Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean

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Edited by David M. Karl, University of Hawaii, Honolulu, HI, and approved March 14, 2014 (received for review January 31, 2014)

Marine dissolved organic carbon (DOC) encompasses one of the largest reservoirs of carbon on Earth. Heterotrophic bacteria are the primary biotic force regulating the fate of this material, yet the capacity of individual strains to significantly contribute to carbon cycling is unknown. Here we quantified the ability of a single *Alteromonas* strain [*Alteromonas* sp. strain Scripps Institution of Oceanography (AltSIO)] to drawdown ambient DOC in a coastal ecosystem. In three experiments, AltSIO alone consumed the entire pool of labile DOC, defined here as the quantity consumed by the submicron size fraction of ambient microbial assemblages within 5 d. These findings demonstrate that complete removal of the labile DOC pool in coastal surface seawater can be achieved by a single taxon. During long-term incubations (>1 y) testing semilabile DOC consumption, AltSIO entered dormancy but remained viable, while the diverse assemblages continued to consume carbon. Given that AltSIO is a large bacterium and thus subject to increased grazing pressure, we sought to determine the ecological relevance of this phenotype. Growth dynamics in natural seawater revealed that AltSIO rapidly outgrew the native bacteria, and despite intense grazing pressure, was never eliminated from the population. A survey in the California Current Ecosystem revealed that large bacteria (≥40 fg C·cell^{-1}) were persistent, accounting for up to 12% of total bacterial abundance and 24% of total bacterial biomass. We conclude that large, rapidly growing bacteria have the potential to disproportionately alter the fate of carbon in the mesotrophic ocean and play an important role in ecosystem function.

dissolved organic matter | ocean carbon cycle | marine biogeochemistry | microbial loop | CCE

Turnover of surface ocean dissolved organic carbon (DOC) by the microbial loop (1) represents the largest flux of C through the ocean reservoir (2). The rate and magnitude of this turnover is influenced in part by microbial community structure (3–5). For example, seasonally accumulated DOC has been shown to resist consumption by depth-stratified surface microbial communities, but is then readily metabolized by phylogenetically distinct mesopelagic populations following annual convective overturn (4). This supports the hypothesis that distinct microbial communities harbor unique metabolic potential adapted to local resource composition (6). It has been hypothesized from experimental metagenomics (7) and transcriptomics (8) that bacterial diversity may enhance the compositional breadth of DOC remineralization as it provides a broader repertoire of enzymes (3, 5, 9) and transporters (10) required for bacterial hydrolysis and uptake of complex macromolecules of varying biochemical reactivity (11).

The notion that DOC degradation may scale with microbial diversity is in general agreement with fundamental biodiversity theory of resource niche partitioning among competing species (12). However, the hypothesis that diversity is required for the degradation of labile DOC has not been explicitly tested. Alternatively, extensive experimental observations suggest that a narrow subset of taxa may disproportionally contribute to the degradation of DOC in the surface ocean. Results from microcosm studies, both nutrient-amended (13–15) and unamended "controls" (4, 16–20), have shown that few taxa, predominantly *Alteromonadales,* rapidly dominate microbial communities. Metagenomic (7) and transcriptomic (8, 21) analyses of DOC-amended microcosms have also shown that *Alteromonadales* accounted for the majority of observed increases in cell abundance and transcriptional activity. Similar results have been observed in experiments tracking species-specific uptake of stable- or radioisotope-labeled phytoplankton exudates (15, 22). Although the manipulation of predator abundance in microcosm studies has been shown to select for some phylotypes (16, 18), in situ field observations have corroborated a high proportional abundance of *Alteromonas* spp. in the natural environment (23, 24). Furthermore, Tada et al. (25) reported that *Alteromonas* spp. accounted for up to 30% of the actively growing bacterial population during a phytoplankton bloom in the western North Pacific.

These findings suggest that single physiotypes, such as *Alteromonas,* may serve as a central conduit for a significant flux of DOC and nutrient mineralization in the upper ocean. In this study, we sought to address one of the most fundamental components required to elicit such observations by quantifying the maximum potential contribution of a single bacterial isolate to DOC degradation, particularly in the absence of other cells that may inhibit or facilitate the growth performance of the individual strain. We tested this by quantifying *Alteromonas*-specific DOC consumption in pure culture and in coculture relative to conventionally assessed DOC drawdown of free-living bacterial seawater communities (26, 27). Thus, our null hypothesis stated that the physiological capacity encoded within a single bacterial genome is not metabolically sufficient to consume ambient DOC to a measurable degree. We interpret our results from three separate DOC drawdown experiments with the single isolate alongside additional

Significance

Primary production generates a reservoir of dissolved organic carbon (DOC) in the ocean as large as the global inventory of atmospheric CO_2. Once formed, DOC accumulates on timescales from less than 1 hour to millennia. Bacteria are important contributors to the respiration of DOC to CO_2 and the conversion of DOC to refractory biopolymers. Yet, the quantitative contribution of individual species within diverse consortia to DOC cycling remains unknown. We report that a single bacterial strain can consume as much DOC as diverse free-living microbial communities. This taxon is commonly observed in seawater when labile carbon is available, and may serve a key ecosystem function by rapidly recycling and regulating the level of DOC while also supporting ocean food webs.

