Dark Carbon Fixation In The Columbia River's Estuarine Turbidity Maxima: Molecular Characterization Of Red-Type Cbbl Genes And Measurement Of DIC Uptake Ratesin Response To Added Electron Donors

By: **S. L. Bräuer** & K. Kranzler & N. Goodson & D. Murphy & H. M. Simon & A. M. Baptista & B. M. Tebo

Abstract

Dark CO2 fixation has been shown to rival the importance of oxygenic photosynthesis in the global carbon cycle, especially in stratified environments, such as salt wedge estuaries. We investigated this process in the Columbia River estuary using a variety of techniques in-cluding functional gene cloning of cbbL (the large subunit of form I RuBisCO), quantitative real-time PCR (qPCR) estimations of cbbL abundance, and analyses of stimulated 14C-bicarbonate assimilation. A diversity of red-type cbbL genes were retrieved from clone libraries, with 28 unique operational taxonomic units determined from 60 sequences. The majority of the sequences formed two clusters that were distinct from the major clusters typically found in soil envi-ronments, revealing the presence of a unique community of autotrophic or facultatively autotrophic/mixotrophic micro-organisms in the Columbia River estuary. qPCR estimates indicated that roughly 0.03–0.15 % of the microbial popu-lation harbored the cbbL gene, with greater numbers of total bacteria and cbbL gene copies found in the estuarine turbid-ity maxima (ETM) compared to non-ETM events. In vitro incubations with radiolabeled bicarbonate indicated maximum stimulation by thiosulfate and also suggested that a diversity of other potential electron donors may stimulate CO2 fixation, including nitrite, ammonium, and Mn(II). Taken together, the-se results highlight the diversity of the microbial metabolic strategies employed and emphasize the importance of dark CO2 fixation in the dynamic waters of the Columbia River estuary despite the abundance of organic material.

S. L. Bräuer & K. Kranzler & N. Goodson & D. Murphy & H. M. Simon & A. M. Baptista & B. M. Tebo (2013) "Dark Carbon Fixation In The Columbia River's Estuarine Turbidity Maxima: Molecular Characterization Of Red-Type Cbbl Genes And Measurement Of DIC Uptake Ratesin Response To Added Electron Donors" Estuarine, Coastal and Shelf Science 36(5):1073-1083. [ISSN: 1559-2731] Version Of Record Available At www.proquest.com

Introduction

Among the most important global biogeochemical processes, CO_2 fixation is generally thought to be dominated by oxygenic photosynthesis carried out by plants, bacteria, and phytoplankton. However, dark CO_2 fixation (including anaplerotic reactions) by obligate and facultative chemolithoautotrophs and mixotrophs is gaining recognition as a widespread and important process that rivals that of oxygenic photosynthesis in freshwater (Jorgensen et al. 1979; Shively et al. 1998; Garcia-Cantizano et al. 2005; Lliros et al. 2011; Casamayor et al. 2012), marine (Tuttle and Jannasch 1977; Indrebø et al. 1979; Taylor et al. 2001; Jost et al. 2008; Glaubitz et al. 2009, 2010; Alonso-Saez et al. 2010; DeLorenzo et al. 2012), estuarine (Casamayor et al. 2001), and soil (Yuan et al. 2012) environments. Among bacteria and archaea, fixation can occur via six major pathways (see review by Hügler and Sievert 2011) as well as by anaplerotic pathways (Moran et al. 2004; González et al. 2008). A variety of reduced

inorganic compounds can be used as electron donors to fuel autotrophic CO_2 uptake, including NH^{4+}, NO^{2-}, $S_2O_3^{2-}$, Fe^{2+}, and, possibly, Mn^{2+}, although isolation of an autotrophic Mn oxidizer remains elusive. Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) is the diagnostic gene for the reductive pentose phosphate cycle (the Calvin–Benson–Basham cycle), and this gene is present among a variety of taxonomic groups including alpha-, beta-, and gamma-proteobacteria as well as firmicutes, cyanobacteria, and chloroflexi (Swingley et al. 2007; Hügler and Sievert 2011). In organic-rich environments such as soils and estuaries, facultatively chemolithoautotrophic members of the alpha- and beta-proteobacteria are likely to be the most abundant, and these organisms usually contain the type IC (and/or sometimes IA) subgroup of red-like RuBisCO genes (Badger and Bek 2008). Additionally, photosynthetic purple sulfur bacteria have been shown to carry out dark carbon fixation in stratified lakes in northeastern Spain (Casamayor et al. 2008).

The Columbia River, including the estuary (our study site), is the largest source of freshwater, by volume (60–90 %), to the California Current System between the San Francisco Bay and the Strait of Juan de Fuca (Barnes et al. 1972), and it also provides a major source of dissolved manganese and nutrients to coastal waters (Aguilar-Islas and Bruland 2006; Bruland et al. 2008). The North and South Channels of the river are dredged to allow shipping (Herfort et al. 2012). Within these channels, water circulation and density stratification, controlled by river discharges, tides, and shelf winds, enable the entrapment of suspended particulate matter in estuarine turbidity maxima (ETM; Gelfenbaum 1983; Jay and Smith 1990; Jay and Musiak 1994; Small and Prahl 2004). These ETM are generated because the net flux of deep water upstream into the estuary allows negatively buoyant particulate matter to remain trapped in the estuary in dense clouds of turbidity (Crump et al. 1998). Estuarine turbidity maxima occur throughout many estuaries in the world, including (but not limited to) the Chesapeake (Schubel 1968), the San Francisco Bay (Jassby and Powell 1994), and the Delaware (Biggs et al. 1983) in the USA; the Yorkshire Ouse, Humber, and Tamar in the UK (Grabemann et al. 1997; Uncles et al. 1998); the Seine, Loire, and Gironde in France (Etcheber et al. 2007); the Elbe and Weser in Germany (Kappenberg and Grabemann 2001); the Guadiana in South Portugal (Garel et al. 2009); the Ariake Bay in Japan (Islam et al. 2006); the Changjiang in China (Li and Zhang 1998); and the Amazon in Brazil (Demaster et al. 1986; Nittrouer et al. 1986; Berhane et al. 1997), to name a few. ETM are hot spots of enzymatic activity and biogeochemical cycling and have been demonstrated to exhibit enhanced bacterial production rates compared to that outside of the ETM, with rates up to 5.6 $\mu g L^{-1} h^{-1}$ in the Columbia River ETM as measured with tritiated thymidine (Crump and Baross 1996). Overall, the presence of zooplankton as well as the microbial growth rates have been strongly correlated with turbidity in the Columbia River, and ETM have been described as having enhanced "microbial loop activities" (Baross et al. 1994). This is true not just for heterotrophic activity but other reactions as well, such as manganese reduction (Klinkhammer and McManus 2001) and oxidation (Bräuer et al. 2011). Thus, we hypothesized that, similar to other microbial processes, autotrophy would be most prominent during an ETM event compared to a non-ETM event. Herein, a polyphasic approach was employed to assess the quantity and diversity of RuBisCO-containing organisms as well as the relative contribution of various electron donors to dark CO_2 fixation in the Columbia River estuary.

