2008

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Received 21 March 2007; revised 31 January 2008; accepted 18 February 2008; published 3 July 2008.

[1] Sedimentary rocks contain the largest mass of organic carbon on Earth, yet these reservoirs are not well integrated into modern carbon budgets. Here we describe the release of dissolved organic matter (DOM) from OM-rich sedimentary rocks under simulated weathering conditions. Results from column experiments demonstrate slow, sustained release of DOM from ancient sedimentary rocks under simulated weathering conditions. ^{1}H-NMR analysis of shale-derived DOM reveals a highly aliphatic, carbohydrate-poor material distinct from other natural DOM pools. Shale-derived DOM is rapidly assimilated and biodegraded by aerobic heterotrophic bacteria. Consequently, no compositional signature of shale-derived DOM other than ^{14}C-depletion is likely to persist in rivers or other surface reservoirs. Combined, these efforts show that dissolution provides a mechanism for the conversion of refractory kerogen into labile biomass, linking rock weathering with sedimentary OM oxidation and the delivery of aged OM to rivers and ocean margins.

Citation: Schillawski, S., and S. Petsch (2008), Release of biodegradable dissolved organic matter from ancient sedimentary rocks, *Global Biogeochem. Cycles*, *22*, GB3002, doi:10.1029/2007GB002980.

1. Introduction

[2] The total inventory of organic carbon (OC) contained in sedimentary rocks ($\sim 10^8$ Pg C) exceeds the combined mass of OC in soils, biomass and nonliving marine OC pools by at least four orders of magnitude [*Berner*, 1987]. The immense size of this reservoir stands in contrast to the relatively small rate of cycling between sedimentary OC and surface carbon pools, estimated to be $\sim$0.1 Pg OC a^{-1} [*Hedges*, 1992; *Siegenthaler and Sarmiento*, 1993; *Houghton et al.*, 2001]; less than 0.0000001% of sedimentary rock OC is oxidized and restored to surficial inorganic carbon pools annually. By some estimates, a smaller flux (0.04–0.08 Pg OC a^{-1}) escapes oxidation during weathering to be reburied as ancient OC in modern sediments [*Blair et al.*, 2003; *Meybeck*, 1993]. Nonetheless, OC in sedimentary rocks constitutes a widespread electron donor that if accessible, may drive metabolic activity in many surface and subsurface environments.

[3] Approximately 95% of organic matter (OM) in sedimentary rocks is kerogen, defined as solvent-insoluble, nonhydrolyzable, macromolecular natural organic matter [*Durand and Monin*, 1980; *Hedges*, 1992]. Kerogen is commonly regarded as highly refractory material rendered inaccessible to biological or chemical attack through inherently recalcitrant composition, physical protection mechanisms and/or isolation in the subsurface away from more reactive Earth surface environments. However, oxidation of ancient sedimentary OM during weathering is a critical component of the global geochemical carbon cycle, restoring CO_2 and removing O_2 from the Earth's atmosphere, helping to maintain equable earth-surface environmental conditions over geologic time [*Berner*, 1987, 1989]. Although numerous feedbacks between atmospheric O_2 and rates of weathering through geologic time have been proposed [*Berner and Canfield*, 1989; *Holland*, 1984; *Chang and Berner*, 1998; *Lasaga and Ohmoto*, 2002; *Holland*, 2003; *Ohmoto*, 2003], no direct mechanism for the transformation of refractory kerogen into remineralized inorganic carbon has been demonstrated.

[4] A common feature of weathering profiles developed on OM-rich sedimentary rocks is the persistence of rock-derived OM in highly weathered regolith and overlying soil [*Petsch et al.*, 2000, 2001a, 2001b, 2003; *Wildman et al.*, 2004; *Blair et al.*, 2003; *Komada et al.*, 2004]. The lack of completely efficient OM oxidation within weathering profiles suggests that ancient, relict OM may be delivered from the weathering environment through erosion into downstream sediment reservoirs. A number of studies have documented the presence of ^{14}C-depleted OM in modern river and marine systems [*Blair et al.*, 2003, 2004; *Dickens et al.*, 2004; *Hedges et al.*, 1986; *Goñi et al.*, 1997, 2000, 2005; *Gordon and Goñi*, 2003, 2004; *Komada et al.*, 2004, 2005; *Leithold et al.*, 2005, 2006; *Kao and Liu*, 1996; *Leithold and Blair*, 2001; *Masiello and Druffel*, 2001; *Raymond and Bauer*, 2001a, 2001b, 2001c; *Raymond et al.*, 2004; *Mayorga et al.*, 2005; *Drenzek et al.*, 2007; *Mitra et al.*, 1999]; however, both the sources and the fate of this ^{14}C-depleted OM remain unclear.

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0886-6236/08/2007GB002980

[5] Although there is considerable debate, many estimates converge on a roughly equal export flux of dissolved OC (DOC) and particulate OC (POC) from land to the oceans by rivers, with a magnitude of ~0.2 to 0.5 Pg C yr^{-1} [*Cole et al.*, 2007; *Richey and Raupach*, 2004; *Richey*, 2004]. Of this, a portion of aged ^{14}C-depleted OC appears to be reburied in modern marine sediments without oxidation [*Blair et al.*, 2003, 2004; *Goñi et al.*, 1997, 2000, 2005]. The remainder of ancient OC lost from rocks, soils and sediments during continental weathering is largely unaccounted for. The work presented here addresses one component of aged OC recycling that has not yet been explored: the release and subsequent biodegradation of DOM from OM-rich sedimentary rocks. Here we report on a laboratory study that investigates: (1) if ancient black shales can release sustained quantities of DOM into aqueous solution, (2) if the composition of shale-derived DOM resembles other natural DOM pools, and (3) if this DOM is readily biodegraded by aerobic heterotrophs.

