COMMENTARY

Microbial diversity and the lability of dissolved organic carbon

Craig E. Nelson[a,1] and Emma K. Wear[b]

[a]*Center for Microbial Oceanography: Research and Education, Department of Oceanography and Sea Grant College Program, University of Hawai'i at Mānoa, Honolulu, HI 96822; and* [b]*Marine Science Institute and Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106*

Marine metagenomics is steadily unveiling the phylogenetic diversity and metabolic potential of the microbial plankton. Our awareness that planktonic Bacteria and Archaea (bacterioplankton) dominate this diversity only underscores our inability to answer a basic ecological question: what are bacterioplankton eating? The majority of these organisms decompose organic matter either as a source of electrons or carbon (often both) and are classified as chemoheterotrophic (like animals and fungi). However, our understanding of the pool of organic compounds in nature remains largely amorphous, stymied by the challenges of resolving the chemical complexity of these heterogenous mixtures (1). At the same time, resolving these linkages is relevant to the global carbon cycle: bacterioplankton are the primary conduit for the massive pool of dissolved organic carbon (DOC) in aquatic ecosystems. Despite steady advances in our understanding of the quantity and quality of marine DOC and how they vary in space and time (2), our ability to directly link diverse heterotrophic microbes to their (presumably) diverse organic resources remains limited. This interaction regulates both the recycling of DOC to higher trophic levels (the microbial loop) and the remineralization of DOC to inorganic constituents, primarily carbon dioxide and other greenhouse gases. In PNAS, Pedler et al. (3) present the results of a study demonstrating that a single bacterial isolate is capable of removing an ecologically relevant pool of ambient DOC, contributing significantly to the growing body of work linking community structure and organic matter lability (4–6).

What is labile DOC, and what is the remainder of the DOC if not labile? The current geochemical conceptualization of DOC divides bulk concentrations into observed reactivity classes for the purposes of more accurately modeling global nutrient cycles (2). In most areas of the global ocean, the pool of labile material comprises compounds rapidly metabolized on the order of hours to days (LDOC); a larger semilabile pool is removed seasonally (SLDOC), and multiple refractory pools are removed slowly at scales ranging from years to millenia. Observable LDOC is a small portion of bulk DOC (typically <2%) (2) because this pool is rapidly metabolized, with bacteria estimated to consume roughly 50% of net primary production on a daily basis (7). The concentrations of various reactivity pools vary widely; in some oligotrophic systems, the tight coupling of production and consumption maintains LDOC stocks below limits of detection, whereas the dynamics of SLDOC are measurable and seasonally predictable (8, 9). In more eutrophic areas, such as upwelling coastal habitats, the spatial and temporal heterogeneity of bulk DOC concentrations, reactive fractions, and microbial responses can be large and dynamic at both diurnal and seasonal scales (Fig. 1) (10).

In contrast, we intuit that lability is not an inherent characteristic of DOC but rather a continuous function of the interaction between chemical composition and the metabolic capacity of the microbial community in a given environment. The empirical foundations of this DOC lability continuum are limited: our strongest evidence comes from observations that DOC persistent at one geographical location or depth can be consumed at another (11). For example, incubating seasonally accumulated DOC and microbes from the surface waters of the Sargasso Sea yields no significant change over weeks, but significant removal occurs when that water is inoculated with microbes (and available nutrients) from the deeper mesopelagic waters (9). This matches field observations of open ocean regions where seasonal mixing occurs, with surface-accumulated DOC becoming bioavailable once exposed to the mesopelagic microbial community and associated nutrient field (8, 12). There is a clear need for more experimental studies resolving interactions between specific components of ambient DOC and specific members of the microbial community that metabolize it; it remains difficult to link microbial community interactions with observational data on DOC reactivity.

