Methane hydrate-bearing seeps as a source of aged dissolved organic carbon to the oceans

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Supplementary Table S1. Sample Location Information

<table>
<thead>
<tr>
<th>Site</th>
<th>Dive-Bottle</th>
<th>Water Depth (m)</th>
<th>Date Collected (DD/MM/YY)</th>
<th>General Description of Area</th>
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<tbody>
<tr>
<td>Barkley Canyon</td>
<td>R676-3</td>
<td>859</td>
<td>08/08/02</td>
<td>Reference Site, Clams and carbonate debris</td>
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<tr>
<td>Barkley Canyon</td>
<td>R676-2</td>
<td>857</td>
<td>08/08/02</td>
<td>Bacterial mat, gas hydrate suspected</td>
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<tr>
<td>Barkley Canyon</td>
<td>R677-3</td>
<td>858</td>
<td>09/08/02</td>
<td>Sediment draped gas hydrate mound</td>
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<tr>
<td>Barkley Canyon</td>
<td>R678-1</td>
<td>868</td>
<td>11/08/02</td>
<td>Exposed Hydrate, thick bacterial mats</td>
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<tr>
<td>Barkley Canyon</td>
<td>R678-2</td>
<td>866</td>
<td>11/08/02</td>
<td>Sediment draped gas hydrate mound</td>
</tr>
<tr>
<td>Barkley Canyon</td>
<td>R678-3</td>
<td>859</td>
<td>10/08/02</td>
<td>Bacterial mat, yellow gas hydrate</td>
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<tr>
<td>Cucumber Ridge</td>
<td>R679-4</td>
<td>1309</td>
<td>12/08/02</td>
<td>Tube worms and clams</td>
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</tbody>
</table>

148° 20' 41" N, 126° 03' 35" W, approximately 3.8 km from the active seepage area
248° 18' 38" N, 126° 04' 00" W, navigation coordinates could not resolve intra-seep site positions
348° 42' 12" N, 126° 55' 00" W
Supplementary Figure S1. Location map of the northern Cascadia margin (NCM), offshore Vancouver Island Canada. Barkley Canyon and Cucumber Ridge are located within a region of the continental slope where widespread subsurface occurrences of gas hydrate (indicated by gray shading) have been seismically inferred. Barkley Canyon has massive fossil and thermogenic-sourced gas hydrate exposed on the seafloor. Cucumber Ridge is a carbonate mound underlain by a gas hydrate acoustic-bottom simulating reflector often associated with the boundary between gas hydrate and underlying free gas. Both sites contain viable chemosynthetic biological communities (Supplementary Fig. S2) frequently associated with hydrocarbon-rich fluid seepage.
Supplementary Figure S1. Location map of the northern Cascadia margin (NCM), offshore Vancouver Island Canada. Barkley Canyon and Cucumber Ridge are located within a region of the continental slope where widespread subsurface occurrences of gas hydrate (indicated by gray shading) have been seismically inferred. Barkley Canyon has massive fossil and thermogenic-sourced gas hydrate exposed on the seafloor. Cucumber Ridge is a carbonate mound underlain by a gas hydrate acoustic-bottom simulating reflector often associated with the boundary between gas hydrate and underlying free gas. Both sites contain viable chemosynthetic biological communities frequently associated with hydrocarbon-rich fluid seepage.

Supplementary Figure S2. Water sampling sites at Barkley Canyon (BC) and Cucumber Ridge (CR). A. R676-3 (859 m depth), BC Reference Site: Seafloor with scattered carbonate debris and clams. No evidence of bacterial mats or gas hydrate. The site is located approximately 3.8 km from the area with active seepage and seafloor gas hydrate outcroppings. B. R676-2 (857 m depth), BC: Large mound partially covered with bacterial mats. C. R677-3 (858 m depth), BC: Exposed yellow gas hydrate draped by thin sediment cover. Some clams and bacterial mats are associated with this feature. D. R678-1 (868 m depth), BC: Massive fragmented accumulations of exposed gas hydrate associated with thick bacterial mats. Clams absent. E. R678-2 (856 m depth), BC: Exposed yellow gas hydrate draped by thin sediment cover. Similar conditions as R677-3 (see above). F. R678-3 (859 m depth), BC: Hummocky mound with extensive bacterial mats and carbonate debris. Massive yellow/orange gas hydrate lies beneath the surface material (see inset). G. R679-4 (1309 m depth), CR: Gently sloping surface with occasional clusters of tube worms and scattered, mostly-empty clam shells.
Supplementary Methods