Materials and Methods

Sample Collection and Storage

Prior to sampling, we utilized data-informed daily forecasts of baroclinic circulation (http://www.stccmop.org/datamart/virtualcolumbiariver/forecasts) to predict salinity intrusion length and variability in the North and South Channels of the estuary. Details of the models (Zhang et al. 2004; Zhang and Baptista 2008) and their simulation skill (Baptista et al. 2005; Burla 2009; Frolov et al. 2009) are described elsewhere. The location of the salt wedge was empirically correlated, via observations of sediment concentrations (http://www.stccmop.org/datamart/observation_network), to the timing of the ETM, which were expected to occur near the front of the salt wedge (B. Crump and L. Herfort, personal communication). Samples were collected in the South Channel on 14th June 2007 and on 17th July 2007 and in both the North and South Channels from 14th through 31st August 2007. Samples were deemed to be either ETM or non-ETM samples by the chief scientist at the time of sampling and in general were defined according to a significant change (greater than 1.5–2-fold) in nephelometric turbidity units or a change in the transmitted or reflected light due to changes in suspended particulate matter in conjunction with the time and location of the predicted occurrence. The maximum turbidity measurements observed roughly correlated with the salt wedge and could be found at the leading edge (low salinity values approx. 5 PSU) or trailing edge (intermediate salinity values approx. 15 PSU) of the salt wedge. Turbidity generally subsided 30 min to 2 h after the salt wedge had passed (for more information, see Bräuer et al. 2011). Samples from the Columbia River estuary (Electronic supplementary material (ESM) Fig. S1) were collected onboard either the R/V *Forerunner* in June and July 2007 or the R/V *Barnes* in August 2007 (Table 1). Water samples collected on the R/V

Table 1 Metadata for samples collected in this study

Sample date	Location	Latitude ±4″	Longitude ±6″	Approximate depth (m)	Oxygen (mg/L)	Analyses conducted
14-Jun-07	Youngs Bay at Skipanon River	46°11′15″	−123°54′3″	9–10	–	qPCR, *cbbL* libraries
17-Jul-07	Smith Point	46° 11’ 37″	−123°51′56″	10	4.71	qPCR
23-Aug-07	Youngs Bay and Tongue Point	46° 12’ 27″	−123°47′58″	10–11	7.02	^{14}C
26-Aug-07	Youngs Bay and Tongue Point	46° 12’28″	−123°47′51″	11–12	6.55	^{14}C

Oxygen concentration data were not collected on 14th June 2007 because the oxygen sensor was not working at that time

Forerunner were retrieved using an air pump and were coordinated with a conductivity, temperature, and depth recorder (CTD). Water samples collected onboard the R/V *Barnes* were retrieved using an SBE Carousel sampler with twelve 10-L Niskin sampling bottles. Cast data were collected using a SeaBird brand CTD equipped with a transmissometer, fluorometer, thermometer, photosynthetically available radiation light sensor, O_2 probe, and altimeter. Microbial cells for molecular assays were concentrated onto triplicate or quadruplicate Sterivex filters (Millipore, Billerica, MA) onboard the ship. The volume filtered was recorded and was approximately 600–900 mL per filter. Filters were stored shipboard in a freezer containing dry ice and were transferred to a −80 °C freezer in the laboratory until further processing. Water for $^{14}CO_2$ assimilation analyses was collected in sterile 1-L bottles, and incubations were initiated on deck immediately after collecting the water (see below).

DNA Extraction, Cloning, and Sequencing

Filters containing microbial cells for DNA extraction were cracked open using sterilized pliers to allow the inner cylinder containing the filter to be removed. The filter was cut loose from the cylinder using a sterile scalpel and was gently folded and cut into tiny pieces using sterile scissors. The filter pieces were allowed to fall directly into bead beating tubes from the FastDNA Spin Kit for Soil (MP Biomedical Sciences, Solon, OH); extraction was carried out according to the manufacturer’s instructions. DNA concentration was determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Wilmington, DE). The *cbbL* gene was then amplified using the primers targeting the red-like RuBisCO genes, *cbbL*R1F and *cbbL*R, as described by the authors (Selesi et al. 2005). PCR products from three to six PCR reactions were pooled and amplicons were cloned into TOPO TA *pCR®2.1* vectors (Invitrogen, Carlsbad, CA). Plasmid DNA from transformants was extracted using the QIAprep Spin Miniprep kit (Qiagen Inc., Valencia, CA) and screened using the M13 forward (−20) primer. Ninety-six-well plates of glycerol stocks were sent to Agencourt (now Beckman Coulter in MA) for the sequencing of clone libraries in both directions using both the M13 forward and reverse primers. Contigs were assembled using Vector NTI (Invitrogen) and alignments (811 bp) were generated using BioEdit (Hall 2001). Twenty-nine operational taxonomic units were identified at a 90 % cutoff for nucleotide data using DOTUR (Schloss and Handelsman 2005). Amino acid alignments were analyzed with DOTUR as well, and 27–29 operational taxonomic units (OTUs) corresponded to approximately a 94–95 % identity cutoff at the amino acid level. Good’s nonparametric coverage was estimated using the equation $[1-(n/N)]\times 100$, where N is the total number of clones evaluated and n is the number of singleton OTUs (Good 1953). Sequences of each unique OTU were deposited into GenBank with accession numbers JQ994305–JQ994332. Phylogenetic trees were constructed using both the neighbor-joining and maximum likelihood algorithms in the PHYLIP software package (Felsenstein 2004).

Real-Time Quantitative PCR

Quantitative real-time PCR (qPCR) was conducted using primers specific for the *cbbL* gene, *cbbL*RF and *cbbL*R1intR (Selesi et al. 2007). Amplifications were performed on triplicate samples in Maxima™ SYBR Green qPCR Master Mix (Fermentas, Glen Burnie, MD) containing 0.2 μM of each primer and were carried out using an Applied Biosystems™ 7300 Real-Time PCR System (Foster City, CA) with the following amplification protocol: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C with 1 min at 60 °C. After amplification, a dissociation protocol was added to verify dissociation profiles. Genomic DNA from *Ralstonia eutropha* H16 (generously provided by Dr. Anne Pohlmann and Dr. Bärbel Friedrich of the Humboldt-Universität zu Berlin) was used in the range of 10^9–10^2 target *ccbL* copies per microliter to generate the calibration curves for quantification as described previously (Fey et al. 2004). Copy number results were expressed on a basis of the amount of DNA extracted per milliliter of water filtered.

Stimulated Dark $^{14}CO_2$ Uptake

^{14}C-bicarbonate assimilation was determined using bottom water collected both during ETM events and non-ETM

events on 23rd and 26th August 2007 (Table 1). Procedures were carried out as described by Nielsen (1952). Briefly, 20 mL of water was added to each serum vial and the vials sealed and crimped. To each of the triplicate samples, 100 μM (final concentration) of either NH^{4+}, NO^{2-}, $S_2O_3^{2-}$, and Fe^{2+}, 10 μM (final concentration) of Mn^{2+}, or no additional electron donor was added, along with 0.1 mL of 20 μCi/mL ^{14}C-bicarbonate. Blanks were prepared by incubating 20 mL of the sample water in the absence of any addition (no ^{14}C-bicarbonate), and these yielded 32–67 CPM (Fig. 2). The vials were incubated in the dark near the in situ temperature of 20 °C for 24 h. Incubation was stopped by filtration onto 0.2-μM Whatman filters that were then fumigated with HCl to remove excess unincorporated bicarbonate prior to scintillation. Filters were then placed in scintillation vials with a scintillation cocktail and decays were measured using a liquid scintillation counter (Packard).