2. Methods

[6] Samples of unweathered New Albany Shale (7.6 % TOC) were obtained from archived core samples collected from a black shale weathering profile located near Clay City, Kentucky, USA. The New Albany Shale is a Late Devonian, OC-rich laminated mudrock. Previous study has shown that kerogen in unweathered New Albany Shale comprises a mixture of mainly aliphatic macromolecular materials that yields a homologous series of C_5-C_{30} alkane/alkene pairs upon pyrolysis, with lesser contributions from aromatic components [*Petsch et al.*, 2000, 2001a, 2001b]. Extractable OM liberated from unweathered New Albany Shale contains abundant aliphatic hydrocarbons (including *n*-alkanes, acyclic isoprenoids, steranes and hopanes) and aromatic hydrocarbons (including aryl isoprenoids and triaromatic steroids) [*Brown and Kenig*, 2004]. Column experiments were developed from these shale samples to quantify liberation of DOM from OM-rich shales. Columns consisted of 30 cm tall, 2.5 cm diameter glass columns filled with either New Albany Shale (hereafter NAS, 82 g shale, 6.23 g TOC), New Albany Shale that had been exhaustively solvent-extracted to remove soluble OM (SE-NAS, 82 g shale, 5.95 g TOC), or precombusted quartz sand. Shale and sand were crushed in a ball mill, dry-sieved, and (SE-NAS only) solvent-extracted in 9:1 dichloromethane:methanol. Extract yield upon solvent extraction was 0.046 $g_{extract}/g_{TOC}$. The 250–500 μm fraction from crushed NAS, SE-NAS and sand were used to fill the columns, so that each column (NAS, SENAS, and sand) contained a similar grain size. This was done to ensure rapid and similar flow in each column without channeling or excessive packing of particles while still providing sufficient surface exposure for dissolution. A common reservoir of deionized water was introduced into each column at controlled flow rate by peristaltic pump via Pt-cured silicone tubing. Flow rates were manipulated during the experiment between 5 and 40 mL h^{-1}. Water exited each column through Pt-cured silicone tubing and were collected into either precombusted 40 mL glass vials or precombusted 4L amber glass bottles. DOC concentrations in each column outflow were determined on a Shimadzu TOC-V carbon analyzer by catalyzed combustion followed by infrared spectrometric detection of resultant CO_2. DOC measured in water exiting the sand column was contributed from the Nannopure water source and from components of the experimental setup, and measured ~0.3 mg L^{-1} throughout much of the experiment. This DOC contribution from water source and experimental setup is considered ''background'' in subsequent discussion. Initially, outflow OC concentrations were measured both before and after filtration through 0.2 μm nitrocellulose fiber filters (Millipore) to evaluate contributions from POC. However, differences in concentration between paired samples were within analytical error indicating no release of POC from columns, and outflow samples obtained after 28 d were not filtered. The concentration of dissolved organic carbon (DOC) in column outflow was monitored over the course of 338 d.

[7] Between days 128 and 166, outflow from each column was collected into capped, precombusted 4 L amber glass jars to obtain sufficient shale-derived DOM for ^1H-NMR. DOM from each column outflow (NAS, SE-NAS, and sand) was recovered by solid-phase extraction using 10 g Bond Elut C18 SPE cartridges (Varian), following a method adapted from *Louchouarn et al.* [2000]. Briefly, outflow samples were acidified to pH 2 and drawn through SPE cartridges using a vacuum manifold. Hydrophobic DOM was sorbed onto the resin, which was rinsed with H_2O (acidified to pH 2 with HCl) then eluted with methanol. DOC concentrations were measured on acidified outflow samples before and after passage through SPE cartridges to estimate SPE extraction efficiency, which averaged 60% for both NAS and SE-NAS outflow samples. [DOC] was at background levels before and after solid-phase extraction of sand column outflow, indicating that the SPE method does not contribute DOC to these waters. SPE C18 extracts recovered by elution with methanol were evaporated to dryness by rotary evaporation under gentle vacuum. Outflow samples from each column were reconstituted in 500 μL D_2O. ^1H NMR spectra of each extract were obtained using a Bruker Avance600 spectrometer operating at 600 MHz, carried out with a 1.5 s recycle delay time and solvent suppression (watergate suppression), and processed with 4 Hz line broadening. A total of 840 scans were averaged for NAS outflow, 713 scans for SE-NAS outflow, and 2500 scans for the sand control column outflow.

[8] Material collected from column experiments on days 167–168 was used in assays of aerobic biodegradation of DOM, modified from protocols developed for studies of drinking water quality. Biodegradation by aerobic heterotrophic prokaryotes was assessed by measuring [DOC] in sterilized column outflow samples during incubation using bacterial inoculum from a local water supply (Quabbin Reservoir intake; [DOC] of Quabbin inoculum below detection limit). Briefly, four replicate volumes (250 mL) of outflow from each column were transferred to precombusted 500 mL amber glass bottles, sterilized by autoclave and cooled to room temperature, after which was added 2.5 mL of Quabbin intake water that had been filtered through a 2.0 μm filter to remove large protozoans and to

isolate a cell size consistent with prokaryotes. Incubations were maintained for 2 weeks at 20°C in the dark (to limit autochthonous production). [DOC] during incubation was measured on each replicate at day 0 (immediately after introduction of inoculum), day 7 and day 14.

[9] Biological assimilation of shale-derived DOC into cellular biomass was assessed using a method adapted from *Van der Kooij* [1990]. Briefly, outflow collected from each column on day 168 was transferred to precombusted 40 mL glass vials, each of which contained 0.1 mL of mineral salts solution (0.7% K_2HPO_4, 0.7% KH_2PO_4, 0.1% $(NH_4)_2SO_4$, 0.01% NaCl, 0.005% $MgCl_2$ and 0.001% $FeSO_4$). After addition of mineral salts, vials were capped, sterilized by autoclave (20 min at 120°C), and cooled to room temperature. Stock cultures of the aerobic heterotrophic bacteria *Pseudomonas fluorescens* P17 and *Spirillum* sp. strain NOX, were obtained from incubations adapted to low-nutrient growth maintained by K. Nüsslein and T. Coneely, Dept. of Microbiology, University of Massachusetts Amherst. Approximately 10^2 cells of each strain were added to each vial of column outflow. Incubations were grown for 9 d at 15°C to achieve stationary-phase cell concentrations. Counts of colony forming units (CFUs) were by plating serially diluted incubations on R_2A agar, incubating for 2 d at 25°C, and counting the number of visually distinct CFUs of P17 and NOX. Average CFU counts were converted into estimates of assimilated carbon mass, following the relationships established by *Van der Kooij* [1990], where 1 μg C (as acetate equivalent) can support 4.1×10^6 CFU of strain P17 and 1.2×10^7 CFU of NOX. One vial from each outflow sample (NAS, SE-NAS and sand) was not inoculated and served as a control to measure background [DOC], and further positive controls were achieved by confirming growth of both strains on acetate-amended mineral salts media.

3. Results

[10] [DOC] in outflow from the NAS and SE-NAS columns were similar and consistently greater than [DOC] in outflow exiting the control sand column (Figure 1). Outflow [DOC] responded to manipulations in flow rate of the water passing through the two shale-filled columns, with lower flow rates generating higher [DOC] (Figure 1a); [DOC] in waters exiting a control column packed with precombusted sand did not respond to flow rate changes. In spite of this response to flow rate during the first 150 d of experiment, the decline in [DOC] obtained at flow rates of 5 mL h^{-1} from days 42–80 to 109–129 to 169–338 indicates that nearly six months were required to achieve near constant [DOC] in this experiment.