I. Determination of methane and SOM-derived DOC $\delta^{13}C$ values

Selecting appropriate $\delta^{13}C$ values for methane-derived DOC and SOM-derived DOC for Eq. 4 is challenging because DOC is composed of numerous compounds produced by multiple biogeochemical processes that may have different isotope fractionation factors. For example, DOC components from below the sulfate reduction zone are produced by fermentation of organic matter and autotrophic acetogenesis. Dissolved organic compounds produced during the fermentation of different saccharides displayed varied fractionation trends relative to the source material. Acetate was $^{13}C$-enriched by as much as 3‰ relative to the source saccharides, whereas lactate, ethanol and formate were slightly $^{13}C$-depleted. As a second example, bulk DOC from the deep biosphere of the northern Cascadia margin was, on average, 3.2‰ more $^{13}C$-enriched than SOM. However, acetate produced by autotrophic acetogenesis immediately below the sulfate reduction zone was ~10‰ more $^{13}C$-depleted than the bulk DOC. Selecting an appropriate fractionation factor for DOC produced from a particular source requires specific knowledge of what compounds comprise the bulk DOC pool, the biogeochemical processes that produce them and the fractionation factor for each process.

There is also considerable uncertainty in the fractionation factors associated with DOC production within the zone of active AOM. Without additional evidence, we presume the methane-derived DOC is produced by biogeochemical processes that directly or indirectly incorporate methane carbon into acetate, other dissolved inorganic compounds (e.g., lactate) or biomass that excretes DOC or is degraded after cellular death. Biomass of methane oxidizing archaea is often, but not always, more $^{13}C$-depleted than the source methane. The DOC excreted by living biomass or released during fermentation of dead biomass is likely to be affected by additional isotopic fractionation.

To circumvent uncertainties in the net isotopic fractionation that occur during DOC production from SOM and methane, we utilized the post-fractionation DOC concentration and isotope data from pore water measurements at Barkley Canyon (Fig. 2b) to obtain estimates for the carbon isotope values of SOM-derived DOC ($I_{SOM}$) and methane-derived DOC ($I_{CH_4}$).

We assume $I_{SOM}$ is the average $\delta^{13}C$ value of the DOC beneath the zone of active AOM (-28.0 ± 1.1‰). This zone is beneath the sulfate reduction zone and is an area where numerous processes including fermentation and acetogenic CO$_2$-reduction are likely to be active. Uniform methane $\delta^{13}C$ values (-49.1 ± 0.4‰) within this zone suggest that methane oxidation is not likely to contribute methane carbon to DOC. Furthermore, the curvature in the sulfate profile is consistent with upward fluid advection which minimizes the chance that methane-derived DOC produced in the AOM zone has been buried.
DOC within the SMT is assumed to be a mixture of SOM-derived DOC and methane-derived DOC. The carbon isotope composition of the methane-derived DOC, $I_{CH_4}$, is determined by rearranging and solving the following isotope mass balance equation:

$$C_{SMT}I_{SMT} = C_{CH_4}I_{CH_4} + C_{SOM}I_{SOM}$$  
(Supp Eq. 1)

where $C$ and $I$ are, respectively, the concentration and δ¹³C values for DOC at the SMT ($C_{SMT}$ and $I_{SMT}$), methane-derived DOC ($C_{CH_4}$ and $I_{CH_4}$) and SOM-derived DOC ($C_{SOM}$ and $I_{SOM}$). $C_{SOM}$ is assumed to be constant throughout the profile and equal to the average concentration of SOM-derived DOC from below the AOM zone (1.2 ± 0.2 mM). Using the measured DOC concentrations from within the SMT, 1.8 mM at 1.5 cm below seafloor (cmbsf) and 3.6 mM at 4.5 cmbsf, and the values provided above, the estimated values of $I_{CH_4}$ at the depth horizons within the primary AOM zone are -46.7‰ at 1.5 cmbsf and -40.4‰ at 4.5 cmbsf. The average value for these estimates is -43.6‰, which is slightly more ¹³C-enriched that the methane below the AOM zone (Fig. 1).