Author contributions: B.E.P. and F.A. designed research; B.E.P. performed research; B.E.P., L.I.A., and F.A. analyzed data; and B.E.P., L.I.A., and F.A. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

Data deposition: The sequence reported in this paper has been deposited in the GenBank databank [accession no. KC758958 (ALT199)].

See Commentary on page 7166.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1401887111/-/DCSupplemental.

competition and grazing studies in size-fractionated natural seawater, and in the context of field measurements of bacterial size frequency distribution and total organic carbon (TOC) concentrations in the California Current Ecosystem (CCE).

Results

Experimental Quality Control. Whole seawater, and filtrate used as ambient dissolved organic matter (DOM) growth media showed a reduction in TOC concentration after each filtration step [0.7 μm nominal glass fiber filter (GF/F), 0.1 μm polyethersulfone (PES)] ensuring contamination-free methodology (Table S1). Concurrent replicated (3×) bacteria-free control incubations showed no growth of bacteria and no change in TOC concentration after 5 d, confirming sterility of controls and the absence of abiotic reductions in DOC (Table S1). In situ seawater conditions including TOC and nutrient concentrations at the time of seawater collection are reported in Table S2.

Cell Retention on GF/Fs and CHN-Derived Cell Carbon and Nitrogen. Cell carbon (C) was directly measured in January 2012. Cell retention was calculated as the difference in cell abundance between whole and GF/F-filtered water and measured 69%, 99%, and 66% for the free-living bacteria seawater community (SWC), *Alteromonas* sp. strain Scripps Institution of Oceanography (AltSIO), and SWC inoculated with AltSIO (SWC+AltSIO) treatments, respectively, after 3 d. After 5 d, cell retention increased to 79%, 99.5%, and 76% for SWC, AltSIO, and SWC + AltSIO, respectively (Table S3). The SWC averaged 19 fg C·cell^{-1}, whereas AltSIO averaged 75 fg C·cell^{-1} on day 3, and decreased slightly to 17.5 and 63.0 fg C·cell^{-1}, respectively on day 5 (Table S3). AltSIO contained 4.4-fold greater cell-specific nitrogen (N) than the average seawater community bacterial cell. C and N values for the SWC are within the range of those reported for coastal bacteria (28, 29).

Bacterial Abundance. Bacterial abundance increased in all treatments in each DOC drawdown experiment (Fig. 1 *A*–*C* and Table S4). In all experiments, the SWC reached the highest cell abundance, followed by SWC + AltSIO. The AltSIO-only treatment consistently showed the lowest maximum cell abundance of ~10^8 cells·L^{-1}, ~10× less than the SWC (Fig. 1 *A*–*C* and Table S4). All treatments yielded greater cell abundance in January relative to May and November. It is possible, but unknown, whether greater incubation volume (20 L vs. 200 mL; *SI Materials and Methods*) contributed to this observation. There was no significant difference at any time point between abundance of AltSIO grown in isolation and that grown in coculture (Table S4; ANOVA, Tukey's $P > 0.05$). AltSIO was inoculated into each coculture at ~1% of the total starting bacterial abundance and reached a final proportional abundance of 17%, 26%, and 30% of the total bacterial assemblage [total 4′,6-diamidino-2-phenylindole (DAPI)-stained cells] in May 2011, November 2011, and January 2012, respectively as determined by fluorescence in situ hybridization (FISH).

Bacterial Biomass. Mean treatment differences in C biomass within each experiment were low relative to treatment-specific differences across experiments (Table S4). The average of all treatments within each experimental date showed the highest C biomass in January 2012 relative to May 2011 and November 2011 (3.87, 2.32, and 1.88 μmol C·L^{-1}, respectively; Fig. 1 and Table S4). The SWC + AltSIO treatment yielded significantly greater bacterial C biomass relative to other treatments in November (Fig. 1 and Table S4) (ANOVA, Tukey's $P < 0.05$). No significant difference was measured in total bacterial C biomass between the SWC and AltSIO-only treatments in May 2011 (Fig. 1 *D*–*F* and Table S4).

