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Thomas S. Bianchi
*University of Florida*

Daniel C.O. Thornton
*Texas A&M University*

Shari A. Yvon-Lewis
*Texas A&M University*

Gary M. King
*Louisiana State University*

Timothy I. Eglinton
*ETH Zürich*

*See next page for additional authors*



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**Key Points:**
- Priming of organic matter exists in aquatic systems
- Ramifications of this work have major implications on greenhouse gas emissions
- First evidence for lab conditions of priming setting stage for more fieldwork

**Supporting Information:**
- Text S1, Table S1 caption, and Figure S1 caption
- Figure S1
- Table S1
- Table S2

**Correspondence to:**
T. S. Bianchi,
tbianchi@ufl.edu

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# Positive priming of terrestrially derived dissolved organic matter in a freshwater microcosm system

**Thomas S. Bianchi[1], Daniel C. O. Thornton[2], Shari A. Yvon-Lewis[2], Gary M. King[3], Timothy I. Eglinton[4], Michael R. Shields[1], Nicholas D. Ward[1], and Jason Curtis[1]**

[1]Department of Geological Sciences, University of Florida, Gainesville, Florida, USA, [2]Department of Oceanography, Texas A&M University, College Station, Texas, USA, [3]Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana, USA, [4]Geologisches Institut, ETH Zürich, Zürich, Switzerland

**Abstract** The role of priming processes in the remineralization of terrestrially derived dissolved organic carbon (TDOC) in aquatic systems has been overlooked. We provide evidence for TDOC priming using a lab-based microcosm experiment in which TDOC was primed by the addition of $^{13}$C-labeled algal dissolved organic carbon (ADOC) or a $^{13}$C-labeled disaccharide (trehalose). The rate of TDOC remineralization to carbon dioxide ($CO_2$) occurred 4.1 ± 0.9 and 1.5 ± 0.3 times more rapidly with the addition of trehalose and ADOC, respectively, relative to experiments with TDOC as the sole carbon source over the course of a 301 h incubation period. Results from these controlled experiments provide fundamental evidence for the occurrence of priming of TDOC by ADOC and a simple disaccharide. We suggest that priming effects on TDOC should be considered in carbon budgets for large-river deltas, estuaries, lakes, hydroelectric reservoirs, and continental shelves.

## 1. Introduction

Although inland waters comprise a small fraction of Earth's surface, they play a critical role in the global C cycle [*Cole et al*., 2007; *Battin et al*., 2008]. Global estimates of the flux of dissolved organic carbon (DOC) from rivers to the ocean range from about 250 to 360 Tg C $y^{-1}$ [*Bauer et al*., 2013]. Notably, only a small fraction of the roughly 2900 Tg C $yr^{-1}$ transported through inland waters, globally, ever reaches the ocean [*Le Quéré et al*., 2014]. Recent observations of immense $CO_2$ evasion from streams and rivers suggests that terrestrially derived DOC (TDOC) is not as recalcitrant as previously thought [*Cole et al*., 2007, and references therein]. For example, it was recently estimated that ~2100 Tg C $yr^{-1}$ of $CO_2$ is outgassed by inland waters globally [*Raymond et al*., 2013]. A key unanswered question is the following: how is this large flux of TDOC processed and "removed" from inland waters? One possible mechanism involves the "priming" of organic matter degradation, a process discovered by *Löhnis* [1926], who revealed that rates of soil humus mineralization were enhanced by the addition of fresh organic residues. While the importance of photodegradation and bacterial consumption of TDOC in freshwaters has been widely investigated, the role of priming processes has been essentially ignored [*Bianchi*, 2011].