[11] Between days 128 and 166, outflow waters were collected for NMR analysis and for biodegradation/bioassimilation experiments during which time [DOC] was not monitored. The final 170 d of column flow revealed a steady DOC concentration of 0.83 ± 0.18 mg L^{-1} (mean of all analyzed samples $\pm 1\sigma$) in water exiting the NAS column and 0.77 ± 0.13 mg L^{-1} exiting the SE-NAS column compared with 0.32 ± 0.10 mg L^{-1} exiting the control column (Figure 1b). Student's t-test analysis indicated that the means of the [DOC] exiting the NAS and SE-NAS between days 169–338 were slightly different ($p = 0.041$), while the means of both shale column [DOC] were significantly different from the sand control column ($p < 10^{-12}$ for either comparison). DOC exiting the sand control column was considered to be background DOC introduced by experimental set up, and was substracted from outflow [DOC] from each shale column. When substracted for contributions from this background, [DOC] exiting the NAS column at steady state (day 167–338, 22 samples) was 0.51 ± 0.28 mg L^{-1}, while the average background-subtracted [DOC] exiting the SE-NAS column at steady state was 0.45 ± 0.23 mg_{DOC} L^{-1}. When integrated over the 338-d course of these experiments, these

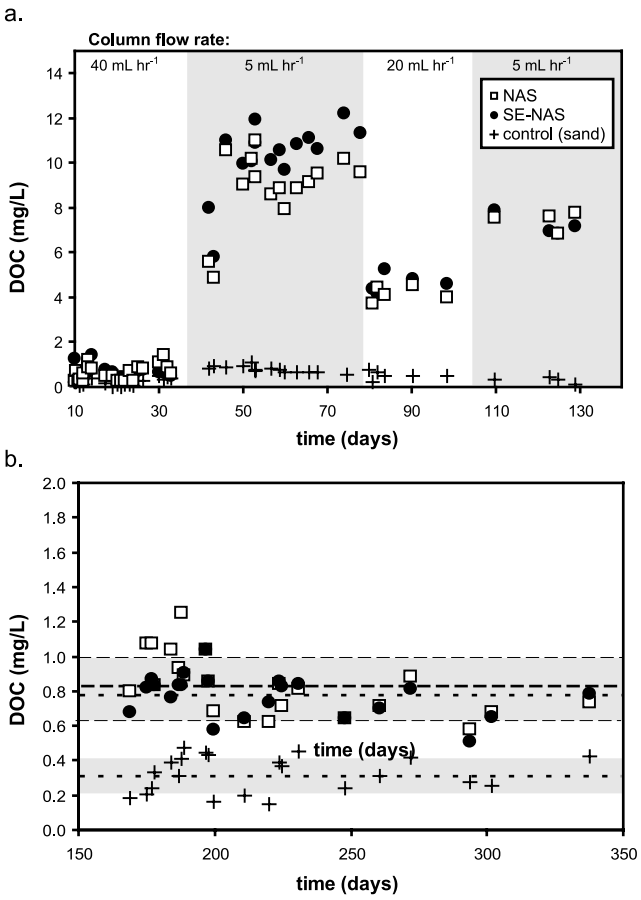

Figure 1. (a) DOC concentration in outflow from NAS, SE-NAS, and sand columns during the first 130 d of experiment, demonstrating response of [DOC] to changes in flow rate of water passing through each column. (b) DOC concentration in outflow from NAS, SE-NAS, and sand columns during the final 200 d of experiment at 5 mL h^{-1}, demonstrating trend toward steady [DOC] exiting each column. Mean [DOC] is shown for the NAS column (wide dashed line), SE-NAS (fine dashed line), and sand control (lower dashed line). Gray horizontal bars indicate range of ± one standard deviation around these means (NAS and SE-NAS ranges overlap).

Table 1. DOC Concentrations (mg L^{-1}) During 14-d Biodegradation Experiment

	NAS Outflow	SE-NAS Outflow	Sand Outflow	NAS–Sand[a]	SE-NAS–Sand[a]
Day 0	0.88 ± 0.01	0.97 ± 0.03	0.29 ± 0.03	0.59 ± 0.04	0.68 ± 0.06
Day 7	0.75 ± 0.02	0.81 ± 0.003	0.30 ± 0.02	0.46 ± 0.03	0.52 ± 0.02
Day 14	0.46 ± 0.01	0.45 ± 0.01	0.34 ± 0.01	0.12 ± 0.02	0.11 ± 0.02
Average [DOC] from sand outflow			0.31 ± 0.03		

[a][DOC] from NAS or SE-NAS outflow biodegradation experiments minus mean [DOC] from sand column measured during biodegradation.

steady state concentrations represent loss of 20.6 mg TOC from the NAS column and 18.2 mg from the SENAS column, or approximately 0.33% to 0.36% loss of TOC from each column, respectively.

[12] Outflow waters collected on days 167–168 used in biodegradation incubations had [DOC] of 0.88 ± 0.01 mg L^{-1} (NAS), 0.97 ± 0.03 mg L^{-1} (SE-NAS) and 0.27 ± 0.03 mg L^{-1} (sand). [DOC] in each incubation after addition of Quabbin Reservoir inoculum were within analytical error of these outflow concentrations, indicating that inoculation did not contribute additional DOC. Over 14 d, [DOC] decreased to 0.46 ± 0.02 mg L^{-1} in NAS outflow waters and to 0.45 ± 0.01 mg L^{-1} in SE-NAS outflow waters, while [DOC] from sand column outflow remained nearly constant (Table 1). These results indicate 80% loss of DOC from NAS outflow and 84% loss of DOC from SE-NAS outflow over 14 d, when contributions from sand column were subtracted as background, nonbioavailable DOC.

[13] While biodegradation assays address remineralization of DOM in a representative heterotrophic bacterial community, these do not address how much new biomass can be supported by DOM. In contrast, assimilable DOM assays indicate the size of a microbial population that can be supported by DOM, i.e., growth of a microbial population. In drinking water quality assessments, waters with assimilable organic carbon concentrations less than 10–20 μg L^{-1} are regarded as biologically stable (i.e., unable to support biomass reproduction) [*Van der Kooij*, 1990; *LeChevallier et al.*, 1993]. Outflow from the control column is at this threshold (12.7 ± 7.8 μg AOC L^{-1}), while both shale columns yield water that is well above this (Table 2). The NAS column exhibited 508 ± 97 μg AOC L^{-1}, while the SE-NAS column exhibited 581 ± 303 μg AOC L^{-1}. The AOC protocol is based on an empirical relationship between growth of CFUs and biological assimilation of acetate. Because the number of CFUs that can be supported per μg of shale-derived DOC is unknown, these values for AOC serve most as a useful comparative guide among samples. Nonetheless, these results indicate that DOM released from OM-rich shales in this experiment would be able to support a population of aerobic heterotrophic bacteria.