DOC Utilization. One of three SWC treatment replicates became contaminated in May 2011 as measured by elevated TOC concentration and was eliminated from all calculations. All other treatments and replicates resulted in a reduction of TOC concentration after 5 d (Fig. 1 and Table S4). Among all experiments, no

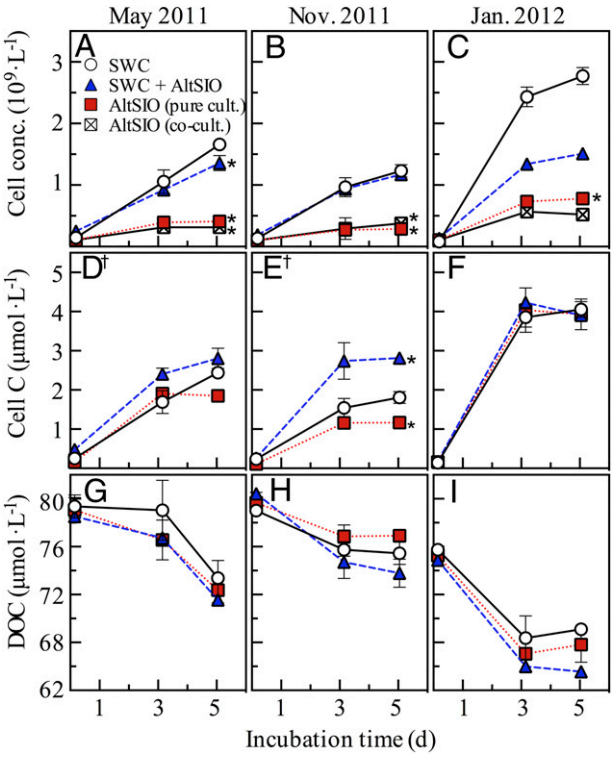

Fig. 1. Bacterial growth, cell C, and DOC consumption of three treatments grown in 0.1 μm filtered coastal North Pacific seawater (*A*–*I*). Treatment abbreviations are as follows: AltSIO (cocult.) represents FISH-derived abundance of AltSIO in coculture, AltSIO (pure cult.) represents *Alteromonas* sp. strain AltSIO pure culture, SWC represents seawater community comprised of 10% GF/F-filtered seawater, and SWC + AltSIO represents coculture of treatments above. All data are mean ± SD of two to three biological replicates. SWC + AltSIO January 2012 was plotted from a single incubation. In *D* and *E*, † represents cell C calculated from direct CHN measurements of each treatment from the January 2012 experiment (Table S4). DOC calculated by subtraction of cell C from TOC. *Significantly different from SWC by ANOVA, Tukey's $P < 0.05$.

significant difference (ANOVA, $P > 0.05$) was measured between treatment-specific means of absolute change in TOC, or biomass-corrected DOC concentration after 5 d (Table S4). Although the SWC + AltSIO treatment consistently resulted in the greatest proportion and rate of DOC drawdown across all experiments (Figs. 1 and 2), significant differences were only detected in November 2011 (Tables S5 and S6). No significant differences were measured in the proportion or rate of DOC drawdown between AltSIO and SWC treatments (Tables S5 and S6).

Long-Term Laboratory Incubation and TOC Drawdown Rates of Coastal Seawater. Incubation and measurement of TOC from the January 2012 experiment was continued for >1 y. No decrease in TOC concentration was detected in the AltSIO-only treatment beyond 3 d (Fig. 3). In contrast, the TOC concentration of the SWC treatment decreased from 70.2 to 57.8 μM between 3 and 379 d and the SWC + AltSIO treatment decreased from 68.1 to 59.6 μM C (Fig. 3). TOC values from the two seawater community incubations, with and without AltSIO, were combined and averaged by time point to determine a mean long-term (>30 d) drawdown rate of 6.5 μmol C·L^{-1}·y^{-1}. Cell abundance (cells·mL^{-1} ± SE) after 379 d of incubation in the SWC, AltSIO-only, and SWC + AltSIO treatments measured $4.2 \times 10^5 \pm 1.1 \times 10^5$, $3.9 \times 10^5 \pm 1.5 \times 10^5$, and $8.6 \times 10^5 \pm 5.3 \times 10^4$, respectively. All treatments and replicates contained viable cells that produced colony-forming units on both 2216 ZoBell agar and noble agar plates without nutrient amendment.