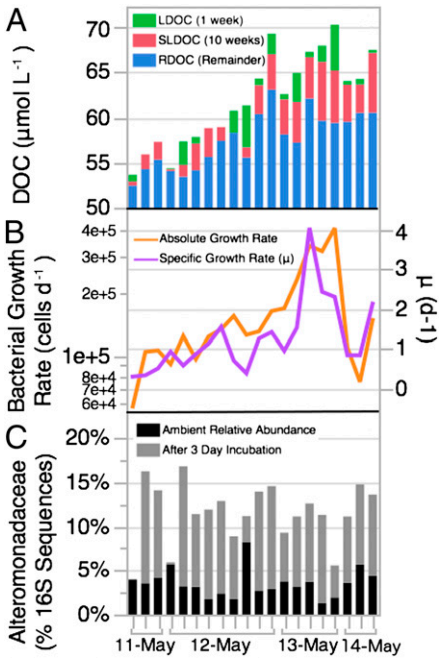

Fig. 1. Spatial and temporal heterogeneity in coastal DOC quantity and quality (*A*), the growth rates of total bacterioplankton (*B*) and enrichment of Alteromonadaceae (*C*) in 20 independent dilution culture incubations. Each *x* axis tick represents 1 of 20 independent dilution culture incubations initiated May 11–14, 2011 from a spatial grid spanning the surface waters of the Santa Barbara Channel, CA. Alteromonadaceae grew rapidly in most incubations, and both ambient (mean 3.5%) and 3-d (mean 11.5%) relative abundances of Alteromonadaceae were relatively stable across a wide range of DOC reactivities. DOC is colored according to reactivity pools to visualize removal over 1 (LDOC) and 10 wk (SLDOC) (*A*). Bacterial growth and specific growth rates are calculated from 5-d log-phase growth curves measured via flow cytometry (*B*). Alteromonadaceae relative abundances are calculated from classification of 16S rRNA gene pyrosequenced amplicons by the SILVA database (*C*). DOC and bacterial concentrations are averages of duplicate incubations; 16S amplicons were generated from a pooled sample.

Pedler et al. (3) contribute to integrating these two complementary conceptualizations of DOC lability. They compare DOC consumption in coastal Southern California seawater

Author contributions: C.E.N. and E.K.W. wrote the paper.

The authors declare no conflict of interest.

See companion article on page 7202.

[1]To whom correspondence should be addressed. E-mail: craig.nelson@hawaii.edu.

in bottles inoculated either with a complex ambient microbial community or a single bacterial strain cultured from these waters. The quantity of DOC removed by the ambient microbial community in the first 5 d (operationally interpreted as LDOC) was matched by the lone isolate, a Gammaproteobacteria in the family Alteromonadaceae. This result serves as an explicit experimental validation of a widely reported observation that incubating seawater containing measurable LDOC (i.e., exhibiting resolvable DOC drawdown on the order of days) fosters the rapid enrichment of ambiently rare opportunistic "copiotrophs," which are generally members of the family Alteromonadaceae (and allied Gammaproteobacteria within a monophyletic clade including Psuedoalteromonadaceae, Idiomarinaceae, and Vibrionaceae) (Fig. 1*C*) (5, 6, 9, 13, 14). The copiotrophic response is commonly observed in even unamended dilution incubations, with a handful of taxa exhibiting a consistently large enrichment independent of natural variability in the quantity of labile DOC and bulk bacterioplankton community response (Fig. 1).

This opportunistic growth suggests a capacity of copiotrophs to use heterogenous resource pools, but because they often emerge from rare ambient seed populations (6), their biogeochemical relevance remains an open question. Although past experiments have largely inferred that copiotrophic taxa are potentially able to rapidly consume LDOC, the results of Pedler et al. (3) make this explicit and demonstrate that a single strain can remove LDOC, implying that the diversity of natural assemblages is extraneous with respect to LDOC cycling. However, these experiments reduce grazing rates and manipulate the ambient community through significant prefiltration, suggesting more work needs to be done to extrapolate to field conditions. We still do not have direct in situ confirmation that copiotrophic taxa such as Alteromonadaceae are significant players in the ambient cycling of LDOC, and the present findings reiterate the importance of pursuing that goal. A key question is whether top-down controls such as viral lysis or grazing, demonstrated clearly by Pedler et al. (3) to be rapid and selective on the large cells of this isolate, limit the extent to which copiotrophic taxa play a significant role in removing LDOC or conversely enhance the removal and transfer of certain LDOC components to higher trophic levels.

By itself, the implication that a single taxon (freed from natural competition and top-down controls) may dominate the rapid dynamics of LDOC raises a suite of fundamental questions about the role of diversity, both phylogenetic and functional, in microbial biogeochemistry. However, Pedler et al. (3) present a second stimulating observation: in continuing to monitor one set of their incubations for a year, they show that after the first 3 d, bottles inoculated only with the cultured isolate ceased to remove DOC (although the cells remained viable), whereas the ambient communities continued to remove DOC (at a steadily slowing pace, eventually removing ∼20% over 12 mo). In contrast to the implications of the short-term incubations, this portion of the experiment agrees with previous observations (9) that additional members of the community are required to remineralize more recalcitrant fractions of the accumulated pool (that which turns over on the scales of weeks to months). Genomic and experimental evidence differentiating copiotrophic and oligotrophic bacterioplankton support a key role for generalist opportunistic copiotrophs in the rapid cycling of LDOC (13–15). By cleanly demonstrating the potential of a cultured copiotroph to remineralize LDOC and further clarifying the necessity of bacterioplankton diversity for remineralizing SLDOC on longer time frames, Pedler et al. (3) go beyond the copiotrophic response. That phylogenetic diversity, and presumably corresponding metabolic diversity, should facilitate the consumption of a broad array of compounds agrees with theories of niche complementarity and experimental validations of the interactions between phylogenetic diversity and the dynamics of resource supply and removal in marine bacterioplankton (16–18).