The first use of the term "priming effect" was introduced by *Bingeman et al*. [1953]. The concept is typically applied to terrestrial ecosystems, whereas the potential for priming in aquatic systems has largely been overlooked until recently [*Guenet et al*., 2010; *Bianchi*, 2011; *Danger et al*., 2013; *Gontikaki et al*., 2013; *Bengtsson et al*., 2014; *Attermeyer et al*., 2015; *Catalán et al*., 2015]. Priming is formally defined by *Kuzyakov et al*. [2000] as "strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of soil." Such treatments typically involve additions of highly biologically labile substrates such as simple sugars and amino acids. There is little agreement between the relatively few studies that have quantified priming effects in aquatic systems. For example, positive priming effects have been observed in sediments [*van Nugteren et al*., 2009] and soils [*Guenet et al*., 2014], whereas no evidence for priming was found in lake waters [*Catalán et al*., 2015], and negative priming effects were observed in NE Atlantic slope sediments [*Gontikaki et al*., 2013]. *Poretsky et al*. [2010] observed shifts in microbial expression of organic carbon (OC)-transporter genes under variable inputs of algal and terrestrially derived OC, providing insight into the biological facilitation of priming processes, but they did not quantitatively address priming per se. To our knowledge, there have been no laboratory-based studies attempting to determine the linkage between the priming of TDOC and $CO_2$ production in aquatic ecosystems.

In this study, we provide new evidence for TDOC priming using a lab-based experiment. The objectives of this work were to determine (1) if $^{13}$C-labeled algal dissolved organic carbon (ADOC) enhanced the microbial consumption of TDOC, (2) whether differences were evident in the priming effects of ADOC versus a $^{13}$C-labeled simple disaccharide substrate (i.e., trehalose) on TDOC, (3) the effect of ADOC and trehalose on microbial enzymatic activity, and (4) how microbial $CO_2$ production responded to priming effects from both trehalose and ADOC additions. In this study, we used a single isolated bacterial culture, TDOC from a single conifer species, priming substrates that were either a simple disaccharide or ADOC (extracted from a single-diatom culture), all mixed respectively in a dilute growth medium in order to conduct a controlled experiment. This contrasts with the limited suite of other studies on aquatic priming that have included complex mixtures of organic carbon substrates and microbial communities [*van Nugteren et al*., 2009; *Guenet et al*., 2014; *Catalán et al*., 2015], complicating assessment of specific linkages between substrate, organism, and decomposition process.

## 2. Methods and Materials

### 2.1. Experimental Lab Design and Sample Collection

The experiment was conducted in laboratory microcosms under highly controlled conditions. Microcosms were set up in 2 L medium bottles (VWR Scientific), with four treatments and four replicates of each treatment. One replicate from each treatment was connected to a recirculating pump and a nondispersive infrared (NDIR) gas analyzer (LI-820 or LI-840 $CO_2$ Analyzer, LI-COR Biosciences) for continuous monitoring of $CO_2$ in the headspace of the microcosm. The remaining three microcosms for each treatment were sealed and were only opened when the bottles were sacrificed for sampling. All microcosms were sampled at $T_0$ (0 h) prior to sealing the bottles. The microcosms connected to NDIR gas analyzers were monitored until the end of the experiment. One replicate from the remaining three microcosms of each treatment was sacrificed at $T_1$ (21 h), $T_2$ (59 h), and $T_3$ (301 h). After removal of the initial $T_0$ samples, each bottle contained 1000 mL of medium with a headspace of 1270 ± 13 mL.

#### 2.1.1. Substrates and Treatments

The TDOC was collected from loblolly pine tree (*Pinus taeda*) needles that were obtained from Free-Air $CO_2$ Enrichment (FACE) experiment from the Duke Forest Site [*Nösberger et al*., 2006]. Pine needles were leached with Milli-Q water following the methods of *Hernes et al*. [2007]; the leachate is herein designated as "FACE." FACE samples were originally selected as these plant materials were grown under controlled $CO_2$ concentrations and isotopic compositions, which should have yielded biomass depleted in $^{13}$C relative to natural modern litter and algae. However, subsequent radiocarbon measurements (Eglinton, unpublished data) did not reveal distinctive isotopic characteristics of the FACE materials. The FACE material had a $^{13}C/^{12}C$ ratio of 0.01084 ± 0.0001 ($\delta^{13}C = -35.70 \pm 0.32‰$). The simple priming substrate consisted of a $^{13}$C-labeled disaccharide, trehalose, in which 99% of the total carbons were labeled with $^{13}$C (Sigma Aldrich). A diatom culture of *Phaeodactylum tricornutum* (CCMP 2561) was obtained from the National Center for Marine Algae and Microbiota. The diatom culture was grown in artificial seawater (see supporting information) in the presence of $^{13}$C-labeled $HCO_3^-$ and harvested in the early stationary phase by centrifugation before being washed once in ultrapure water to remove excess salts and dissolved nutrients. The resulting diatom concentrate was frozen, freeze-dried, and extracted to yield a "diatom" leachate, described here as ADOC, using the same method of *Hernes et al*. [2007]. The diatom leachate had a $^{13}C/^{12}C$ ratio of 0.7209 ± 0.0281 ($\delta^{13}C = 63124.3 \pm 1469.9‰$).