Results

RuBisCO Gene Diversity

A total of 60 *cbbL* gene clones were obtained, representing 28 unique OTUs at the 95 % identity cutoff. Rarefaction analyses indicated that the sampling depth was sufficient to cover the bulk of the diversity in these samples, as indicated by the asymptotic shape of the curve (ESM Fig. S2). Several clones affiliated with sequences from a previous study by Selesi et al. (2005) that examined *cbbL* diversity in agricultural soil. A few (6 %) grouped in cluster RI with clone HNPKR97, and a few more (8 %) grouped in cluster RIII with clone HKOR89 and an *Arthobacter* isolate R45 (Fig. 1). Three of the 60 clone sequences (two OTUs) grouped with *Rhodobacter sphaeroides*. However, the majority of the clones fell into two clades, herein named CRI and CRII: 29, or roughly half of the sequences, clustered with clone HSMR12 (Selesi et al. 2005), whereas 16 (or roughly a quarter) of the sequences clustered with sequences of the *cbbL* genes of *Bacillus* and *Arthobacter* species isolated from agricultural soil (Selesi et al. 2005), R32c and R33c (Fig. 1). Good's nonparametric coverage estimator indicated approximately 76 % coverage of the OTU diversity.

RuBisCO Gene Abundance

Quantitative real-time PCR analyses indicated that anywhere from 40 up to 2,000 copies/mL of the *cbbL* gene were present during the times of sampling (Table 2). The copy numbers found in ETM were consistently higher than those found during non-ETM events, with 451 versus 38 copies in June and 2,254 versus 131 in July. According to *t* tests, the difference between the ETM and non-ETM samples was only significant in July (P=0.02) and not in June (P=0.06). Compared to the total number of estimated bacteria in these same samples, reported in a previous study (Bräuer et al. 2011), the percentages of those carrying the *cbbL* gene were 0.06 and 0.03 % for the ETM and non-ETM samples in June and 0.15 and 0.03 % for the ETM and non-ETM samples in July, respectively (Table 2).

$^{14}CO_2$ Uptake

A goal of this study was to elucidate the potential contribution of nitrite, thiosulfate, and ammonium to dark CO_2 fixation in the Columbia River estuary and to test the hypothesis that Mn(II) may serve as an electron donor in fueling autotrophic or mixotrophic reactions. Relative to no addition, all donors stimulated ^{14}C uptake during ETM events (Fig. 2, solid squares). However, outside of ETM events, there was little to no stimulation of C fixation by most of the donors compared to no addition (Fig. 2, open circles). CO_2 uptake was stimulated the greatest by thiosulfate during the second sampling on 26th August (Fig. 2b); however, during the first sampling, stimulation by ammonium and nitrite was also significant (Fig. 2a). Interestingly, the uptake of ^{14}C-bicarbonate in the presence of Mn(II) was greater than the control during the ETM events.

Discussion

RuBisCO Gene Diversity

The phylogenetic distribution of *cbbL* genes found here appears to be distinct from that found in either soil (Selesi et al. 2005) or groundwater (Alfreider et al. 2009; Kellermann et al. 2012). A few of the clones grouped with *R. sphaeroides*, which is especially noteworthy since several species of *Rhodobacter* were isolated from the Columbia River estuary in a previous study (Bräuer et al. 2011). However, *cbbL* genes were not detected among any of the nine isolates from that study that were tested using four different primer sets (Brauer and Xu, unpublished data), suggesting that those isolates were not capable of CO_2 fixation using RuBisCO. The majority of the clones fell into two clades, herein named CRI and CRII. In contrast to our findings, sequences from both of these clusters were much less abundant in a study of agricultural soil (Selesi et al. 2005), with only 5 of the 46 distinct clones (based on RFLP patterns) from each of two treatment plots affiliating with clusters CRI and CRII. In fact, of the four clusters identified in the agricultural soil by Selesi et al., only two of those clusters—RI and RIII—were identified here, and cluster RB and RII were not detected, likely reflecting the unique environmental conditions of estuarine waters compared to agricultural soil. Certainly, microbial community structure is

Fig. 1 Neighbor-joining tree inferring the phylogenetic relationships of red-like *cbbL* sequences found in clone libraries of water from the Columbia River in this study and sequences from top blast hits as well as a broad range of described species. Alignments were generated using BioEdit (Hall 2001) and dendrograms were calculated using PHYLIP (Felsenstein 2004). Bootstrapping values are shown only for nodes that were supported by both maximum-likelihood and neighbor-joining analyses and which were supported >60 % of the time. The number of the sequences from the library that represented a particular OTU is given in *parentheses*. *Pyrococcus furiosus* was used as the outgroup

Table 2 Quantitative real-time data comparing *cbbL* gene copies obtained in this study to total bacterial cell estimates (SSU gene copies/4.09) determined in a previous study (Bräuer et al. 2011)

Sample date	Turbidity status	*cbbL* gene copies/mL	SE	SSU rRNA gene copies/mL	SE	Percentage carrying RuBisCO
14-Jun-07	ETM	451	80.0	7.56E+05	8.06E+04	0.06
	Non-ETM	38.34	4.08	1.52E+05	3.57E+04	0.03
17-Jul-07	ETM	2250	278	1.55E+06	3.64E+04	0.15
	Non-ETM	131	7.46	3.82E+05	4.28E+04	0.03

SE standard error for triplicate samples

known to reflect the geochemical environment in the Columbia River (Fortunato et al. 2012) and other environments (Horner-Devine et al. 2007); thus, the differences observed here are most likely due to distinct environmental differences rather than stochastic effects related to the time of sampling, DNA extraction, or other experimental procedures. A diversity of *cbbL* genes have also been found in other organic-rich environments such as soils (Selesi et al. 2005; Tolli and King 2005; Videmšek et al. 2009), groundwater (Alfreider et al. 2009; Kellermann et al. 2012), and marine environments (Elsaied and Naganuma 2001; Nigro and King 2007), among others. Moreover, *cbbL* genes have been found to be not only abundant (Selesi et al. 2007) but also highly active in soils (Yuan et al. 2012); thus, the organisms harboring them are playing an important role in the global carbon cycle.