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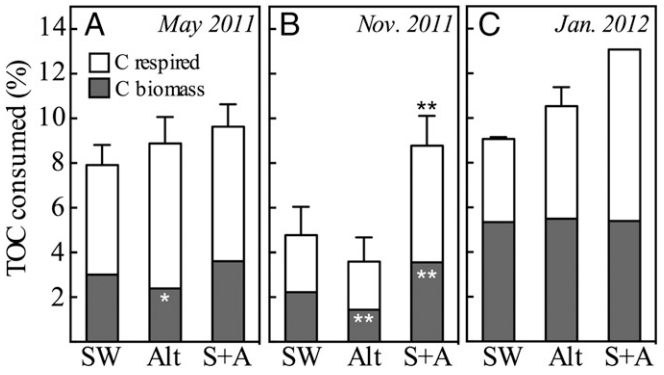

Fig. 2. Bacterial partitioning of coastal surface ocean DOC during three incubation experiments (*A–C*). Stacked bar graph of bacterial biomass (lower bar), C respired (upper bar), and total C consumed (sum of both bars) displayed as a percentage of initial TOC concentration. Alt, *Alteromonas* sp. AltSIO pure culture; SW, seawater community; S + A, coculture of SWC and AltSIO. All values are mean ± SD of two to three biological replicates. January 2012 S + A is a single incubation. Significant differences relative to SWC (ANOVA, Tukey's) were detected in bacterial biomass in May and November, but no differences were detected in the proportion of C respired between treatments in any experiment. The S + A treatment in November 2011 was the only to show a significant difference in total C consumed. $*P < 0.05$; $**P < 0.01$.

***Alteromonas* sp. AltSIO Survival in Size-Fractionated Seawater.** AltSIO was grown in size-fractionated natural seawater collected from the Ellen Browning Scripps Memorial Pier (hereafter "Scripps Pier"), Scripps Institution of Oceanography, to determine growth and survival when faced with bottom-up and top-down control. The <0.2 μm size fraction was intended to serve as a bacteria-free control to gauge the growth of AltSIO unchallenged by competition or grazing mortality, but was found to contain 1.5×10^5 cells·mL^{-1} after filtration of natural seawater. Thus, this treatment instead served to test the ability of AltSIO to compete with the smallest filterable size class of bacteria (30). AltSIO abundance increased from 10^4 to 10^5 cells·mL^{-1} within 24 h in both treatments without grazers (<0.2- and <0.7-μm size fractions) (Fig. 4 *A*, *B*, *D*, and *E*). After 24 h, the proportion of AltSIO to total bacterial cell abundance increased from 8% to 68% in the <0.2-μm size fraction, and from 0.4% to 3.0% in the <0.7-μm size fraction (Fig. 4 *A* and *B,* respectively). Within the same time period, AltSIO abundance decreased nearly 10-fold in the treatment with grazers, whereas protist abundance increased (Fig. 4*C*). AltSIO abundance then increased 25-fold following a decrease in protist abundance (Fig. 4*C*). By the end of the experiment AltSIO comprised 19%, 1%, and 0.05% of the total bacterial abundance in the <0.2-μm, <0.7-μm, and <50-μm treatments, respectively (Fig. 4).

Biovolume of AltSIO and Cell Size Distribution Frequency in the California Current Ecosystem. AltSIO measured 0.72 μm^3 and 72 fg C·cell^{-1}. The average of 17,388 individual cells from 29 samples from the CCE measured 0.13 μm^3 and 21 fg C·cell^{-1}, respectively, and ranged in volume from 0.02 to 1.79 μm^3, and C content from 6.6 to 107.6 fg C·cell^{-1}. Averaged within samples, mean values between samples ranged from 0.08 to 0.18 μm^3 and from 16.2 to 26.0 fg C·cell^{-1}. The distribution frequency of cell biovolume of each sample was binned into 0.2-μm^3 increments (Fig. S1*A*). Across all samples, the smallest size class (0–0.2 μm^3) accounted for 69–95% of total bacterial abundance (Fig. S1*A*). Large cells, defined here as ≥0.3355 μm^3 or ≥40 fg C·cell^{-1}, comprised up to 12% of the total bacterial abundance and up to 24% of the total bacterial C biomass (Fig. S1*B*). In all 29 samples from the CCE, large cells comprised ≥1% of the total bacterial community abundance (Fig. S1*B*). We note the strong agreement between microscopy-derived estimates of bacterial C and those derived from direct CHN measurements for both AltSIO and the mixed bacterial assemblages.

Discussion

A Single Bacterial Taxon Is Capable of Complete Drawdown of the Labile DOC Pool. We hypothesized that owing to the sheer chemical complexity of DOM, heterotrophic bacterial consumption of DOC may be dependent on the iterative processing by multiple species bearing complimentary metabolic capacities. Specifically, we tested the hypothesis that a single bacterial strain, alone, cannot consume a measureable degree of ambient DOC during nonbloom (phytoplankton), nonamended conditions. Here, we explicitly define "labile" and "semilabile" DOC fractions in our microcosms as those that are degraded by the free-living bacterial community (SWC treatment) in the short term (0–5 d), and long-term (>30 d), respectively. This operational nomenclature is in accordance with the universal definitions proposed by Hansell (2) based on apparent decay rates of major bulk DOC fractions.