For our bacterial source, *Acinetobacter* sp. MR1 (an isolate most closely related to *Acinetobacter bouveti* [*Carr et al*., 2003]) was obtained from the lower Mississippi River using a bacterial exopolymer for enrichment; the isolate was identified by its 16S rRNA gene sequence using standard methods for genomic extraction, polymerase chain reaction (PCR) amplification and amplicons sequencing [e.g., *King and King*, 2014; 16S rRNA gene sequence accession number KP860050]. *Acinetobacter* sp. MR1 was sent to Texas A&M University where the culture was maintained on a basal medium as described by *Hardy and King* [2001].

The dissolved organic matter (DOM) extracts were used to make the following treatments, which each contained a background DOC level of 41 μM C from the bacterial inoculation and media salts: (1) 100% added carbon as FACE (68 μM C), (2) 72% added carbon as FACE (68 μM C) + 28% $^{13}$C ADOC (27 μM C), (3) 64% added carbon as FACE (68 μM C) + 36% $^{13}$C trehalose (38 μM C), and (4) medium control (no added

organic materials, see Table S1 in the supporting information). The medium control enabled us to determine whether the transferred bacteria were capable of growing without the addition of organic carbon (irrespective of carbon source). The DOM sources were 0.2 μm filtered prior to addition to the microcosms and added to make a total final concentration of 1.2 mg C $L^{-1}$, which is of the same order of as many aquatic ecosystems [*Spencer et al*., 2012]. The actual concentrations were much greater (up to 4.8 mg C $L^{-1}$) at the start of the experiment, mainly due to carry over from the lysed bacterial cells (see Figure S1 and explanation in the supporting information). However, this carry-over was accounted for with the medium control. Microcosms were maintained at a constant 26 to 28°C in a temperature-controlled room. Cultures were maintained in the dark except when sampling. A record was kept of room temperature, pressure, and humidity from the VWR thermometer/barometer in the room. Temperature accuracy was checked with a National Institute of Standards and Technology Traceable Thermistor and was found to vary within less than 0.9°C over the course of the experiments.

During each sampling, headspace and water were distributed for the following analyses: 100 mL gas from headspace for $^{13}C$ of $CO_2$; at the start of the experiment (0 h) replicate samples were taken of the room air for $\delta^{13}C$; 12 mL of the water was placed in a Labco 12 mL Exetainer for $^{13}C$ dissolved inorganic carbon (DIC) analyses, 140 mL for DIC concentrations, and 400 mL for total organic carbon. Samples at 0 h were taken from each bottle to provide a baseline for that bottle (see supporting information). Subsequent samples were collected by sacrificing one bottle from each treatment; this meant that the experiment would be sampled at 3 time points (21 h, 59 h, and 301 h. The sampling times for glucosidase activity were at 192 and 301 h. The water in the bottles attached to the NDIR gas analyzers was not sampled. Sampling times were determined based on changes in $CO_2$ concentrations in the headspaces of the LiCor bottles.