cbbL Gene Abundance

The copy numbers determined here may seem low by volume; however, bacterial numbers in estuaries are typically around 10^6 or 10^7 cells/mL (Crump and Baross 1996; Cochran and Paul 1998; Cottrell et al. 2010; Bräuer et al. 2011). Thus, when calculated as a percentage of the total bacterial cells, our numbers are comparable to other studies in the literature, which found that *cbbL* gene copies represented from <0.01 % to 1.0 % of the total estimated bacteria. For example, Selesi et al. (2005) found 10^6–10^7 copies of the *cbbL* gene per gram of agricultural soil. Using the estimate of Whitman et al. (1998) of 10^9 cells per gram of soil in the top meter, the estimates of Selesi et al. represent roughly 0.1–1 % of cells, while our estimates ranged from 0.03 to 0.15 % of cells. Similar *cbbL* gene copy number estimates were also found in sediments from the Black Sea (Schippers et al. 2012) and Chesapeake Bay (Breuker et al. 2011) and in groundwater (Kellermann et al. 2012), whereas relative copy number was possibly less abundant in sediments of the Benguela upwelling (Schippers et al. 2012). These estimates of *cbbL* abundance are consistent with that of other functional genes in soil. For example, *nirK* (nitrate reductase) was estimated to occur in the range of 10^4–10^6 copies per gram of soil (Henry et al. 2004), or roughly 0.01 %, while *nosZ* (nitrous oxide reductase) was estimated at 10^5–10^7 copies per gram, or 0.1–0.5 % of the estimated SSU rRNA copy number that was measured (Henry et al. 2006). Some other functional genes can represent a much larger percentage of the total cell number. For example, some soils have been found to contain up to 4×10^6 copies per gram (dry weight) of the ammonia-monooxygenase gene, *amoA* (Okano et al. 2004). However, it is important to keep in mind that microbial abundance does not always correlate with activity or ecological significance, especially since many members of a microbial community may be dormant or inactive (Jones and Lennon 2010; Lennon and Jones 2011). In fact, rare members of microbial communities can be important constituents of the functional diversity of an environment (Sogin et al. 2006; Huber et al. 2007).

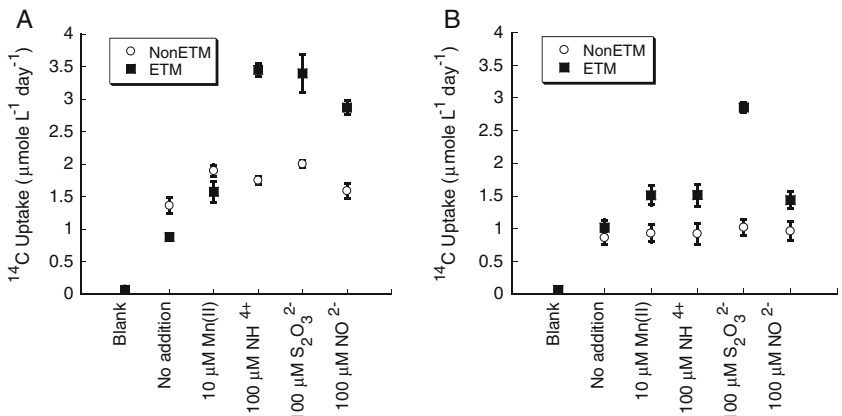

Fig 2 Comparison of total $^{14}CO_2$ fixation from filtered samples for samples amended with ^{14}C-bicarbonate and 100 µM nitrite, thiosulfate, ammonium, 10 µM Mn(II), or no addition. Control samples of estuary water were incubated and filtered for comparison. Samples were collected on 23rd August (**a**) and 26th August (**b**) 2007 during both an ETM and a non-ETM event. Incubations were conducted in triplicate. *Bars* represent standard deviation

$^{14}CO_2$ Uptake

Dark CO_2 uptake rates measured in the absence of added electron donors ranged from 0.9 to 1.4 $\mu mol L^{-1} day^{-1}$ (to 72–114 $mg C m^{-3} day^{-1}$; Fig. 2) and closely mirrored the maximum values (0.7 and 1.4 $\mu mol L^{-1} day^{-1}$) found in the sulfidic zones of the Central Baltic Sea (Labrenz et al. 2005), in the oxic/anoxic interface of the Ebro River salt wedge (5.5–124 $mg C m^{-3} day^{-1}$; Casamayor et al. 2001), and in the Black Sea (0.32–1.5 $\mu M C day^{-1}$; Taylor et al. 2001 and references therein). More data are needed to draw robust conclusions; however, data in the literature suggest higher average dark CO_2 fixation rates in estuarine environments compared to that found in marine environments such as the Black Sea, Baltic Sea, Cariaco Basin, and Saanich Inlet (see Table 4 published in Casamayor et al. 2001). Although Columbia River Estuarine waters are known to experience hypoxia (Roegner et al. 2011), it is important to note that the samples analyzed here were normoxic (6–7 mg/L; Table 1) and that higher uptake rates may occur in anoxic waters, as has been demonstrated in karstic lakes in Spain (Casamayor et al. 2012). However, due to the enhanced turbidity and abundance of particulate matter, anaerobic pockets were likely abundant in these samples. Indeed, ETM waters in the Columbia River Estuary that are usually normoxic appear to support reductive reactions such as Mn reduction (Klinkhammer and McManus 2001; Bräuer et al. 2011).

$^{14}CO_2$ Uptake in Response to Electron Donors

CO_2 uptake was stimulated the greatest by thiosulfate and reached maximum values of 3.4 $\mu M day^{-1}$, similar to studies of the Baltic Sea where dissolved inorganic carbon (DIC) uptake in response to thiosulfate approached 10 $\mu M day^{-1}$ (Labrenz et al. 2005). Sulfur-oxidizing bacteria have also been implicated as contributors to dark CO_2 fixation in the Ebro River (Casamayor et al. 2001), the Black Sea (Sorokin 1972; Jørgensen et al. 1991), and the Cariaco Basin (Taylor et al. 2001, 2006). Interestingly, in our study, ammonium and nitrite also stimulated CO_2 uptake, but only on the 23rd and not on the 26th of August. This is likely due to spatial differences in microbial populations (Fortunato et al. 2012) and fluctuations in nutrient concentrations. Although nitrite concentrations are generally very low, other nitrogen species can vary temporally, spatially, and/or seasonally, perhaps due to changes in nitrogen availability and/or oxidation and reduction rates. For example, nitrate concentrations in the Columbia River estuary varied from 5 to 15 μM in August 2007 samplings (Smith et al. 2010) and reached high values in the 50-μM range in winter, with heavier rainfall (Colbert and McManus 2003; Bruland et al. 2008; Smith et al. 2010), while concentrations of reduced ammonium were found to be highest in summertime (from remineralization), averaging around 15 μM and reaching as high as 60 μM (Gilbert et al. 2013). However, it is important to keep in mind that the turnover rates are ultimately more important than concentrations, and ETM have been demonstrated to have extremely high turnover rates (Baross et al. 1994); thus, even chemicals in low abundance, such as nitrite, may rapidly cycle in the ETM.

In contrast to studies conducted in the Baltic Sea (Labrenz et al. 2005), the uptake of ^{14}C-bicarbonate in the presence of Mn(II) was greater than the control during the ETM events. Manganese cycling (oxidation and reduction) are an important process in both systems, with particulate manganese oxide concentrations in the range of approx. 300 nM in the Baltic Sea (Neretin et al. 2003) and 600 nM in Columbia River ETM (Bräuer et al. 2011). Dissolved Mn(II) concentrations ranged from 14 to 50 nM in oxic waters or up to 18 μM in bottom anoxic waters of the Baltic (Neretin et al. 2003), but are usually below 200 nM in the Columbia River (Klinkhammer et al. 1997; Klinkhammer and McManus 2001; Aguilar-Islas and Bruland 2006; Bräuer et al. 2011). While our data suggest that Mn(II) may possibly serve as an energy source, stimulation of anaplerotic or mixotrophic reactions by heterotrophic bacteria is also a possibility. Autotrophic growth using Mn(II) as an energy source has not yet been demonstrated unequivocally despite the predicted thermodynamic favorability of the reaction (Tebo et al. 2005). To date, all known Mn(II)-oxidizing isolates are heterotrophic, although one isolate is known to contain genes for CO_2 fixation using the CBB pathway (Caspi et al. 1996; Dick et al. 2008).

