboratory. The estimated environmental fluid endmember is overlaid as a black dotted line with 99% confidence intervals in grey.

Figure 3: **Schematic of dissolved organic matter dynamics in hydrothermal vent environments.**

Seawater in the oceanic lithosphere is heated to >400°C for a few years before emission, removing ~94% of the deep-ocean SPE-DOC. Non-extractable low molecular weight carbon may be formed abiotically or after mobilisation of particulate organic carbon from buried sediments. Diffuse vents form, allowing increased abiotic formation of formate and microbial metabolism of fluids during mixing with seawater, further increasing bulk DOC.

*Methods*

*Sample collection*Samples were collected by remotely operated vehicle (ROV: *Isis, Jason 2, Quest and Kiel 6000*) in the period 2009-2013 from seven vent sites with different types of sampling devices as summarized in SI Table 1. Some devices have been described and previously evaluated for suitability for DOC sampling7. For the sampling at the Endeavour and Axial vent sites during Atlantis cruise AT18-08 with the Jason ROV, our primary sampling device was the HFPS31. This sampler pulls hydrothermal fluids through a titanium intake nozzle, then through a Teflon-lined hose and titanium tubing connected to collapsible-bag samplers (made of 2-mil Tedlar or 4-mil Teflon, PMC Bag) or piston samplers made of titanium or PVC. Bag sampling was evaluated for DOC sampling by Lang et al. (2006) and found to have undetectable levels of DOC contamination when exposed to de-ionized water for 8 hours. For this study, we evaluated the piston-type sampler blanks by cleaning and assembling the samplers and then filling them with a 2% HCl solution and allowing to stand for 6 hours, then processing the samples and analysing for DOC. This acid treatment procedure should leach more DOC than a typical sample with pH in the range of 3.5 to 7. The sampler blanks were elevated on average 10.2 µmol/kg (n=4) above the 2% HCl starting solution. For sampling at E2 during James Cook cruise JC080 with the Isis ROV, Titanium major samplers were used (NOCS, UK), as described previously by Lang et al. (2006). The process blank for shipboard ultrapure water was found to be <3 µM. For sampling at the Von Damm and Beebe vent fields during James Cook cruise JC082 with the Isis ROV, we used gas tight samplers (Ifremer, France). We tested the procedural blank by filling the samplers with IAPSO reference surface water, which had similar concentrations before and after contact with the sampler material (75µM vs 67-69µM, n=3). The KIPS sampler system, which was used at the Menez Gwen and Logatchev vent sites using the Kiel 6000 and Quest ROVs is made from perfluoralkoxy (PFA), polyetheretherketone, polytetrafluorethylene and high-purity titanium. This sampler was not tested for procedural blank, but successfully sampled high purity vent fluid from the Menez Gwen vent site with bulk DOC < 7µM.

*SPE-DOC extraction*Samples were filtered (ashed glass fibre filters, Whatman, 0.7 µm) and acidified with ultrapure HCl to pH 2. An aliquot of the sample (25-580 ml) was passed through pre-cleaned (MeOH and pH 2 water) PPL32,33 (styrene-divinyl benzene polymer) solid phase extraction cartridges (Varian) at <10 ml min-1. The cartridges were thoroughly rinsed of salts with pH 2 deionized water, dried with argon and the SPE-DOC was extracted with HPLC-grade methanol (0.6 or 6 ml for 0.1 or 1 g cartridges; Sigma-Aldrich). An aliquot of methanol (0.1-1.0 ml) was dried overnight at 50 °C and the SPE-DOC re-dissolved with 10 ml acidified (pH 2) deionized water.

*Analysis of DOC concentrations*All DOC concentrations (bulk DOC and SPE-DOC from the environment and experiments) were analysed using a Shimadzu TOC-V high temperature catalytic combustion instrument, and deep-water reference material (Hansell, University of Miami) was measured at least every 10 samples to monitor instrument performance. For some high-temperature fluid samples, which often had lower SPE-DOC concentrations and usually lower extraction volumes, SPE-DOC was analysed in a more concentrated 1 ml sample34.