### 2.2. Bulk Organic Carbon Analyses

Water samples (400 mL) were filtered through combusted and preweighed 25 mm glass fiber filter (Whatman GF/F, 0.7 μm pore size) for particulate organic carbon (POC) analyses and the filtrate was used to measure dissolved organic carbon (DOC). POC samples were prepared for analysis via acid fumigation (12 N HCl) for 6 h to remove carbonates, dried at 50° for 12 h, and wrapped in a tin boat. The wrapped POC filters were analyzed on a Carlo Erba NA1500 Carbon-Nitrogen-Sulfur (CNS) elemental analyzer. DOC concentration was determined via high-temperature catalytic oxidation with a Shimadzu TOCV-CSN, according to the method of [*Guo et al*., 1994]. The detection limit on the instrument is 50 μg C $L^{-1}$, with a precision within 2%, based on the coefficient of variation.

### 2.3. $^{13}C$ Analyses

The $^{13/12}C$ ratio of the FACE and diatom leachates were analyzed by mixing 10.0 mL of a 105.12 mg C $L^{-1}$ lab standard (calibrated against a beta-alanine standard at $-26.2 \pm 0.1$ per mil versus Vienna Peedee belemnite, $n > 300$ [*Lalonde et al*., 2014]) with 5 μL of the leachate to be analyzed. Seventy and 100 μL of this mix was then added to a silver boat and gently dried on a low-temperature hot plate (~50°C). The silver boat was then folded, wrapped in a tin boat, and analyzed by a Eurovector Elemental Analyzer coupled to an Isoprime Stable Isotope Ratio Mass Spectrometer at Concordia University, Montreal. Triplicate analyses were completed for each leachate.

The $^{13/12}C$ ratio of $CO_2$ and $^{13/12}C$ ratio of DIC was measured using a Thermo Finnigan DeltaPlus XL isotope ratio mass spectrometer (IRMS) with a GasBench II universal online gas preparation device. The IRMS was outfitted with both high-ohm and low-ohm collector resistors so that low-isotopic enrichment samples (natural abundance) and highly $^{13}C$-enriched samples can be measured. For $\delta^{13}C$ measurements of DIC samples water was injected into septum top vials that contained three drops of 85% phosphoric acid and were filled with helium. Acidified $CO_2$ and He gases were transferred from the GasBench II to the IRMS.

### 2.4. Glucosidase Activity

Exoenzyme activity was measured using the fluorimetric method of *Hoppe* [1983] as modified by *Marx et al*. [2001] to facilitate assays with a 96-well plate format. The substrate analog for β-glucosidase activity was 4-methylumbelliferone-β-D-glucoside (Sigma-Aldrich), which is cleaved by β-glucosidase to 4-methylumbelliferone (MUF). The liberated MUF is fluorescent and therefore an increase in fluorescence over time is directly proportional to enzyme velocity. The substrate was dissolved in sterile

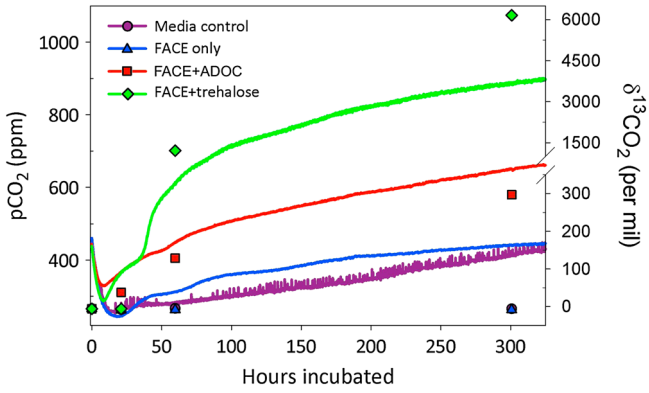


**Figure 1.** Continuously measured concentrations of $CO_2$ over the sampling time of 0 to 301 h, along with $\delta^{13}C$ values (permil versus Vienna Pee Dee Belemnite (V-PDB)) of the $CO_2$. These were the following four treatments: (1) 100% added OC as FACE (68 μM C), (2) 72% added carbon as FACE (68 μM C) + 28% $^{13}C$ ADOC (27 μM C), (3) 64% added carbon as FACE (68 μM C) + 36% $^{13}C$ trehalose (38 μM C), and (4) medium control (no added organic materials, see Table S1). The medium control enabled us to determine the rate of respiration of OC transferred with the bacterial inoculant.

ultrapure water (autoclaved and 0.2 μm filtered) and stored frozen in the dark. Each assay (200 μL) consisted of 100 μL of culture, 50 μL of substrate, and 50 μL of sterile 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES) buffer at pH 7.75 (0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid dissolved in basal medium) [*Marx et al.*, 2001]. More details of the methods of spectrofluorometric instrumentation, measurements, and quantification are provided in the supporting information.