Additionally, at least two studies have demonstrated broad zones of dark CO_2 fixation that were not correlated with mixing zones or a supply of reduced chemicals to fuel chemoautotrophic reactions in the Black Sea (Karl and Knauer 1991) and in the Elbro River Estuary (Casamayor et al. 2001). Thus, it is likely that a variety of microbial metabolic processes may simultaneously contribute to dark CO_2 fixation, at least in some environments. Chemoautotrophic reactions are notoriously low-yielding, resulting in extremely low growth rates and low biomass (Shively et al. 1998; Kelly and Wood 2006). Thus, heterotrophic reactions are thought to dominate wherever labile organic matter is present. The Columbia River estuary is known to have multiple sources of organic matter including marine, freshwater, and autochthonous contributions; to contain high concentrations of particulate organic carbon and particulate nitrogen (Herfort et al. 2011); and to support high rates of heterotrophic activity (Crump et al. 1998; Crump and Baross 2000), especially during ETM. For example, the rates of [^{3}H]thymidine incorporation, a proxy for bacterial growth rates, among particle attached bacteria in the Columbia River estuary

averaged from 7.7 up to 57.4 pmol$L^{-1}h^{-1}$ (Crump et al. 1999). Bulk CO_2 fixation prohibits the distinction of true autotrophy from mixotrophic and heterotrophic CO_2 uptake, an important consideration given that anaplerotic CO_2 fixation can account for upwards of 10 % of the total cell C during heterotrophic growth (Perez and Matin 1982) and 32–47 % when cultures are grown mixotrophically (Perez and Matin 1982) or on C1 compounds (Doronia and Trotsenko 1985). Overall, based on the environmental conditions as well as the diversity of type IC RuBisCO genes of the red cluster, it can be inferred that, similar to findings in oxic marine waters (Alonso-Saez et al. 2010), dark CO_2 fixation by facultative autotrophs, mixotrophs, and/or heterotrophs may predominate over that of obligate autotrophs in the Columbia River estuary.

Conclusions

The scientific community has made recent strides in understanding estuarine and marine carbon cycling, in particular the importance of dark CO_2 fixation. The research presented here demonstrates that the diversity and abundance of *cbbL* genes encoding type I RuBisCO provide potential for significant dark CO_2 fixation in the Columbia River. Furthermore, ETM samples were significantly stimulated by the addition of a variety of electron donors, suggesting that the microbial community is extremely versatile and can utilize a variety of metabolic strategies for C uptake in addition to heterotrophy. Knowledge of the ecology and activity of autotrophic and mixotrophic bacteria provided in this report is critical to our understanding of carbon cycling from the microbial to the global scale. Overall, this work contributes to a growing body of evidence demonstrating the global significance of dark CO_2 fixation and helps provide a more comprehensive understanding of this “missing sink” in the global carbon cycle.

Acknowledgments The authors would like to thank the captains and crews of the R/V *Barnes* and R/V *Forerunner* for assistance with sampling. We thank the chief scientists, especially Lydie Herfort and Byron Crump, as well as the computer analysts who gave us insight into predicting and capturing samples during ETM events. We also thank Dr. Anne Pohlman and Dr. Bärbel Friedrich of Humboldt-Universität zu Berlin for their generous donation of *Ralstonia eutropha* H16 cultures. We also thank R. Davis for helpful discussion of RuBisCO genes. Partial support was provided through the following National Science Foundation, Division of Ocean Sciences grants: The Science and Technology Center for Coastal Margin Observation and Prediction (0424602) awarded to A. Baptista and an Ocean Sciences Postdoctoral Fellowship (0935270) awarded to S. Bräuer. Support to B. Tebo was provided by an Oregon Opportunity grant and grant no. P42ES010337 from the National Institute of Environmental Health Sciences (NIEHS).

References

Aguilar-Islas, A.M., and K.W. Bruland. 2006. Dissolved manganese and silicic acid in the Columbia River plume: a major source to the California current and coastal waters off Washington and Oregon. *Marine Chemistry* 101: 233–247.

Alfreider, A., C. Vogt, M. Geiger-Kaiser, and R. Psenner. 2009. Distribution and diversity of autotrophic bacteria in groundwater systems based on the analysis of RubisCO genotypes. *Systematic and Applied Microbiology* 32: 140–150.

Alonso-Saez, L., P.E. Galand, E.O. Casamayor, C. Pedros-Alio, and S. Bertilsson. 2010. High bicarbonate assimilation in the dark by Arctic bacteria. *ISME Journal* 4: 1581–1590.

Badger, M.R., and E.J. Bek. 2008. Multiple Rubisco forms in proteobacteria: their functional significance in relation to CO_2 acquisition by the CBB cycle. *Journal of Experimental Botany* 59: 1525–1541.

Baptista, A.M., Y.L. Zhang, A. Chawla, M. Zulauf, C. Seaton, E.P. Myers, J. Kindle, M. Wilkin, M. Burla, and P.J. Turner. 2005. A cross-scale model for 3D baroclinic circulation in estuary–plume–shelf systems: II. Application to the Columbia River. *Continental Shelf Research* 25: 935–972.

Barnes, C., A. Duxbury, and B. Morse. 1972. Circulation and selected properties of the Columbia River effluent at sea. In *The Columbia River estuary and adjacent ocean waters*, ed. A. Pruter and D. Alverson, 41–80. Seattle, WA: University of Washington Press.

Baross, J., B. Crump, and C.A. Simenstad. 1994. Elevated 'microbial loop' activities in the Columbia River estuary turbidity maximum. In *Changing particle fluxes in estuaries: implications from science to management. ESCAERF22 Symposium*, ed. K. Dyer and R. Orth, 459–464. Fiedensborg, Denmark: Olsen & Olsen.

Berhane, I., R.W. Sternberg, G.C. Kineke, T.G. Milligan, and K. Kranck. 1997. The variability of suspended aggregates on the Amazon Continental Shelf. *Continental Shelf Research* 17: 267–285.

Biggs, R.B., J.H. Sharp, T.M. Church, and J.M. Tramontano. 1983. Optical-properties, suspended sediments, and chemistry associated with the turbidity maxima of the Delaware Estuary. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 172–179.

Bräuer, S.L., C. Adams, K. Kranzler, D. Murphy, M. Xu, P. Zuber, H.M. Simon, A.M. Baptista, and B.M. Tebo. 2011. Culturable *Rhodobacter* and *Shewanella* species are abundant in estuarine turbidity maxima of the Columbia River. *Environmental Microbiology* 13: 589–603.

Breuker, A., G. Koeweker, A. Blazejak, and A. Schippers. 2011. The deep biosphere in terrestrial sediments in the Chesapeake Bay area, Virginia, USA. *Frontiers in Microbiology* 2: 156. doi:10.3389/fmicb.2011.00156.