*Extraction efficiency tests*Extraction efficiency (SPE-DOC/bulk DOC) averaged 55.5±8.8% (mean ± standard deviation) in the six samples taken farthest from hydrothermal vent influence, with a highest value of 66%. The extraction efficiency decreased dramatically in samples with greater influence from hydrothermal fluids due to the increase in non-extractable DOC. SPE-DOC concentrations are reported after subtraction of procedural blank values. Blank contamination may come from the SPE columns despite vigorous cleaning or from non-volatile contaminants in the elution methanol. The blank value averaged 9.6±6.6% of the sample values, and the error associated with subtraction of the blank is reflected in the error bars of Figure 2 in the main manuscript, along with instrumental precision. Samples with SPE-DOC below a detection limit of 2x blank standard deviation are shown as zero in Figure 2 in the main manuscript.

In order to test the extraction efficiency of some low-carbon number compounds, solutions of organic acids (formate & acetate), amino acids (glutamic & aspartic) and methanol were prepared to 500 µM C (similar to concentrations found at the Von Damm vent field) and were analysed for bulk DOC and SPE-DOC concentrations after extraction of 10 ml solution. Results of this test are presented in the supplementary figures and are discussed in the main manuscript.

*Laboratory hydrothermal degradation experiment*Hydrothermal degradation of marine dissolved organic matter (extracted dissolved organic matter from North Equatorial Pacific Intermediate Water32 in artificial seawater35) was conducted in a modified version of a Dickson-type hydrothermal apparatus. A flexible gold bag (Vtotal ≈ 100 ml) with a titanium closure and access tube was encased in a stainless steel pressure vessel filled with distilled water, which was used to apply adjustable isostatic pressure on the reaction cell. Simultaneously, the vessel was heated to designated temperature levels by an electric ceramic heater arrangement (Parr Instruments Company). Fluid samples could be taken from the gold cell during reaction via the titanium access tube through a 2 µm titanium frit (Vici) and an attached custom-fitted valve while keeping pressure and temperature nearly constant. Accounting for estimated volume expansion at experimental conditions, 40-70 ml sample was transferred into the pre-conditioned gold bag, and after closure of the apparatus the remaining air headspace was removed through the valve by pressurisation. The sample access valve was then closed, the sample pressurised to 400 bar, and the vessel was finally taken to reaction temperature (300 or 380 °C). The temperature was monitored by thermocouple reaching into the vessel directly adjacent to the reaction cell. Consecutive samples were taken over the course of 4 days. Artificial seawater and dissolved organic matter extract were taken as blank and control samples. Samples (3 ml) were taken into sterile plastic syringes after removal of 1.5 ml dead volume from the titanium tubes. Each sample was weighed, acidified to pH 2, cooled down to room temperature, diluted ~10x and pre-concentrated with PPL sorbent as above.

Our initial experiment was at 300 °C, as an average value of the venting measured in the environment, and the second experiment was run at the highest temperature possible for our experimental set up, 380 °C. The longer timescale of the second experiment was designed to ensure maximum SPE-DOC removal was achieved. The rate of reaction was increased because of the increased concentration, meaning a greater absolute loss of SPE-DOC over the time of the experiment, which is useful for the measurement of changing concentration after dilution of the samples. However, if we assume that each component of SPE-DOC (of which there are thousands) is lost by a first order reaction mechanism, then the half-life (t1/2) of loss is independent of concentration, and is only dependant on the reaction rate coefficient, *k*, as t1/2 = ln(2)/*k*. 93% loss should be achieved over the same timescale regardless of the starting concentration, assuming first order kinetics. The discussion on whether DOM degradation is subject to first order kinetics is outside the scope of this manuscript, but it is probably a reasonable assumption as water derived protons and hydroxide ions, which are the other major participants in the reaction, are in large excess over the DOC components.

*Analysis of dissolved magnesium*Dissolved magnesium concentrations were measured from acidified samples by ICP-OES after dilution in 2% HCl. Dissolved Mg in samples from Endeavour and Axial were measured by ion chromatography. These values were then used to calculate the percentage of hydrothermal fluid in each sample based on the ambient magnesium concentration in seawater (~54 mM) and an assumed value of zero magnesium in pure hydrothermal fluids.

*Error analysis and statistical processing of results*

Data were extrapolated to a value of 1.6 ± 1.2 µM (93.5 ± 4.7% degradation) in pure vent fluid using the robust linear regression method in the statistical software R (robustbase::lmrob). This error associated with this value represents the 99% confidence interval of the standard error (SEx = SDx/√n). This result is generated from 86 independent measurements, 84 of which fall into a normal distribution around the line presented in Figure 2A of the manuscript. The two high values from the Beebe vents at around 30 µM are clear outliers from this normal distribution.

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