[14] ^{1}H NMR spectra of DOM isolated from outflow from the NAS and SE-NAS columns by C18 solid phase extraction (Figure 2) reveal a composition dominated by protons bound to various aliphatic carbons with resonances between ~1–3 ppm, especially methylene protons ($-CH_2-$, 1.2–1.4 ppm) with lesser contributions from methyl ($-CH_3$, ~0.9 ppm) and methine (–CH<, 1.6–1.8 ppm) protons. Protons from methyl groups adjacent to aromatic and/or olefinic carbons are also apparent (2–3 ppm). Contributions from protons associated with ether, ester, hydoxyl and amino carbons are present in limited amounts. Minor differences are observed between DOM isolated from the SE-NAS (Figure 2b) and NAS (Figure 2c) columns, with SE-NAS DOM exhibiting lower contributions from carbonyl, ester, aromatic and/or olefinnic carbons. ^{1}H NMR analysis of C18 SPE extract of outflow from the sand column revealed only a baseline signal of resonances from CH_3OH and HOD (Figure 2a), effectively demonstrating that the DOC characterized by NMR in this study derives from OM in black shales and not from carbon-bearing components of the experimental set-up.

4. Discussion

4.1. Implications for Global DOM Release From Sedimentary Rocks

[15] Both solvent-extracted and untreated NAS yield sustained DOC in these column experiments. While solvent extraction removes lower molecular weight components, it is not immediately clear how this transfers to solubility of sedimentary OM in aqueous media. It appears that kerogen too can be solubilized in water. One implication of the similar release of DOC from both columns is that it is kerogen, not solvent-soluble bitumen, that provides most shale-derived DOC.

[16] The background-subtracted [DOC] recovered from the shale columns at steady state (after experiment day 167)

Table 2. Colony Forming Units and Assimilable OC Within Shale-Derived DOM

	P. fluorescens P17 10^6 CFU/mL	*Spirillum* NOX 10^6 CFU/mL	Total AOC [a]μg/L	Shale-Derived AOC μg/L
NAS outflow	1.61 ± 0.31	1.37 ± 0.74	508 ± 97	495 ± 97
SE-NAS outflow	1.78 ± 1.23	1.76 ± 0.41	581 ± 303	568 ± 303
Sand outflow	0.04 ± 0.03	0.35 ± 0.12	12.7 ± 7.8	0.0

[a]Total AOC (assimilable organic carbon) is calculated from the method of Van der Kooij [1992], in which 1 μg of acetate-C supports 4.2×10^6 CFU of P17 and 1.2×10^7 CFU of NOX.

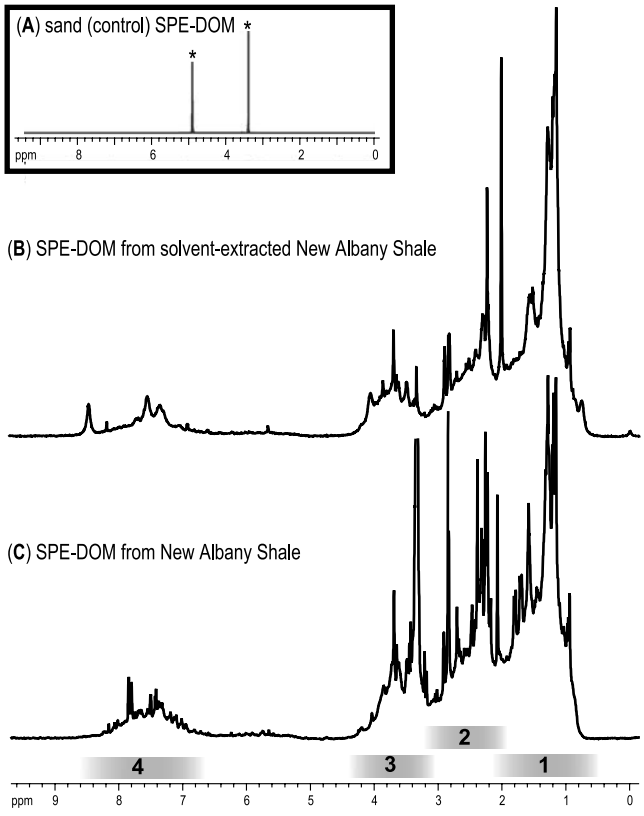

Figure 2. ^{1}H-NMR spectra obtained on dissolved material recovered by solid phase extraction. Bands of resonances at bottom correspond to: (1) aliphatic H (0–2 ppm); (2) -CH_3 and –CH_2- on aromatic carbon (1.8–3 ppm); (3) ester, ether, amino, and hydroxyl H (3–4.5 ppm); and (4) aromatic H (6.5–9 ppm). (a) Spectrum obtained from 2500 scans of solid-phase extracted material from sand (control) column outflow between experiment days 128 and 166. Note lack of signal from sand column outflow compared with NAS and SE-NAS outflow, indicating lack of contribution of DOM due to experimental set-up. Asterisk indicates resonances from HOD (~4.8 ppm) and CH_3OH (~3.4 ppm). (b) Spectrum obtained from 840 scans of solid-phase extracted DOM isolated from SE-NAS column outflow between experiment days 128 and 166; (c) Spectrum obtained from 713 scans of solid-phase extracted DOM isolated from NAS column outflow between experiment days 128 and 166.

can be used to estimate the flux of DOC and rates of kerogen dissolution in these column experiments. At steady state, the NAS shale column would produce 22.3 mg_{DOC} a^{-1} and the SE-NAS column would produce 19.7 mg_{DOC} a^{-1}. If this simple rate is applied to the entire columns, these fluxes suggest 279 years to remove all OC from the NAS column and 302 years for the SE-NAS column. As each column was loosely filled with 25 cm of crushed shale, the results also imply a crude estimate of linear propagation of column "weathering fronts" at between 0.83–0.90 mm a^{-1}. Field and modeling studies show that in the absence of significant erosion rates, weathering fronts showing TOC loss penetrate into black shales at rates between 10–100 cm ka^{-1} [*Petsch et al.*, 2000; *Bolton et al.*, 2006], suggesting that these columns provide a reasonable analog model for shale weathering.