Bruland, K.W., M.C. Lohan, A.M. Aguilar-Islas, G.J. Smith, B. Sohst, and A. Baptista. 2008. Factors influencing the chemistry of the near-field Columbia River plume: nitrate, silicic acid, dissolved Fe, and dissolved Mn. *Journal of Geophysical Research Oceans* 113: C00B02. doi:10.1029/2007JC004702.

Burla M. 2009. The Columbia River estuary and plume: natural variability, anthropogenic change and physical habitat for salmon. PhD thesis, Oregon Health & Science University, Beaverton, OR

Casamayor, E.O., J. Garcia-Cantizano, and C. Pedros-Alio. 2008. Carbon dioxide fixation in the dark by photosynthetic bacteria in sulfide-rich stratified lakes with oxic–anoxic interfaces. *Limnology and Oceanography* 53: 1193–1203.

Casamayor, E.O., J. Garcia-Cantizano, J. Mas, and C. Pedros-Alio. 2001. Primary production in estuarine oxic/anoxic interfaces: contribution of microbial dark CO_2 fixation in the Ebro River salt wedge estuary. *Marine Ecology Progress Series* 215: 49–56.

Casamayor, E.O., M. Lliros, A. Picazo, A. Barberan, C.M. Borrego, and A. Camacho. 2012. Contribution of deep dark fixation

processes to overall CO_2 incorporation and large vertical changes of microbial populations in stratified karstic lakes. *Aquatic Sciences* 74: 61–75.

Caspi, R., M.G. Haygood, and B.M. Tebo. 1996. Unusual ribulose-1,5-bisphosphate carboxylase/oxygenase genes from a marine manganese-oxidizing bacterium. *Microbiology* 142: 2549–2559.

Cochran, P.K., and J.H. Paul. 1998. Seasonal abundance of lysogenic bacteria in a subtropical estuary. *Applied and Environmental Microbiology* 64: 2308–2312.

Colbert, D., and J. McManus. 2003. Nutrient biogeochemistry in an upwelling-influenced estuary of the Pacific Northwest (Tillamook Bay, Oregon, USA). *Estuaries and Coasts* 26: 1205–1219.

Cottrell, M.T., J. Ras, and D.L. Kirchman. 2010. Bacteriochlorophyll and community structure of aerobic anoxygenic phototrophic bacteria in a particle-rich estuary. *The ISME Journal* 4: 945–954.

Crump, B.C., and J.A. Baross. 1996. Particle-attached bacteria and heterotrophic plankton associated with the Columbia River estuarine turbidity maxima. *Marine Ecology Progress Series* 138: 265–273.

Crump, B.C., and J.A. Baross. 2000. Characterization of the bacterially-active particle fraction in the Columbia River estuary. *Marine Ecology Progress Series* 206: 13–22.

Crump, B.C., J.A. Baross, and C.A. Simenstad. 1998. Dominance of particle-attached bacteria in the Columbia River estuary, USA. *Aquatic Microbial Ecology* 14: 7–18.

Crump, B.C., E.V. Armbrust, and J.A. Baross. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia River, its estuary, and the adjacent coastal ocean. *Applied and Environmental Microbiology* 65: 3192–3204.

DeLorenzo, S., S.L. Bräuer, C.A. Edgmont, L. Herfort, B.M. Tebo, and P. Zuber. 2012. Ubiquitous dissolved inorganic carbon assimilation by marine bacteria in the Pacific Northwest coastal ocean as determined by stable isotope probing. *PLoS One* 7: e46695.

Demaster, D.J., S.A. Kuehl, and C.A. Nittrouer. 1986. Effects of suspended sediments on geochemical processes near the mouth of the Amazon River—examination of biological silica uptake and the fate of particle-reactive elements. *Continental Shelf Research* 6: 107–125.

Dick, G.J., S. Podell, H.A. Johnson, Y. Rivera-Espinoza, R. Bernier-Latmani, J.K. McCarthy, J.W. Torpey, B.G. Clement, T. Gaasterland, and B.M. Tebo. 2008. Genomic insights into Mn (II) oxidation by the marine alphaproteobacterium *Aurantimonas* sp. strain SI85-9A1. *Applied and Environmental Microbiology* 74: 2646–2658.

Doronia, N.V., and Y.A. Trotsenko. 1985. Levels of carbon dioxide assimilation in bacteria with different pathways of C1 metabolism. *Mikrobiologiya* 53: 885–889.

Elsaied, H., and T. Naganuma. 2001. Phylogenetic diversity of ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit genes from deep-sea microorganisms. *Applied and Environmental Microbiology* 67: 1751–1765.

Etcheber, H., A. Taillez, G. Abril, J. Garnier, P. Servais, F. Moatar, and M.V. Commarieu. 2007. Particulate organic carbon in the estuarine turbidity maxima of the Gironde, Loire and Seine estuaries: origin and lability. *Hydrobiologia* 588: 245–259.

Felsenstein J. 2004. PHYLIP (Phylogeny Inference Package) version 3.68. *Department of Genome Sciences*, *University of Washington*, *Seattle* (distributed by the author).

Fey, A., S. Eichler, S. Flavier, R. Christen, M.G. Hofle, and C.A. Guzman. 2004. Establishment of a real-time PCR-based approach for accurate quantification of bacterial RNA targets in water, using *Salmonella* as a model organism. *Applied and Environmental Microbiology* 70: 3618–3623.

Fortunato, C.S., L. Herfort, P. Zuber, A.M. Baptista, and B.C. Crump. 2012. Spatial variability overwhelms seasonal patterns in bacterioplankton communities across a river to ocean gradient. *The ISME Journal* 6: 554–563.

Frolov, S., A.M. Baptista, Y.L. Zhang, and C. Seaton. 2009. Estimation of ecologically significant circulation features of the Columbia River estuary and plume using a reduced-dimension Kalman filter. *Continental Shelf Research* 29: 456–466.

Garcia-Cantizano, J., E.O. Casamayor, J.M. Gasol, R. Guerrero, and C. Pedros-Alio. 2005. Partitioning of CO_2 incorporation among planktonic microbial guilds and estimation of in situ specific growth rates. *Microbial Ecology* 50: 230–241.

Garel, E., L. Pinto, A. Santos, and O. Ferreira. 2009. Tidal and river discharge forcing upon water and sediment circulation at a rock-bound estuary (Guadiana estuary, Portugal). *Estuarine, Coastal and Shelf Science* 84: 269–281.

Gelfenbaum, G. 1983. Suspended sediment response to semidiurnal and fortnightly tidal variations in a mesotidal estuary—Columbia River, USA. *Marine Geology* 52: 39–57.

Gilbert, M., Needoba, J.N., Koch, C., Barnard, A., and Baptista A.M. (2013). Nutrient loading and transformations in the Columbia River estuary determined by high resolution in situ sensors. *Estuaries and Coasts*. doi:10.1007/s12237-013-9597-0.

Glaubitz, S., M. Labrenz, G. Jost, and K. Jürgens. 2010. Diversity of active chemolithoautotrophic prokaryotes in the sulfidic zone of a Black Sea pelagic redoxcline as determined by rRNA-based stable isotope probing. *FEMS Microbiology Ecology* 74: 32–41.