Our findings demonstrate, unequivocally, that a single bacterial strain does in fact have the capacity to consume the labile DOC pool without the assistance of other members of the microbial community. This finding is derived from the repeated observations that a single strain consumed an equivalent magnitude of ambient DOC as three separate free-living microbial assemblages sampled over three seasons (Fig. 1). We note that these data cannot determine whether a single taxon necessarily remineralized the same chemical components of the DOM pool as complex microbial communities. In each experiment, the microbial and chemical community species composition was justifiably presumed different, yet the response of each treatment was consistent and robust. These data provide conclusive evidence that key individual taxa have the potential to exhaust the extant labile DOC pool in coastal surface seawater. This demonstrates that the fundamental biogeochemical process of labile DOC turnover does not require a complex community. This finding contributes to the ongoing discussion of functional redundancy within microbial ecosystems (31–33), and has

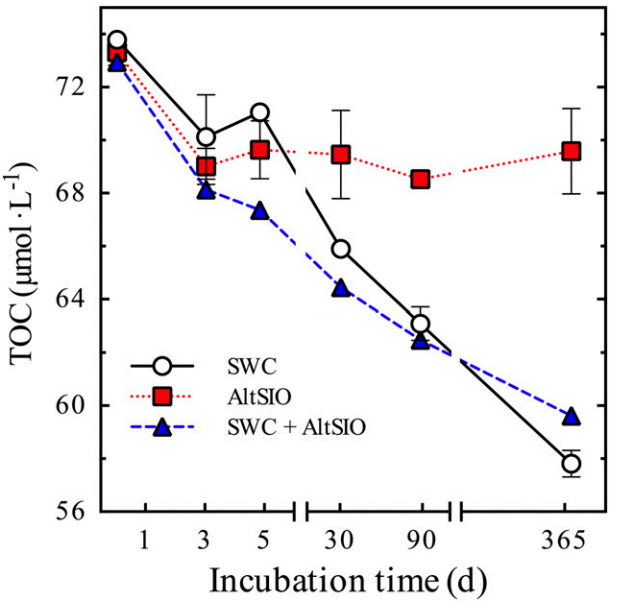

Fig. 3. Long-term carbon drawdown of January 2012 incubation. Data are mean ± SD of two biological replicates. SWC + AltSIO is a single incubation. TOC concentrations of AltSIO are significantly different from SWC and SWC + AltSIO on and after time points 31 and 89, respectively [repeated-measures two-way ANOVA, Sidak's multiple comparison post hoc test $P < 0.05$ (Table S7)]. Except on day 5, no significant difference was detected between SWC and SWC + AltSIO treatments.

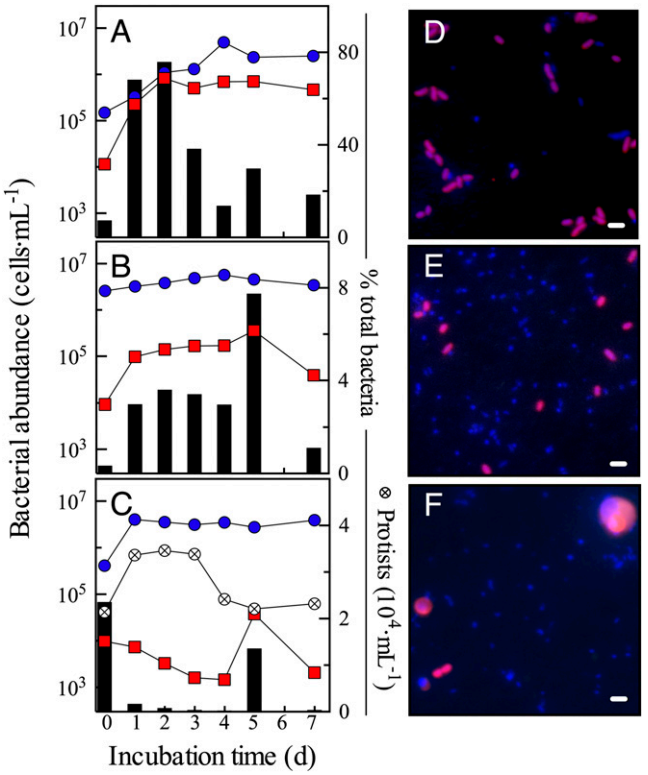

Fig. 4. Growth dynamics of *Alteromonas* sp. strain AltSIO cocultured with native microbial assemblages in size-fractionated coastal seawater: (*A* and *D*) <0.2 μm FSW, (*B* and *E*) <0.8 μm FSW, and (*C* and *F*) <50 μm FSW. (*A*–*C*) Abundances of AltSIO (red squares) and total bacteria (blue circles) are shown on the left *y* axis. Black bars are the proportion of AltSIO to total bacterial abundance (right *y* axis). Crosshatched circles (in *C*) show protist abundance (right *y* axis). *D*–*F* are representative images from day 3. Each image is a composite of three epifluorescence micrographs (1,000× magnification) with exposure/emission for Cyanine 3 (Cy3; FISH probe), DAPI (DNA), and FITC (autofluorescent photopigments). AltSIO-specific FISH probe-labeled cells appear red/cyan. A protist is imaged in the upper right corner of *F*. (Scale bar: 2 μm.)

important implications for our understanding of the relationship between bacterial community composition and the ocean's role in global carbon cycling.