### 2.5. $CO_2$ Measurements

$CO_2$ was monitored, continuously, in the headspace of one bottle from each set of replicates. For each one, a diaphragm pump pulled headspace from the bottle, pushed it through an NDIR detector (LiCor-820 or LiCor-840), and then back into the headspace of the bottle. The detectors were zeroed and spanned just prior to the start of the experiment and exhibited no drift during the experiment.

### 2.6. End-Member Mixing Model

The amount of each OC substrate that was remineralized to $CO_2$ during the experiment, including OC transferred with the bacterial inoculant, was determined based on changes in the concentration and stable isotopic composition of headspace $CO_2$ for each treatment. Although both DIC and $CO_2$ were monitored throughout the experiment, $CO_2$ values were used in the calculations described here. The ionic composition of medium that was used made interpretations of carbonate equilibrium complicated, whereas $CO_2$ values provided a more direct measurement of experimental conditions. The following equation describes a simple mass balance of the conversion of each OC type to $CO_2$ between two time intervals (e.g., $T_x$ to $T_{x+1}$):

$$C_f = C_i + \Delta C_M + \Delta C_{FACE} + \Delta C_P \quad (1)$$

where $C_i$ and $C_f$ are the initial and final concentrations of $CO_2$ in the experimental vessel's headspace for the timeframe being examined. The $C_i$ value used for $T_0$ was the minimum $pCO_2$ value observed for each treatment after headspace equilibration occurred (Figure 1). $\Delta C_M$ is the change in $CO_2$ concentration in the headspace of the medium control experiment during the time interval, which was used to account for the baseline respiration of OC transferred with the bacterial inoculant. $\Delta C_{FACE}$ and $\Delta C_P$ are the amount of FACE leachate and priming substrate (e.g., trehalose or ADOC) converted to $CO_2$ during the time interval, respectively.

The following stable isotopic end member mixing model was used to solve for the unknown variables in equation (1), $\Delta C_{FACE}$ and $\Delta C_P$:

$$R_f \times C_f = R_i \times C_i + R_M \times \Delta C_M + R_{FACE} \times \Delta C_{FACE} + R_P \times \Delta C_P \quad (2)$$

where $R_i$ and $R_f$ are the initial and final $^{13/12}C$ ratios of $CO_2$ measured from the experimental vessel's headspace for the timeframe being examined. $R_M$ is the $^{13/12}C$ ratio of $CO_2$ for the medium control experiment at the final time point of the period being examined; see more details in the supporting information on the model calculations.

## 3. Results and Discussion

The bulk DOC concentrations in all four treatments from 0 to 301 h ranged from 5.7 to 0.34 mg C $L^{-1}$ (Figure S1 in the supporting information). The DOC concentration in the FACE-only treatment increased during the first 24 h to the highest DOC concentration observed over the entire experiment, which may have been due to lysis of a significant proportion of the bacteria added in the inoculum. Over the next 24 h, the DOC concentrations of