Glaubitz, S., T. Lueders, W.-R. Abraham, G. Jost, K. Jürgens, and M. Labrenz. 2009. ^{13}C-isotope analyses reveal that chemolithoautotrophic gamma- and epsilonproteobacteria feed a microbial food web in a pelagic redoxcline of the Central Baltic Sea. *Environmental Microbiology* 11: 326–337.

González, J.M., B. Fernández-Gómez, A. Fernàndez-Guerra, et al. 2008. Genome analysis of the proteorhodopsin-containing marine bacterium *Polaribacter* sp. MED152 (Flavobacteria). *Proceedings of the National Academy of Sciences* 105: 8724–8729.

Good, I.J. 1953. The population frequencies of species and the estimation of population parameters. *Biometrika* 40: 237–264.

Grabemann, I., R.J. Uncles, G. Krause, and J.A. Stephens. 1997. Behaviour of turbidity maxima in the Tamar (UK) and Weser (FRG) estuaries. *Estuarine, Coastal and Shelf Science* 45: 235–246.

Hall T. 2001. BioEdit version 5.0.6. . *North Carolina State University*, *Department of Microbiology*. http://www.mbio.ncsu.edu/bioedit/bioedit.html/.

Henry, S., D. Bru, B. Stres, S. Hallet, and L. Philippot. 2006. Quantitative detection of the *nosZ* gene, encoding nitrous oxide reductase, and comparison of the abundances of 16S rRNA, *narG*, *nirK*, and *nosZ* genes in soils. *Applied and Environmental Microbiology* 72: 5181–5189.

Henry, S., E. Baudoin, J.C. López-Gutiérrez, F. Martin-Laurent, A. Brauman, and L. Philippot. 2004. Quantification of denitrifying bacteria in soils by *nirK* gene targeted real-time PCR. *Journal of Microbiological Methods* 59: 327–335.

Herfort, L., T. Peterson, L. McCue, and P. Zuber. 2011. Protist 18S rRNA gene sequence analysis reveals multiple sources of organic matter contributing to turbidity maxima of the Columbia River estuary. *Marine Ecology Progress Series* 438: 19–31.

Herfort, L., T. Peterson, F. Prahl, L. McCue, J. Needoba, B. Crump, G. Roegner, V. Campbell, and P. Zuber. 2012. Red waters of *Myrionecta rubra* are biogeochemical hotspots for the Columbia River estuary with impacts on primary/secondary productions and nutrient cycles. *Estuaries and Coasts* 35: 878–891.

Horner-Devine, M.C., J.M. Silver, M.A. Leibold, et al. 2007. A comparison of taxon co-occurrence patterns for macro- and microorganisms. *Ecology* 88: 1345–1353.

Huber, J.A., D.B. Mark Welch, H.G. Morrison, S.M. Huse, P.R. Neal, D.A. Butterfield, and M.L. Sogin. 2007. Microbial population structures in the deep marine biosphere. *Science* 318: 97–100.

Hügler, M., and S.M. Sievert. 2011. Beyond the Calvin cycle: autotrophic carbon fixation in the ocean. *Annual Review of Marine Science* 3: 261–289.

Indrebø, G., B. Pengerud, and I. Dundas. 1979. Microbial activities in a permanently stratified estuary. II. Microbial activities at the oxic–anoxic interface. *Marine Biology* 51: 305–309.

Islam, M.S., H. Ueda, and M. Tanaka. 2006. Spatial and seasonal variations in copepod communities related to turbidity maximum along the Chikugo estuarine gradient in the Upper Ariake Bay, Japan. *Estuarine, Coastal and Shelf Science* 68: 113–126.

Jassby, A.D., and T.M. Powell. 1994. Hydrodynamic influences on interannual chlorophyll variability in an estuary—Upper San-Francisco Bay Delta (California, USA). *Estuarine, Coastal and Shelf Science* 39: 595–618.

Jay, D.A., and J.D. Smith. 1990. Circulation, density distribution and neap-spring transitions in the Columbia River estuary. *Progress in Oceanography* 25: 81–112.

Jay, D.A., and J.D. Musiak. 1994. Particle trapping in estuarine tidal flows. *Journal of Geophysical Research* 99: 20445–20461.

Jones, S.E., and J.T. Lennon. 2010. Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Sciences* 107: 5881–5886.

Jorgensen, B.B., J.G. Kuenen, and Y. Cohen. 1979. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). *Limnology and Oceanography* 24: 799–822.

Jørgensen, B.B., H. Fossing, C.O. Wirsen, and H.W. Jannasch. 1991. Sulfide oxidation in the anoxic Black Sea chemocline. *Deep Sea Research Part A. Oceanographic Research Papers* 38(Supplement 2): S1083–S1103.

Jost, G., M. Zubkov, E. Yakushev, M. Labrenz, and K. Jurgens. 2008. High abundance and dark CO_2 fixation of chemolithoautotrophic prokaryotes in anoxic waters of the Baltic Sea. *Limnology and Oceanography* 53: 14–22.

Kappenberg, J., and I. Grabemann. 2001. Variability of the mixing zones and estuarine turbidity maxima in the Elbe and Weser estuaries. *Estuaries* 24: 699–706.

Karl, D.M., and G.A. Knauer. 1991. Microbial production and particle flux in the upper 350 m of the Black Sea. *Deep Sea Research Part A. Oceanographic Research Papers* 38(Supplement 2): S921–S942.

Kellermann, C., D. Selesi, N. Lee, M. Hügler, J. Esperschütz, A. Hartmann, and C. Griebler. 2012. Microbial CO_2 fixation potential in a tar-oil-contaminated porous aquifer. *FEMS Microbiology Ecology* 81: 172–187.

Kelly D. P. and A. P. Wood. 2006. The chemolithotrophic prokaryotes. In *The prokaryotes*: *ecophysiology and biochemistry*, *vol. 2*, edited by Dworkin M., E. Rosenberg and K.-H. Schleifer, 441–456. New York: Springer.

Klinkhammer, G.P., and J. McManus. 2001. Dissolved manganese in the Columbia River estuary: production in the water column. *Geochimica Et Cosmochimica Acta* 65: 2835–2841.

Klinkhammer, G.P., C.S. Chin, C. Wilson, M.D. Rudnicki, and C.R. German. 1997. Distributions of dissolved manganese and fluorescent dissolved organic matter in the Columbia River estuary and plume as determined by in situ measurement. *Marine Chemistry* 56: 1–14.

Labrenz, M., G. Jost, C. Pohl, S. Beckmann, W. Martens-Habbena, and K. Jurgens. 2005. Impact of different in vitro electron donor/acceptor conditions on potential chemolithoautotrophic communities from marine pelagic redoxclines. *Applied and Environmental Microbiology* 71: 6664–6672.

Lennon, J.T., and S.E. Jones. 2011. Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology* 9: 119–130.

Li, J.F., and C. Zhang. 1998. Sediment resuspension and implications for turbidity maximum in the Changjiang Estuary. *Marine Geology* 148: 117–124.