[17] There are several ways in which DOC yields from column experiments may be extended to larger-scale systems. In one approach, annual DOC fluxes can be normalized to the TOC mass in each column, which suggest 3.6 mg_{DOC} g_{TOC}^{-1} a^{-1} from the NAS column and 3.3 mg_{DOC} g_{TOC}^{-1} a^{-1} from the SE-NAS column, or rates of 0.0033–0.0036 a^{-1}. This approach does not reflect processes that may inhibit dissolution in real weathering profiles such as saturation effects and limited water flow. It is likely that only a small fraction of the OM in each column is exposed to water that is undersaturated with shale-derived DOC, and thus normalizing to total column TOC content is inaccurate. This rate does however conform to observations of rapid, recent OM leaching of small hand specimens of OM-rich rock found in spoil piles of quarries [*Lo and Cardott*, 1995] and roadcuts [*Littke et al.*, 1991; *Fischer and Gaupp*, 2005; S. T. Petsch, personal communication, 2003]. If the length of the column exceeds the length-scale of DOC-undersaturation during column flow (which is confirmed by the response of [DOC] to flow rate), then perhaps the simplest approach to a rate may also be the most applicable to natural systems. The cross-sectional column area of these experiments is 4.9×10^{-4} m^2, which can be used to generate a rate of DOC release based purely on cross-sectional area: 45.5 g m^{-2} a^{-1} (NAS column) and 40.2 g m^{-2} a^{-1} (SE-NAS column).

[18] This rate can be used to estimate DOC yields from continental surfaces. When extrapolated to the global distribution of land area underlain by OM-rich sedimentary rocks [*Di-Giovanni et al.*, 2002], the rate of DOC yield obtained from the columns extrapolates to global generation of 1.3–1.5 Pg_{DOC} a^{-1} from sedimentary rocks. Although greater than the estimated flux of 0.1 Pg C a^{-1} associated with weathering/oxidation of ancient sedimentary OM [*Hedges*, 1992; *Siegenthaler and Sarmiento*, 1993; *Houghton et al.*, 2001], this experiment shows that simple dissolution may provide the initial step in liberation of ancient OM from sedimentary rocks during weathering.

4.2. Implications for DOM Character in Natural Waters

[19] The great abundance of aliphatic resonances and deficit in carbohydrate-like material observed in ^{1}H-NMR spectra of shale-derived DOM generated in this study does not resemble the composition of DOM recovered from marine and freshwater settings [*Aluwihare et al.*, 1997; *Repeta et al.*, 2002; *Brown et al.*, 2004; *Kaiser et al.*, 2003; *Kim et al.*, 2003; *Mash et al.*, 2004; *Schwede-Thomas et al.*, 2005], which generally exhibits a more "carbohydrate" -like, less aliphatic-rich composition. However, this interpretation is complicated by the multiple DOM isolation methods and spectroscopic techniques in studies of natural DOM. Intriguingly, DOM recovered from the shale columns does resemble aliphatic-rich, carbohydrate/aromatic-poor DOM recovered from oil refinery wastewaters [*Li et al.*, 2005], but as this study employed XAD resin and ^{13}C NMR, direct comparison is not possible. It is unclear whether C18 SPE

preferentially recovers a hydrophobic, aliphatic-rich component of DOM at the expense of polar biochemical structures such as aromatics, amino acids and carbohydrates. Using ^{13}C NMR, *Kaiser et al.* [2003] suggested that SPE of DOM from the Tagliamento River, Italy, isolated a fraction of DOM that is enriched in aliphatic material (average aliphatic/aromatic ratio = 4.3) compared with material recovered by ultrafiltration (average aliphatic/aromatic ratio = 2.2). *Schwede-Thomas et al.* [2005] compared DOM from the Suwannee River (GA), McDonald's Branch (NJ) and Pony Lake (Antarctica) extracted by XAD and SPE resins and by ultrafiltration, and characterized using ^{13}C NMR, UV adsorption and fluorescence spectroscopy, and observed similar apparent enrichment in aliphatic components in SPE-DOM ^{13}C-NMR spectra compared with other isolation methods. Nonetheless, comparison of the ^{1}H NMR spectra of SPE-isolated shale-derived DOM presented here with ^{1}H NMR spectra of ultra-filtered, lyophilized DOM from seawater, pond, river and estuary DOM [*Repeta et al.*, 2002] and ^{1}H NMR spectra of SPE-DOM from the Tagliamento River [*Kaiser et al.*, 2003] support the conclusion the shale-derived DOM is distinct from other aquatic DOM. The analyses presented here reveal a composition of C18 SPE shale-derived DOM that is similar to the highly aliphatic, oxygen-poor kerogens found in Late Devonian black shales [*Petsch et al.*, 2001a, 2001b].

[20] The results presented here indicate that OM-rich sedimentary rocks may provide a slow but steady source of DOM to natural waters wherever particles and surfaces of shale are exposed. In this regard, kerogen dissolution during weathering is unlikely to provide a point-source of aged DOM in river systems. Physical weathering of sedimentary rocks at an outcrop may deliver particulates to rivers that contain shale OM. Given the slow rate of dissolution, kerogen-bearing particulates and sediments may generate shale-derived DOM during long expanses of transport, and also during storage within floodplain and coastal sediments. DOM inputs from other, more modern sources such as vegetation, young soils and autochthonous production will dilute this contribution, masking simple interpretation using bulk DOC $\Delta^{14}C$ ratios.

[21] These results also indicate that shale-derived DOM is rapidly biodegraded and can support both microbial heterotrophy and population growth. Indeed, the extent of growth and biodegradation was surprising given the kerogen is nominally a refractory, macromolecular substance of limited bioavailability. In contrast with estuarine/marine DOM biodegradation experiments that extend over months [i.e., *Raymond and Bauer*, 2001b], here over 70% of shale-derived DOM was biodegraded within 14 d. These results, based on the portion of DOM generated in these column experiments, suggest that there is nothing inherently resistant about the composition of shale-derived DOM that may promote preservation of this pool within the modern carbon cycle. Rather, lack of degradation is likely associated with protection afforded to material trapped within the rock matrix, limiting exposure to dissolution and degradation conditions. When liberated from the rock matrix by dissolution, this material is rapidly biodegraded.