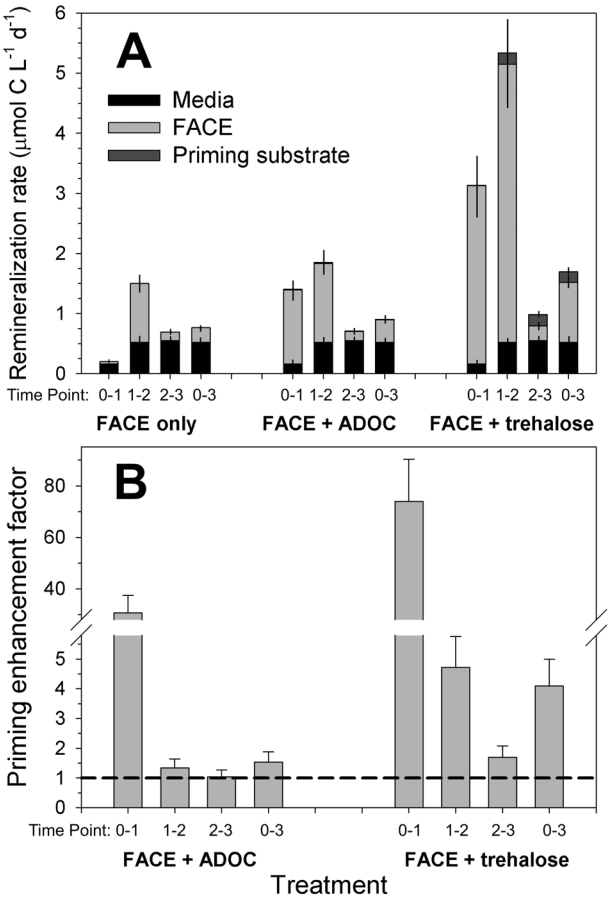


**Figure 2.** (a) Rates of remineralization of FACE leachate, priming substrates (e.g., trehalose and ADOC), and organic carbon present in the media control calculated with the end member mixing model for each treatment. Calculations were made for each time step (i.e., $T_0 - T_1 = 0$–21 h, $T_1 - T_2 = 21$–60 h, $T_2 - T_3 = 60$–301 h) and for the entire duration of the experiment (i.e., $T_0 - T_3 = 0$–301 h). (b) The proportional increase in FACE remineralization rates in the FACE + trehalose and FACE + ADOC treatments relative to the FACE-only treatment. The priming enhancement factor represents the FACE remineralization rate in the primed treatments divided by the FACE remineralization rate in the FACE-only treatment.

all four treatments converged to concentrations between 0.6 and 1.2 mg C $L^{-1}$. The most significant decrease in DOC concentration over a 24 h period occurred in the FACE-only treatment. These values remained relatively constant for the next approximately 10 days, suggestive of a baseline range of values after the initial consumption of the most bioavailable DOC was consumed by bacteria in all treatments. The POC concentrations of these treatments from 0 to 301 h ranged from 0.1 to 1.0 mg C $L^{-1}$ (Figure S1 in the supporting information). As expected, the lowest POC values were always found in the medium control treatment. In all treatments, except the FACE-only, the POC values increased over the first 24 h. This is consistent with the DOC results and suggests that the POC increases over the first 24 h likely represented conversion of DOC into bacterial biomass. DOC increased in the FACE-only treatment, where there was a loss of POC, suggesting that some of the POC loss was transferred into the DOC pool. The highest abundance of POC occurred in the FACE + trehalose treatment followed by the FACE + ADOC treatment at 59 h. At 301 h there was a loss of POC in these two treatments. The difference in POC concentrations between the primed and FACE-only treatments indicate that the diatom and trehalose priming substrates were assimilated as microbial biomass along with the FACE material.

In the first 24 h there was a significant decrease in $CO_2$ concentrations in all treatments (Figure 1). This likely occurred due to uptake of $CO_2$ from the headspace as the medium may have been slightly undersaturated with respect to $CO_2$, despite the bubbling. The trehalose and ADOC treated experiments did not reach as low of a minimum $pCO_2$ value as the media control and FACE-only experiments, presumably due to more rapid remineralization of OC to $CO_2$ during this initial period. Since minimum $pCO_2$ values for each treatment were used for calculations in the first time interval (e.g., 0–21 h), estimations of FACE remineralization were likely underestimated for the ADOC and trehalose treated experiments for this interval.

The FACE + trehalose microcosm showed much higher $CO_2$ concentrations than any of the other treatments by the end of the experiment, with the FACE + ADOC treatment also exceeding the FACE-only treatment. The rate at which FACE and priming substrates were converted to $CO_2$ was calculated based on equations (1) and (2) for each time interval and the duration of the entire experiment.