Lliros, M., L. Alonso-Saez, F. Gich, A. Plasencia, O. Auguet, E.O. Casamayor, and C.M. Borrego. 2011. Active bacteria and archaea cells fixing bicarbonate in the dark along the water column of a stratified eutrophic lagoon. *FEMS Microbiology Ecology* 77: 370–384.

Moran, M.A., A. Buchan, J.M. Gonzalez, et al. 2004. Genome sequence of *Silicibacter pomeroyi* reveals adaptations to the marine environment. *Nature* 432: 910–913.

Neretin, L.N., C. Pohl, G. Jost, T. Leipe, and F. Pollehne. 2003. Manganese cycling in the Gotland Deep, Baltic Sea. *Marine Chemistry* 82: 125–143.

Nielsen, E.S. 1952. The use of radioactive carbon (C14) for measuring organic production in the sea. *Journal du Conseil* 18: 117–140.

Nigro, L.M., and G.M. King. 2007. Disparate distributions of chemolithotrophs containing form IA or IC large subunit genes for ribulose-1,5-bisphosphate carboxylase/oxygenase in intertidal marine and littoral lake sediments. *FEMS Microbiology Ecology* 60: 113–125.

Nittrouer, C.A., T.B. Curtin, and D.J. Demaster. 1986. Concentration and flux of suspended sediment on the Amazon Continental-Shelf. *Continental Shelf Research* 6: 151–174.

Okano, Y., K.R. Hristova, C.M. Leutenegger, L.E. Jackson, R.F. Denison, B. Gebreyesus, D. Lebauer, and K.M. Scow. 2004. Application of real-time PCR to study effects of ammonium on population size of ammonia-oxidizing bacteria in soil. *Applied and Environmental Microbiology* 70: 1008–1016.

Perez, R.C., and A. Matin. 1982. Carbon dioxide assimilation by *Thiobacillus novellus* under nutrient-limited mixotrophic conditions. *Journal of Bacteriology* 150: 46–51.

Roegner, G.C., J.A. Needoba, and A.M. Baptista. 2011. Coastal upwelling supplies oxygen-depleted water to the Columbia River estuary. *PLoS One* 6.

Schippers, A., D. Kock, C. Höft, G. Köweker, and M. Siegert. 2012. Quantification of microbial communities in subsurface marine sediments of the Black Sea and off Namibia. *Frontiers in Microbiology* 3: 16. doi:10.3389/fmicb.2012.00016.

Schloss, P.D., and J. Handelsman. 2005. Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Applied and Environmental Microbiology* 71: 1501–1506.

Schubel, J.R. 1968. Turbidity maximum of northern Chesapeake Bay. *Science* 161: 1013.

Selesi, D., M. Schmid, and A. Hartmann. 2005. Diversity of green-like and red-like ribulose-1,5-bisphosphate carboxylase/oxygenase large-subunit genes (*cbbL*) in differently managed agricultural soils. *Applied and Environmental Microbiology* 71: 175–184.

Selesi, D., I. Pattis, M. Schmid, E. Kandeler, and A. Hartmann. 2007. Quantification of bacterial RubisCO genes in soils by *cbbL* targeted real-time PCR. *Journal of Microbiological Methods* 69: 497–503.

Shively, J.M., G. van Keulen, and W.G. Meijer. 1998. Something from almost nothing: carbon dioxide fixation in chemoautotrophs. *Annual Review of Microbiology* 52: 191–230.

Small, L.F., and F.G. Prahl. 2004. A particle conveyor belt process in the Columbia River estuary: evidence from chlorophyll *a* and particulate organic carbon. *Estuaries* 27: 999–1013.

Smith, M.W., L. Herfort, K. Tyrol, D. Suciu, V. Campbell, B.C. Crump, T.D. Peterson, P. Zuber, A.M. Baptista, and H.M. Simon. 2010. Seasonal changes in bacterial and archaeal gene expression patterns across salinity gradients in the Columbia River coastal margin. *PLoS One* 5: e13312.

Sogin, M.L., H.G. Morrison, J.A. Huber, D.M. Welch, S.M. Huse, P.R. Neal, J.M. Arrieta, and G.J. Herndl. 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". *Proceedings of the National Academy of Sciences* 103: 12115–12120.

Sorokin, Y.I. 1972. The bacterial population and the processes of hydrogen sulphide oxidation in the Black Sea. *Journal du Conseil* 34: 423–454.

Swingley, W.D., S. Sadekar, S.D. Mastrian, et al. 2007. The complete genome sequence of *Roseobacter denitrificans* reveals a mixotrophic rather than photosynthetic metabolism. *Journal of Bacteriology* 189: 683–690.

Taylor, G.T., M. Iabichella-Armas, R. Varela, F. Müller-Karger, X. Lin, and M.I. Scranton. 2006. Microbial ecology of the Cariaco Basin's redoxcline: the US–Venezuela Cariaco times series program. *Past and Present Water Column Anoxia* 64: 471–499.

Taylor, G.T., M. Iabichella, T.Y. Ho, M.I. Scranton, R.C. Thunell, F. Muller-Karger, and R. Varela. 2001. Chemoautotrophy in the redox transition zone of the Cariaco Basin: a significant midwater source of organic carbon production. *Limnology and Oceanography* 46: 148–163.

Tebo, B.M., H.A. Johnson, J.K. McCarthy, and A.S. Templeton. 2005. Geomicrobiology of manganese(II) oxidation. *Trends in Microbiology* 13: 421–428.

Tolli, J., and G.M. King. 2005. Diversity and structure of bacterial chemolithotrophic communities in pine forest and agroecosystem soils. *Applied and Environmental Microbiology* 71: 8411–8418.

Tuttle, J.H., and H.W. Jannasch. 1977. Thiosulfate stimulation of microbial dark stimulation of carbon dioxide in shallow marine waters. *Microbial Ecology* 4: 9–25.

Uncles, R.J., R.J.M. Howland, A.E. Easton, M.L. Griffiths, C. Harris, R.S. King, A.W. Morris, D.H. Plummer, and E.M.S. Woodward. 1998. Seasonal variability of dissolved nutrients in the Humber-Ouse Estuary, UK. *Marine Pollution Bulletin* 37: 234–246.

Videmšek, U., A. Hagn, M. Suhadolc, V. Radl, H. Knicker, M. Schloter, and D. Vodnik. 2009. Abundance and diversity of CO_2-fixing bacteria in grassland soils close to natural carbon dioxide springs. *Microbial Ecology* 58: 1–9.

Whitman, W.B., D.C. Coleman, and W.J. Wiebe. 1998. Prokaryotes: the unseen majority. *Proceedings of the National Academy of Sciences* 95: 6578–6583.

Yuan, H.,T. Ge, C. Chen, A. G. O'Donnell, and J. Wu 2012. Significant role for microbial autotrophy in the sequestration of soil carbon. *Applied and Environmental Microbiology* 78:2328–2336.

Zhang, Y.L., and A.M. Baptista. 2008. SELFE: a semi-implicit Eulerian–Lagrangian finite-element model for cross-scale ocean circulation. *Ocean Modelling* 21: 71–96.

Zhang, Y.L., A.M. Baptista, and E.P. Myers. 2004. A cross-scale model for 3D baroclinic circulation in estuary–plume–shelf systems: I. Formulation and skill assessment. *Continental Shelf Research* 24: 2187–2214.