[22] The rapid biodegradation of shale-derived DOM furthermore suggests that a distinct composition of DOM is not likely to be observed in natural river systems dominated by shale sources, except as isotopic signatures such as ^{13}C and especially ^{14}C. These results shows that shale-derived DOM can be rapidly incorporated into the microbial foodweb of rivers, estuaries or marine systems, and suggest that it can be readily transformed through biological processing into more carbohydrate- and protein-like material. The highly aliphatic composition observed in ^{1}H NMR analysis is thus not likely to be preserved except in systems where microbial heterotrophy is not active. The more polar (ester-, ether-, and amino-rich) composition of DOM pools observed in many settings are thus not inconsistent with contributions from highly aged rock OM sources. Instead, shale-derived DOM will quickly be transformed in rivers, estuaries, and marine systems to resemble other DOM pools derived largely from microbial heterotrophy. Thus the contribution of rock weathering to the organic carbon pools of rivers and the coastal ocean may be easily overlooked in studies that examine the composition of DOM. Furthermore, $\delta^{13}C$ signatures of kerogen largely overlap with those of modern C3 vegetation, limiting the use of this isotopic tracer, while differences in $\Delta^{14}C$ signatures between ancient soils and ancient rocks are easily obscured when masked by the overwhelming contributions from modern allochthonous and autochthonous OM inputs.

[23] Dissolution provides a simple mechanism that converts solid-phase kerogen into labile biomass. Direct evidence for oxidation of kerogen by molecular O_2 to inorganic carbon is currently lacking, although numerous lines of evidence indicate that overall conversion of sedimentary organic carbon to inorganic carbon (CO_2 + DIC) must occur. There is limited evidence suggesting that microorganisms can incorporate ^{14}C-depleted sedimentary OM into cellular biomass [*Wengel et al.*, 2006; *Chabbi et al.*, 2006; *Wakeham et al.*, 2006; *Slater et al.*, 2006; *Krumholz et al.*, 2002; *Coolen et al.*, 2002; *Petsch et al.*, 2001a, 2001b], although whether this occurs as a solid phase reaction or proceeds via an aqueous intermediate has not been determined. Demonstrating that kerogen can rapidly dissolve under laboratory conditions provides the link between field-scale shale weathering and global-scale carbon cycling. New, shale-derived DOM may be generated within shale weathering profiles or liberated from shale fragments in regolith or soil, providing a source of rapidly degraded DOM to groundwaters. Shale-derived DOM may also be generated within aquatic systems that receive the products of shale erosion, as shale-derived particulate matter slowly releases DOM within river suspended load and bed load resuspension events. Shale-derived DOM may be generated far downstream from shale exposures and outcrops, and may be created far removed in time from when particulate matter was released by erosion.

5. Conclusions

[24] Column experiments show that OM-rich sedimentary rocks can generate a sustained supply of dissolved organic matter. The composition of C18 solid-phase extract of this material is revealed to be highly aliphatic and relatively poor in carbohydrate, protein and aromatic resonances

compared with many surface DOM pools. DOM released from shales is also found to be readily biodegraded and assimilated into microbial biomass. Dissolution may be the rate limiting step controlling the conversion of sedimentary OM to inorganic carbon, which ultimately controls atmospheric O_2 consumption and CO_2 release.

[25] **Acknowledgments.** K. Nüsslein and T. Coneely are acknowledged for providing P17 and NOX strains used in DOC assimilation, and for assistance with biodegradation assays. Assistance with ^{1}H NMR was provided courtesy of C. Dickinson (UMass MSREC, retired). This manuscript was improved through the careful comments of M. Formolo, E. Gordon, and two anonymous reviewers. Support for this research is provided through the NSF Integrated Carbon Cycle Research program (EAR-0403960). S.S. was partially supported by the UMass Geosciences Elinor Fierman Memorial Fund.

References

Aluwihare, L. I., D. J. Repeta, and R. F. Chen (1997), A major biopolymeric component to dissolved organic carbon in surface sea water, *Nature*, *387*(6629), 166–169.

Berner, R. A. (1987), Models for carbon and sulfur cycles and atmospheric oxygen: Application to Paleozoic geologic history, *Am. J. Sci.*, *287*, 177–196.

Berner, R. A. (1989), Biogeochemical cycles of carbon and sulfur and their effect on atmospheric oxygen over Phanerozoic time, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *75*, 97–122.

Berner, R. A., and D. E. Canfield (1989), A new model for atmospheric oxygen over Phanerozoic time, *Am. J. Sci.*, *289*, 333–361.

Blair, N. E., E. L. Leithold, S. T. Ford, K. A. Peeler, J. C. Holmes, and D. W. Perkey (2003), The persistence of memory: The fate of ancient sedimentary organic carbon in a modern sedimentary system, *Geochim. Cosmochim. Acta*, *67*, 63–73.

Blair, N. E., E. L. Leithold, and R. C. Aller (2004), From bedrock to burial: The evolution of particulate organic carbon across coupled watershed-continental margin systems, *Mar. Chem.*, *92*(1–4), 141–156.

Bolton, E. W., R. A. Berner, and S. T. Petsch (2006), The weathering of sedimentary organic matter as a control on atmospheric O-2. Part II: Theoretical modeling, *Am. J. Sci.*, *306*(8), 575–615.

Brown, T. C., and F. Kenig (2004), Water column structure during deposition of Middle Devonian-Lower Mississippian black and green/gray shales of the Illionois and Michigan Basins: A biomarker approach, *Palaeogeogr. Palaeoclimatol. Palaeoecol.*, *215*, 59–85.

Brown, A., D. M. McKnight, Y. P. Chin, E. C. Roberts, and M. Uhle (2004), Chemical characterization of dissolved organic material in Pony Lake, a saline coastal pond in Antarctica, *Mar. Chem.*, *89*(1–4), 327–337.

Chabbi, A., C. Rumpel, P. M. Grootes, J. A. Gonzalez-Perez, R. D. Delaune, F. Gonzalez-Vila, B. Nixdorf, and R. F. Huttl (2006), Lignite degradation and mineralization in lignite-containing mine sediment as revealed by C-14 activity measurements and molecular analysis, *Org. Geochem.*, *37*(8), 957–976.

Chang, S., and R. A. Berner (1998), Humic substance formation via the oxidative weathering of coal, *Environ. Sci. Technol.*, *32*, 2883–2886.

Cole, J. J., et al. (2007), Plumbing the global carbon cycle: Integrating inland waters in the terrestrial carbon budget, *Ecosystems*, *10*, 172–185.

Coolen, M. J. L., H. Cypionka, A. M. Sass, H. Sass, and J. Overmann (2002), Ongoing modification of Mediterranean Pleistocene Sapropels mediated by prokaryotes, *Science*, *296*, 2407–2410.