During the entire 301 h incubation period, the rate of FACE leachate remineralization in the FACE-only experiment was $0.24 \pm 0.04$ μmol C $L^{-1}$ $d^{-1}$ as opposed to $1.00 \pm 0.17$ μmol C and $0.38 \pm 0.06$ μmol C $L^{-1}$ $d^{-1}$ in the FACE + trehalose and FACE + ADOC treatments, respectively (Figure 2). This equates to a priming enhancement factor of $4.1 \pm 0.9$ and $1.5 \pm 0.3$ for the FACE + trehalose and FACE + ADOC treatments,

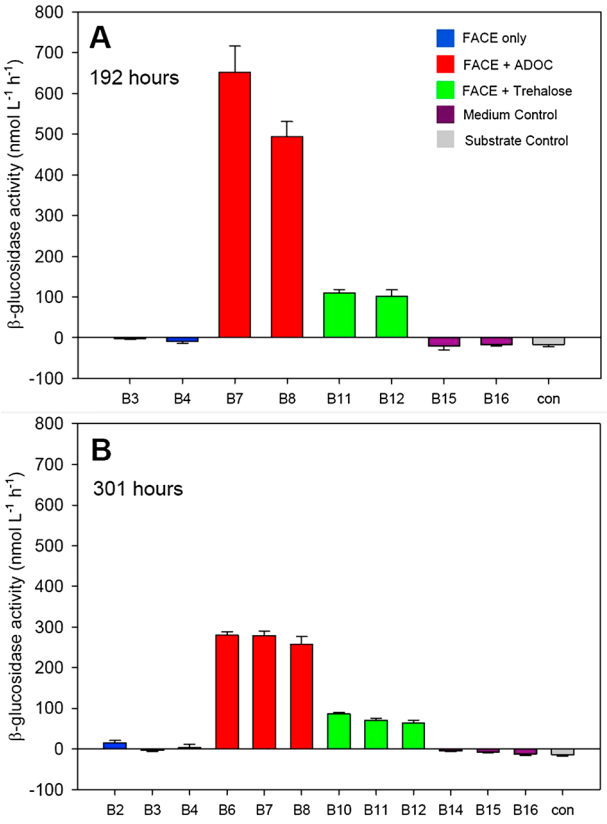


**Figure 3.** Glucosidase activity in different bottles at different time during the experiment: (a) 192 h and (b) 301 h. For logistical reasons, it was not possible to align enzyme sampling with the main sampling events. Note that the number of bottles increases over time as bottles could only be sampled for enzyme activity after they had been sacrificed for headspace sampling. The *x* axis labels refer to individual bottles (B1 to B16) and their treatment is shown by the color coding key on the figure. The grey bar (substrate control) was a methodological control assayed during the glucosidase measurements and therefore does not refer to a bottle treatment. Negative values (e.g., substrate controls) are an artifact resulting from decay of the fluorescent product. Bars show mean + SD ($n = 5$) of the measurements from each bottle.

respectively, over the 301 h period. The largest difference between the primed and nonprimed treatments occurred during the first 21 h, when TDOC was converted to $CO_2$ 74 ± 16 and 31 ± 7 times more rapidly with the addition of trehalose and algal DOC, respectively (Figure 2). In the FACE + ADOC treatment, priming was only evident during the first 21 h; the priming enhancement factor was 1.3 ± 0.3 and 1.0 ± 0.2 (e.g., the FACE remineralization rate was equal to the FACE-only treatment) during the 21–60 h and 60–301 h time intervals, respectively (Figure 2). In the FACE + trehalose treatment, on the other hand, priming continued with enhancement factors of 4.7 ± 1.0 and 1.7 ± 0.4 during the 21–60 h and 60–301 h time intervals, respectively.

In addition to elevated priming effects, a greater amount of trehalose was converted to $CO_2$ relative to ADOC. For example, trehalose was converted to $CO_2$ at a rate of 0.18 ± 0.03 μmol C $L^{-1}$ $d^{-1}$ throughout the 301 h incubation period compared to 0.011 ± 0.002 μmol C $L^{-1}$ $d^{-1}$ for the ADOC (Figure 2). It is likely that a large fraction of OC in the diatom leachate was assimilated into bacterial biomass, whereas the trehalose was mostly respired to $CO_2$.