Semilabile DOC Decay May Require Bacterial Community Diversity. During long-term incubation (>1 y) of coastal seawater, AltSIO unexpectedly ceased to consume DOC beyond 3 d (Fig. 3). This lack of measurable C consumption, despite maintenance of a relatively high abundance of viable cells, suggests that AltSIO entered a state of prolonged physiological dormancy (34). Our data do not support the hypothesis that growth became limited by N or P availability as dissolved nutrients were in excess of its cellular requirement (*SI Text* and Tables S2 and S3). This leaves open the possibility of an undetermined micronutrient requirement, or bioavailable-C limitation. In support of the latter, some gammaproteobacteria with similar physiological traits have been found to exhibit varying concentration threshold dependencies for metabolic activation following reintroduction to substrates after prolonged C starvation (16). With respect to DOC quality, Cherrier and Bauer (35) concluded from short-term incubations that the growth of bacterial populations in the eastern North Pacific became limited by the availability of readily catabolized sources of energy. Whether AltSIO growth became limited by bulk DOC concentration or quality is not known. In either case, that AltSIO remained culturable points to its ability to emerge from dormancy following increased nutrient supply, and supports the notion that similar "feast and famine" strategists (16, 36) may persist in the environment by virtue of such physiological capacity (37, 38). Lastly, the functional inability of AltSIO to consume DOC after 3 d, while parallel incubations of mixed microbial assemblages continued to consume DOC for >1 y (Fig. 3) suggests that substantial semilabile DOC decay may require broader community diversity than that required for labile DOC turnover. Although AltSIO appears incapable of consuming operationally defined semilabile DOC on its own, it is unknown whether other isolates, e.g., from the mesopelagic, possess such capacity.

DOC Concentration Thresholds and Apparent Decay in the CCE. A minimum average concentration of ~54 μmol C·kg^{-1} was associated with upwelling waters in the near shore region of the CCE (<210 km from shore) (Table S8). We interpret this data as representing the region-specific maximum DOC drawdown potential of resident microbial communities in the surface ocean (0–200 m), i.e., 54 μM C operationally demarks the transition between the semilabile and semirefractory DOC fraction in the CCE as defined by Hansell (2). Within this context, given that the experimental seawater community microcosms consumed carbon to 58 μM after 1 y (Fig. 3), we conclude that the field equivalent of the operationally defined semilabile DOC pool was nearly exhausted. Others have shown that depth stratified bacterial communities have specific limitations for DOC drawdown capability (4). If the same constraints hold true here, it would suggest that the microcosms populated by consortia originally collected from coastal surface waters may not have the capacity to consume DOC below ~54 μM. This prediction assumes that the physiological limitation of the community is sculpted by the chemical composition and concentration of the local nutrient supply.

Large Bacteria Are Important Players in the Ocean Carbon Cycle. *Alteromonas* sp. strain AltSIO is a model organism that represents an ecologically adaptive strategy for survival in the ocean: It is a large, fast-growing bacterium capable of rapidly responding to increased substrate supply, reduced competition, or reduced grazing pressure. In general, large cells are characterized by high nucleic acid content (39), high frequency of division (40), and exhibit high apparent growth and C production rates (41, 42).

The nearly fourfold greater C content of AltSIO (Table S3) led to a C demand equal to the free-living bacteria community (Table S5). Competition for resources did not have a measurable impact on the growth of AltSIO, as it rapidly outgrew the free-living bacteria seawater community and the smallest-sized bacteria in both DOC drawdown and grazing/competition microcosm experiments (Fig. 4 *A* and *D*), despite the theoretical competitive advantage of smaller bacteria to acquire dissolved nutrients (43). When added to size-fractionated natural seawater communities (Fig. 4), AltSIO retained its large size, yet was never eliminated from the population and exhibited an increase in abundance following a decrease in protist abundance (Fig. 4*C*). This suggests that preferential feeding of protists on a single morphotype may be prey density threshold-dependent, as has been observed in some freshwater heterotrophic nanoflagellages when feeding exclusively on large bacteria (44). However, even preferred prey populations have been shown to survive and persist during sustained grazing, albeit at low levels, during tight coupling between predator and prey populations (45). Thus, despite elevated grazing pressures, large bacteria such as AltSIO with similar survival strategies theoretically remain among those best poised to consume ephemeral substrate pulses following temporary release from top-down control. During periods of high predation they serve a critical ecosystem function as a substantial nutrient link via protozoa to higher trophic levels (46).