Dickens, A. F., Y. Gelinas, C. A. Masiello, S. Wakeham, and J. I. Hedges (2004), Reburial of fossil organic carbon in marine sediments, *Nature*, *427*(6972), 336–339.

Di-Giovanni, C. C., J. R. Disnar, and J. J. Macaire (2002), Estimation of the annual yield of organic carbon released from carbonates and shales by chemical weathering, *Global Planet. Change*, *32*, 195–210.

Drenzek, N. J., D. B. Montluçon, M. B. Yunker, R. W. Macdonald, and T. I. Eglinton (2007), Constraints on the origin of sedimentary organic carbon in the Beaufort Sea from coupled molecular C-13 and C-14 measurements, *Mar. Chem.*, *103*(1–2), 146–162.

Durand, B., and J. C. Monin (1980), Elemental analysis of kerogens (C, H, O, N, S, Fe), in *Kerogen*, edited by B. Durand, pp. 113–142, Editions Technip, France.

Fischer, C., and R. Gaupp (2005), Change of black shale organic material surface area during oxidative weathering: Implications for rock-water surface evolution, *Geochim. Cosmochim. Acta*, *69*(5), 1213–1224.

Goñi, A. M., K. C. Ruttenberg, and T. I. Eglinton (1997), Sources and contribution of terrigenous organic carbon to surface sediments in the Gulf of Mexico, *Nature*, *389*, 275–278.

Goñi, M. A., M. B. Yunker, R. W. Macdonald, and T. I. Eglinton (2000), Distribution and sources of organic biomarkers in Arctic sediments from the Mackenzie River and Beaufort Shelf, *Mar. Chem.*, *71*, 23–51.

Goñi, M. A., M. B. Yunker, R. W. Macdonald, and T. I. Eglinton (2005), The supply and preservation of ancient and modem components of organic carbon in the Canadian Beaufort Shelf of the Arctic Ocean, *Mar. Chem.*, *93*(1), 53–73.

Gordon, E. S., and M. A. Goñi (2003), Sources and distribution of terrigenous organic matter delivered by the Atchafalaya River to sediments in the northern Gulf of Mexico, *Geochim. Cosmochim. Acta*, *67*(13), 2359–2375.

Gordon, E. S., and M. A. Goñi (2004), Controls on the distribution and accumulation of terrigenous organic matter in sediments from the Mississippi and Atchafalaya river margin, *Mar. Chem.*, *92*(1–4), 331–352.

Hedges, J. I. (1992), Global biogeochemical cycles: Progress and problems, *Mar. Chem.*, *39*, 67–93.

Hedges, J. I., W. A. Clark, P. D. Quay, J. E. Richey, A. H. Devol, and U. D. Santos (1986), Compositions and fluxes of particulate organic material in the Amazon River, *Limnol. Oceanogr.*, *31*(4), 717–738.

Holland, H. D. (1984), *The Chemical Evolution of the Atmosphere and Oceans*, Princeton Univ. Press, Princeton, N. J.

Holland, H. D. (2003), Discussion of the article by A. C. Lasaga and H. Ohmoto on "The oxygen geochemical cycle: Dynamics and stability," Geochim. Cosmochim. Acta 66, 361–381, 2002, *Geochim. Cosmochim. Acta*, *67*(4), 787–789.

Houghton, J. T., Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, and D. Xiaosu (2001), Climate change 2001: The scientific basis, in *Third Assessment Report to the Intergovernmental Panel on Climate Change (IPCC)*, p. 881, Cambridge Univ. Press, N.Y.

Kaiser, E., A. J. Simpson, K. J. Dria, B. Sulzberger, and P. G. Hatcher (2003), Solid-state and multi-dimensional solution-state NMR of solid phase extracted and ultrafiltered riverine dissolved organic matter, *Environ. Sci. Technol.*, *37*, 2929–2935.

Kao, S. J., and K. K. Liu (1996), Particulate organic carbon export from a subtropical mountainous river (Lanyang Hsi) in Taiwan, *Limnol. Oceanogr.*, *41*(8), 1749–1757.

Kim, S., A. J. Simpson, E. B. Kujawinski, M. A. Freitas, and P. G. Hatcher (2003), High resolution electrospray ionization mass spectrometry and 2D solution NMR for the analysis of DOM extracted by C18 solid phase disk, *Org. Geochem.*, *34*, 1325–1335.

Komada, T., E. R. M. Druffel, and S. E. Trumbore (2004), Oceanic export of relict carbon by small mountainous rivers, *Geophys. Res. Lett.*, *31*(7), LO7504, doi:10.1029/2004GL019512.

Komada, T., E. R. M. Druffel, and J. Hwang (2005), Sedimentary rocks as sources of ancient organic carbon to the ocean: An investigation through Delta C-14 and delta C-13 signatures of organic compound classes, *Global Biogeochem. Cycles*, *19*(2), GB2017, doi:10.1029/2004GB002347.

Krumholz, L. R., S. J. Harris, and J. M. Suflita (2002), Anaerobic microbial growth from components of Cretaceous shales, *Geomicro. J.*, *19*, 593–602.

Lasaga, A. C., and H. Ohmoto (2002), The oxygen geochemical cycle: Dynamics and stability, *Geochim. Cosmochim. Acta*, *66*, 361–381.

LeChevallier, M. W., N. E. Shaw, L. A. Kaplan, and T. L. Bott (1993), Development of a rapid assimilable organic-carbon method for water, *Appl. Environ. Micro.*, *59*(5), 1526–1531.

Leithold, E. L., and N. E. Blair (2001), Watershed control on the carbon loading of marine sedimentary particles, *Geochim. Cosmochim. Acta*, *65*(14), 2231–2240.

Leithold, E. L., D. W. Perkey, N. E. Blair, and T. N. Creamer (2005), Sedimentation and carbon burial on the northern California continental shelf: the signatures of land-use change, *Cont. Shelf Res.*, *25*(3), 349–371.

Leithold, E. L., N. E. Blair, and D. W. Perkey (2006), Geomorphologic controls on the age of particulate organic carbon from small mountainous and upland rivers, *Global Biogeochem. Cycles*, *20*(3), GB3022, doi:10.1029/2005GB002677.

Li, L. B., S. Yan, C. B. Han, and G. B. Shan (2005), Comprehensive characterization of oil refinery effluent-derived humic substances using various spectroscopic approaches, *Chemosphere*, *60*(4), 467–476.