It is important to note that while there was likely bacterial lysis when the treatments were inoculated, which added to the DOC in the treatments, there was only one combination of FACE/priming substrate remineralization rates that could have resulted in the $^{13}CO_2$ values we observed (assuming the amount of $CO_2$ produced in the media control is a representative of how much media OC was converted to $CO_2$ in the other treatments). Thus, while there may have been a "slight amount of priming" in the FACE-only treatment due to bacterial lysis (see supporting information), the model results here clearly indicate that more FACE was consumed in both the diatom and trehalose experiments.

Glucosidase activity was used as an additional measure of "biological activity" in this experiment (Figure 3). The glucosidase activity was highest in the cultures that grew the fastest and had carbohydrates added to them (i.e., diatom biomass (used to make our ADOC) is composed of ~40% carbohydrate, and trehalose is a carbohydrate) (Figure 3). The pattern of activity was FACE + ADOC > FACE + trehalose > FACE > medium control, with all rates decreasing over time. Rates in the FACE and medium were below detection most of the time. It is logical that the FACE + ADOC experiment would have the highest rates of activity as this treatment contained complex glucose-based polymers. Also, since trehalose is an alpha-linked disaccharide it does not serve as a substrate for the enzyme measured (beta-glucosidase), so there was clearly a bias toward the ADOC priming substrate. Finally, it is also important to note that some of the bottles yielded negative rates. We can only speculate that this phenomenon may have occurred due to an experimental artifact. Thus, in our data interpretation, we only considered rates that were greater than that of the substrate control.

## 4. Conclusions and Possible Implications

The results from this laboratory experiment provide unequivocal evidence for the occurrence of priming of TDOC by ADOC and a simple sugar. While this work provides the basis for linking how TDOC can be primed under very simple lab-based conditions, there are certainly limitations in this approach. These experiments used a single bacterial strain and two known carbon sources to document priming processes. This will hopefully provide a path to studies that may further document priming under more a complicated set of biogeochemical conditions, with more details on roles of microbial consortia and a more complex suite of enzymes and substrates involved in priming. To understand ecosystem-level implications of the priming effect, we propose that more work should be conducted in natural systems along the freshwater to ocean continuum that likely serve as "hot spots" for priming to occur. These hot spots include lakes/reservoirs, estuaries, floodplains, tributaries, and continental shelves with high ADOC production [*Bianchi*, 2011].

In particular, priming effects on TDOC should be considered in light of the many past and ongoing environmental perturbations in the Anthropocene. For example, hydroelectric reservoirs may serve as excellent "mixing chambers" for priming because of enhanced phytoplankton growth (relative to upstream conditions), inputs of terrestrially derived organic matter from the watershed, and enhanced water residence times. These conditions could potentially lead to enhanced remineralization rates of TDOC and, in most cases, higher GHG production. Similarly, plumes in large-river delta-front estuaries may also serve as important sites for priming of TDOC [*Bianchi*, 2011]. These plumes are key interfaces where the major TDOC inputs to global ocean occur [*Bauer et al*., 2013] and commonly mix with waters that have high primary productivity.

Finally, in the context of carbon cycling in soils, sediments, freshwaters, and oceanic waters, there appears to be an emerging consensus that organic matter "quality," often described in terms of words such as "recalcitrant" and "labile" in the context of food resources for heterotrophs, may only be valid for a specific environment. Instead, "reactivity" of organic compounds is increasingly recognized as being a function of ecosystem properties rather than being related to intrinsic chemical characteristics [*Schmidt et al*., 2011]. Moreover, the potential assimilation capability of the local microbial community, where such substrates may reside in very low concentrations like the deep ocean, also needs to be considered [see *Arrieta et al*., 2015]. While there is clearly much more work needed, our observations reinforce this emerging view of controls on organic matter reactivity and stability in the aquatic environment.

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