In the CCE, large cells (≥40 fg C·cell^{-1}) comprised 1–12% of the total bacterial abundance, but accounted for as much as 24% of the total bacterial carbon biomass (Fig. S1). Furthermore, large cells never decreased below 1% of total bacterial abundance (Fig. S1*B*). Field and mesocosm data presented here, suggest that large pelagic bacteria may at times achieve a refuge in rarity, thereby

persisting in the environment by function of decreased predator encounter rates as has been predicted for the rare biosphere (33). The range in the proportional frequency of large bacteria in the CCE also highlights the fact that bacterivorous grazer populations are themselves highly variable and tightly regulated by top-down control (47, 48). Given the inherent fluctuations in microbial predator–prey populations, the competitive advantage for the consumption of labile substrates remains with those whose physiology enables the most rapid response following decoupling from top-down control. The genomic basis enabling such physioptypes to rapidly mobilize from numerical obscurity to dominance has been described (49, 50). Furthermore, several recent studies have shown that typically rare members of the heterotrophic microbial assemblage are often highly active, suggesting a decoupling between abundance and specific activity in the environment and highlighting the importance of "rare" species in geochemical cycling and ecosystem function (51–53).

Conclusion

The labile DOC pool encompasses the single greatest flux of carbon, up to ~25 Pg C·y^{-1}, through the DOM reservoir in the global ocean (2). The majority of this reduced carbon is processed through the microbial loop in the upper ocean. Although the role of microbial community structure in carbon cycling has been of interest for decades, the quantitative contribution of individual bacterial strains remains almost entirely unknown. We have provided quantitative evidence that a single bacterial strain, or physiotype, can consume as much DOC as the diverse submicron size fraction of the ambient microbial assemblage within 5 d. Longer incubations showed that microbial diversity, although not required for labile DOM consumption, may instead be central to the recycling of semilabile DOM. These data imply that biotic degradation of more recalcitrant fractions of the DOM pool may scale with bacterial diversity and associated metabolic potential, but remains open for further investigation. Microcosm experiments and field data of bacterial size frequency in the California Current Ecosystem both showed that although large bacteria are preferentially grazed, they persist and contribute a significant proportion of total bacterial carbon in the environment. Together, these findings suggest that rarely dominant bacteria, otherwise intensely grazed or in a temporary survival state, may be responsible for a disproportionately large fraction of DOM recycling. Their outsized potential to influence the fate of carbon in the surface ocean suggests that rapidly growing large bacteria play an important role in ecosystem function and should be considered in global models of ocean carbon cycling (31).

Materials and Methods

Sampling, Experimental Setup, and *Alteromonas* sp. AltSIO Isolation. AltSIO (deposited with GenBank accession no. KC758958) was isolated by plating whole seawater collected from Scripps Pier (32° 52.02′ N, 117° 15.43′ W) onto noble agar [15 g·L^{-1} GF/F-filtered seawater (FSW)]. AltSIO shares ~99% 16S ribosomal DNA (rDNA) sequence homology with *Alteromonas macleodii*, a globally cosmopolitan gammaproteobacteria described as an opportunistic copiotroph (54) with wide strain-specific niche specialization (55).

DOC drawdown experiments were conducted with 0.1 μm filtered Scripps Pier seawater collected at ~1 m depth. Filtration used PES membranes (Supor-100; PALL) previously flushed with 2 L MilliQ (Millipore) water then 0.5 L seawater to remove leachable carbon (*SI Materials and Methods*). TOC concentration was measured in three treatments: (*i*) seawater community (SWC) (10% GF/F-FSW; 90% 0.1 μm FSW), (*ii*) AltSIO in pure culture, and (*iii*) SWC inoculated with AltSIO (SWC + AltSIO). Filter-sterilized seawater served as a control to test abiotic effects on TOC concentration. All experiments were conducted in the dark at ambient temperature (14 ± 1 °C to 16 ± 1 °C).

Microbial Abundance. Bacteria and protists were enumerated by epifluorescence microscopy using the nucleic acid stain DAPI (56). An *Alteromonas* sp. AltSIO strain-specific rDNA FISH probe was designed, tested, and optimized as described (57). Absolute concentration of AltSIO in cocultures was determined by multiplying the relative proportion of probe-positive cells within FISH-processed samples by total bacterial abundance determined from non-FISH–processed filters as described (57).