Littke, R., U. Klussmann, B. Krooss, and D. Leythäuser (1991), Quantification of loss of calcite, pyrite and organic matter due to weathering of Toarcian black shales and effects on kerogen and bitumen characteristics, *Geochim. Cosmochim. Acta*, *55*, 3369–3378.

Lo, H. B., and B. J. Cardott (1995), Detection of natural weathering of Upper McAlester coal and Woodford Shale, Oklahoma, USA, *Org. Geochem.*, *22*, 73–83.

Louchouarn, P., S. Opsahl, and R. Benner (2000), Isolation and quantification of dissolved lignin from natural waters using solid-phase extraction and GC/MS, *Anal. Chem.*, *72*, 2780–2787.

Mash, H., P. K. Westerhoff, L. A. Baker, R. A. Nieman, and M. L. Nguyen (2004), Dissolved organic matter in Arizona reservoirs: Assessment of carbonaceous sources, *Org. Geochem.*, *35*(7), 831–843.

Masiello, C. A., and E. R. M. Druffel (2001), Carbon isotope geochemistry of the Santa Clara River, *Global Biogeochem. Cycles*, *15*(2), 407–416.

Mayorga, E., A. Aufdenkampe, C. A. Masiello, A. V. Krusche, J. Hedges, P. D. Quay, J. E. Richey, and T. A. Brown (2005), Young organic matter as a source of carbon dioxide outgassing from Amazonian rivers, *Nature*, *436*, 538–541.

Meybeck, M. (1993), C, N, P and S in rivers: From sources to global inputs, in *Interaction of C, N, P, and S, Biogeochemical Cycles and Global Change*, edited by R. Wollast, F. T. McKenzie, and L. Chou, pp. 163–193, Springer, New York.

Mitra, S., T. S. Bianchi, L. Guo, and P. H. Santschi (1999), Terrestrially derived dissolved organic matter in the Chesapeake Bay in the Middle Atlantic Bight, *Geochim. Cosmochim. Acta*, *64*, 3547–3557.

Ohmoto, H. (2003), Reply to comments by H.D. Holland on ''The oxygen geochemical cycle: Dynamics and stability,'' Geochim. Cosmochim. Acta 66, 361-381, 2002, *Geochim. Cosmochim. Acta*, *67*(4), 791–795.

Petsch, S. T., R. A. Berner, and T. I. Eglinton (2000), A field study of the chemical weathering of ancient sedimentary organic matter, *Org. Geochem.*, *31*, 475–487.

Petsch, S. T., T. I. Eglinton, and K. J. Edwards (2001a), ^{14}C-dead living biomass: Evidence for microbial assimilation of ancient organic matter during shale weathering, *Science*, *292*, 1127–1131.

Petsch, S. T., R. J. Smernik, T. I. Eglinton, and J. M. Oades (2001b), A solid state ^{13}C-NMR study of kerogen degradation during black shale weathering, *Geochim. Cosmochim. Acta*, *65*, 1867–1882.

Petsch, S. T., K. J. Edwards, and T. I. Eglinton (2003), Abundance, distribution and δ^{13}C analysis of microbial phospholipid-derived fatty acids in a black shale weathering profiles, *Org. Geochem.*, *34*, 731–743.

Raymond, P. A., and J. E. Bauer (2001a), Riverine export of aged terrestrial organic matter to the North Atlantic Ocean, *Nature*, *409*, 497–500.

Raymond, P. A., and J. E. Bauer (2001b), Use of C-14 and C-13 natural abundances for evaluating riverine, estuarine, and coastal DOC and POC sources and cycling: A review and synthesis, *Org. Geochem.*, *32*(4), 469–485.

Raymond, P. A., and J. E. Bauer (2001c), DOC cycling in a temperate estuary: A mass balance approach using natural ^{14}C and ^{13}C isotopes, *Limnol. Oceanogr.*, *46*, 655–667.

Raymond, P. A., J. E. Bauer, N. F. Caraco, J. J. Cole, B. Longworth, and S. T. Petsch (2004), Controls on the variability of organic matter and dissolved inorganic carbon ages in northeast US rivers, *Mar. Chem.*, *92*(1–4), 353–366.

Repeta, D. J., T. M. Quan, L. I. Aluwihare, and A. Accardi (2002), Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters, *Geochim. Cosmochim. Acta*, *66*, 955–962.

Richey, J. E. (2004), Emission of CO_2 from riverine systems, in *Global Change and the Earth System: A Planet Under Pressure*, edited by W. Steffan, pp. 172–173, Springer, New York.

Richey, J. E., and M. R. Raupach (2004), Pathways of atmospheric CO_2 through fluvial systems, in *Toward CO_2 Stabilization: Issues, Strategies and Consequences, a SCOPE/GCP Rapid Assessment Project*, edited by C. Fields, pp. 329–340, Island Press, Washington, D.C.

Schwede-Thomas, S. B., Y. P. Chin, K. J. Dria, P. Hatcher, E. Kaiser, and B. Sulzberger (2005), Characterizing the properties of dissolved organic matter isolated by XAD and C-18 solid phase extraction and ultrafiltration, *Aquat. Sci.*, *67*(1), 61–71.

Siegenthaler, U., and J. L. Sarmiento (1993), Atmospheric carbon dioxide and the ocean, *Nature*, *365*, 119–125.

Slater, G. F., R. K. Nelson, B. M. Kile, and C. M. Reddy (2006), Intrinsic bacterial biodegradation of petroleum contamination demonstrated in situ using natural abundance, molecular-level C-14 analysis, *Org. Geochem.*, *37*(9), 981–989.

Van der Kooij, D. (1990), Assimilable organic carbon (AOC) in drinking water, in *Drinking Water Microbiology: Progress and Recent Developments*, edited by G. A. McFeters, pp. 57–87, Springer, New York.

Wakeham, S. G., A. P. McNichol, J. E. Kostka, and T. K. Pease (2006), Natural-abundance radiocarbon as a tracer of assimilation of petroleum carbon by bacteria in salt marsh sediments, *Geochim. Cosmochim. Acta*, *70*(7), 1761–1771.

Wengel, M., E. Kothe, C. M. Schmidt, K. Heide, and G. Gleixner (2006), Degradation of organic matter from black shales and charcoal by the wood-rotting fungus Schizophyllum commune and release of DOC and heavy metals in the aqueous phase, *Sci. Total Environ.*, *367*(1), 383–393.

Wildman, R. A., R. A. Berner, S. T. Petsch, E. W. Bolton, J. O. Eckert, U. Mok, and J. B. Evans (2004), The weathering of sedimentary organic matter as a control on atmospheric O_2. Part I: Analysis of a black shale, *Am. J. Sci.*, *304*, 234–249.

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