The average values for $I_{SOM}$ and $I_{CH_4}$ calculated above are utilized in Eq. 4 to calculate the fraction of deep seawater DOC derived from SOM ($f_{SOM}$) and methane ($f_{CH_4}$), as described in the text.

II. DOC$_{MD}$ flux calculation for the Barkley Canyon seep

For a steady-state system, the flux of methane-derived DOC (DOC$_{MD}$) from the seafloor must balance losses resulting from bottom-water currents with background DOC concentrations that flush the seep area. The flux of DOC$_{MD}$ required to maintain the observed DOC$_{MD}$ concentrations at Barkley Canyon is calculated as:

$$DOC_{MD} \text{ flux} = \frac{DOC \text{ Inventory} (moles)}{Flushing \text{ Time} (hr)}$$  
(Supp Eq. 2)

where DOC$_{MD}$ Inventory is the quantity of DOC$_{MD}$ present in the bottom water of the seep area and Flushing Time is the time required for bottom water currents to replace the volume of water overlying the seep.

The DOC$_{MD}$ Inventory (moles) is calculated as:

$$DOC_{MD} \text{ Inventory} = BW \text{ volume} (L) \times [DOC_{MD}](moles \, L^{-1})$$  
(Supp Eq. 3)

where BW volume is the bottom water volume overlying the 0.5 km² Barkley Canyon seep to a height of 2 m above the seafloor (1 x 10⁹ L), and [DOC$_{MD}$] is the concentration of the DOC$_{MD}$ within that volume. A 2 m water column height was selected because that is the volume from where the bottom water samples for this study were collected. The average DOC$_{MD}$ concentration in that region is 19 ± 9 µM (See
Table below). Thus, the estimated steady-state $DOC_{MD} \text{Inventory}$ in the 2 m of seawater overlying Barkley Canyon is $1.9 \pm 0.9 \times 10^4$ moles.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$^{2}DOC_{\text{meas}}$ (µM)</th>
<th>$f_{\text{CH}_4}$</th>
<th>$^{3}DOC_{MD}$ (µM)</th>
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<tbody>
<tr>
<td>R676-3</td>
<td>49</td>
<td>0.00</td>
<td>0</td>
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<tr>
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<tr>
<td></td>
<td><strong>Average</strong></td>
<td><strong>19 ± 9</strong></td>
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</tr>
</tbody>
</table>

1. Reference sample (collected outside the active seep) not included in average
2. Measured deep-seawater DOC
3. $[DOC_{MD}] = [DOC]_{\text{meas}} \times f_{\text{CH}_4}$

The *Flushing Time* is calculated as:

$$Flushing \text{ Time} = \frac{Distance \ across \ Seep (m)}{BW \ current \ velocity \ (m \ s^{-1})}, \quad \text{(Supp Eq. 4)}$$

where, in this case, the *Distance across Seep* is ~700 m and the *BW current velocity* is estimated to be $2 \pm 1$ cm s$^{-1}$. By this calculation, $9.8 \pm 4.9$ hrs are required to replace the bottom water overlying the Barkley Canyon seep. A similar approach was utilized to obtain the flushing time of deep seawater overlying mud volcanoes off the Costa Rican convergent margin$^{20}$. Estimates of bottom water current velocities from analysis of video tapes recorded during *ROPOS* dives when the Barkley Canyon samples were collected ranged from 2 to 25 cm s$^{-1}$. The current velocity of $2 \pm 1$ cm s$^{-1}$ selected for this calculation is the conservative end of that range and is comparable to Acoustic Doppler Current Profiler determined current velocities of 2.4 to 3.2 cm s$^{-1}$ at the Costa Rican mud volcanoes$^{20}$.

Using the calculated ‘$DOC_{MD} \text{Inventory}$’ of $1.9 \pm 0.9 \times 10^4$ moles and ‘Flushing Time’ of $9.8 \pm 4.9$ hrs, as calculated by Supp. Eqs. 2 and 3, respectively, a conservative estimate for the net annual ‘$DOC_{MD} \text{flux}$’ (Supp Eq. 1) from the Barkley Canyon seep is $1.7 \pm 1.2 \times 10^7$ mol yr$^{-1}$. 
References for Supplementary Information.