Direct Measure of Bacterial Cell Carbon and Nitrogen by CHN Analysis. January 2012 samples (~2L) were gravity filtered onto precombusted 25-mm GF/Fs, acidified in a desiccator to remove inorganic carbonates, then dried at 50 °C. CHN was measured by high-temperature combustion using an organic elemental analyzer (*SI Materials and Methods*). Bacterial biomass C in SWC and AltSIO treatments was determined from direct measurement of treatment-specific cell C (Table S2) multiplied by respective cell abundance. Bacterial biomass C of the combined SWC + AltSIO treatment was calculated from the proportional abundance and cell-specific C content of AltSIO and SWC at each time point as determined by FISH. Because all samples were inoculated into 0.1-μm FSW, nonbacterial particulate organic C was negligible.

TOC and DOC. Incubation carboys were sampled in a laminar flow hood using aseptic technique. TOC concentrations were measured by high-temperature combustion using a Shimadzu 500 V-CSN/TNM-1 TOC analyzer as described (58), and calculated from the average of five sample injections (100 μL) per two to three replicate vials. Samples from each experiment were analyzed in a single run to limit instrument variability, and interspersed with low DOC deep seawater reference material (59) (*SI Materials and Methods*). Following standard convention (4, 20, 26), DOC concentration from incubation experiments was calculated by subtracting bacterial biomass C (Table S4) from TOC measurements of unfiltered samples to avoid contamination from sample handling. Assuming a single C conversion factor for all bacteria (e.g., 20 fg C·cell^{-1}) proved erroneous owing to the significantly greater C content of AltSIO (Table S3). Thus, direct measurement of cell C proved critical to accurately measure treatment-specific DOC consumption. TOC included bacterial C and therefore measured decreases were directly attributed to bacterial respiration of C. Likewise, DOC consumption reported here is the cumulative of C respired and C incorporated into biomass, and thus reflects total bacterial C demand (60).

Frequency Distribution of Bacterial Cell Volume in the California Current Ecosystem. Samples (n = 29) were collected from 6 depths (0–100 m) from 5 conductivity, temperature, density rosette hydrocasts across a 29-km transect in the California Current (58). Length and width of 17,388 bacterial cells were measured by DAPI fluorescence and used to calculate biovolume (61), and cell-specific C content (62) as described (58). Autofluorescent cyanobacteria were manually eliminated from all measurements. Biovolume of AltSIO cells (n = 79) was measured by atomic force microscopy (AFM) as described for dry AFM samples (63).

***Alteromonas* sp. AltSIO Survival in Size-Fractionated Seawater.** Whole seawater was collected from Scripps Pier, passed through 50 μm Nytex nylon mesh, 0.7 μm GF/Fs, and 0.2 μm nuclepore polycarbonate filters, respectively. AltSIO was added to each size fraction at ~10^4 cells·mL^{-1} and incubated at 15 °C on a 12-h light:dark cycle at a light level of 100 μEinstein·m^{-2}·s^{-1}, and sampled daily for microbial abundance.

Statistical Analysis. Statistical analyses were preformed using GraphPad Prism for Mac OS X Version 6.0d (GraphPad Software, Inc.). Significant differences between treatments were tested for in May and November 2011 data by conducting analysis of variance (one-way ANOVA) and Tukey's post hoc multiple comparison tests at the 95% confidence interval (α = 0.05) on delta TOC values between days 0 and 5. Insufficient biological replication precluded statistical analysis on short-term (5-d) January 2012 data. Long-term (379 d) DOC drawdown data from January 2012 was analyzed by repeated measures two-way ANOVA and Sidak's multiple comparison test (α = 0.05) comparing the change in TOC concentration between AltSIO and AltSIO + SWC treatments individually against the SWC treatment over time.

ACKNOWLEDGMENTS. We thank M. Porrachia, B. Stephens, G. I. Ball, and C. Carlson for analytical support and technical advice; and T. Samo for providing raw bacterial size data from the California Current Ecosystem. We are grateful to K. Barott, T. Samo, and three anonymous reviewers for substantially improving this manuscript. This work was supported by the National Science Foundation (NSF) Graduate Research Fellowship Program (B.E.P.), Grant NSF OCE0962721 (to F.A.), the Gordon and Betty Moore Foundation Marine Microbial Initiative (F.A.), and NSF CAREER OCE-05-28475 (to L.I.A.). Field sampling was supported by NSF OCE 04-17616 and 10-26607 for the CCE Long-Term Ecological Research Program, and made possible by the captain and crew of the R/V *Melville*. Nutrient data was provided by the Pier Based Regional Harmful Algal Bloom Program with funding through the Southern California Coastal Ocean Observing Systems from US National Oceanic and Atmospheric Administration Grant NA11NOS0120029.

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