Pedler et al. present the results of a study demonstrating that a single bacterial isolate is capable of removing an ecologically relevant pool of ambient DOC.

The questions raised by this study and others touch on the forces shaping diversity in the bacterioplankton. When faced with the inexplicable diversity of photoautotrophic plankton, all of which ostensibly persisted on a simple diet of sunlight, water, carbon dioxide, and a narrow suite of mineral nutrients, Hutchinson coined the "Paradox of the Plankton" (19), codifying a concept that has stimulated decades of advances in ecological theories on the interplay between diversity and resource utilization. An analogous paradox is not a prominent part of the discussion surrounding the current explosion of culture-independent discovery of Bacterial and Archaeal diversity, but perhaps it should be. Our implicit assumption is that the phylogenetic diversity of microbes in soils and seas is maintained primarily by metabolic niche specialization of bacterioplankton on the diverse organic compounds left to decompose in ecosystems (a concept perhaps intuitively comfortable for mammals capable of smelling 10^{12} different compounds) (20). The results of Pedler et al. (3) cast new light on this assumption and urge us to delve more deeply into the linkages among the uncultured microbial majority and the composition of DOC to better grasp how bacterioplankton eating habits may shape the cycling of carbon in the biosphere.

1 Kujawinski EB (2011) The impact of microbial metabolism on marine dissolved organic matter. *Annu Rev Mar Sci* 3(1): 567–599.

2 Hansell DA, Carlson CA (2014) *Biogeochemistry of Marine Dissolved Organic Matter* (Elsevier, New York), 2nd Ed.

3 Pedler BE, Aluwihare LI, Azam F (2014) Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. *Proc Natl Acad Sci USA* 111:7202–7207.

4 Cottrell MT, Kirchman DL (2003) Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. *Limnol Oceanogr* 48(1): 168–178.

5 Gómez-Consarnau L, Lindh MV, Gasol JM, Pinhassi J (2012) Structuring of bacterioplankton communities by specific dissolved organic carbon compounds. *Environ Microbiol* 14(9):2361–2378.

6 Nelson CE, Carlson CA (2012) Tracking differential incorporation of dissolved organic carbon types among diverse lineages of Sargasso Sea bacterioplankton. *Environ Microbiol* 14(6):1500–1516.

7 Ducklow HW (1999) The bacterial component of the oceanic euphotic zone. *FEMS Microbiol Ecol* 30(1):1–10.

8 Carlson CA, Ducklow HW, Michaels AF (1994) Annual flux of dissolved organic-carbon from the euphotic zone in the Northwestern Sargasso Sea. *Nature* 371(6496):405–408.

9 Carlson CA, et al. (2004) Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. *Limnol Oceanogr* 49(4):1073–1083.

10 Halewood ER, Carlson CA, Brzezinski MA, Reed DC, Goodman J (2012) Annual cycle of organic matter partitioning and its availability to bacteria across the Santa Barbara Channel continental shelf. *Aquat Microb Ecol* 67(3):189–209.

11 Carlson CA, Hansell DA, Tamburini C (2011) DOC persistence and its fate after export within the ocean interior. *Microbial Carbon Pump in the Ocean* (American Association for the Advancement of Science, Washington, DC), pp 57–59.

12 Goldberg SJ, Carlson CA, Hansell DA, Nelson NB, Siegel DA (2009) Temporal dynamics of dissolved combined neutral sugars and the quality of dissolved organic matter in the Northwestern Sargasso Sea. *Deep-Sea Res Pt I* 56(5):672–685.

13 McCarren J, et al. (2010) Microbial community transcriptomes reveal microbes and metabolic pathways associated with dissolved organic matter turnover in the sea. *Proc Natl Acad Sci USA* 107(38):16420–16427.

14 Mou X, Sun S, Edwards RA, Hodson RE, Moran MA (2008) Bacterial carbon processing by generalist species in the coastal ocean. *Nature* 451(7179):708–711.

15 Lauro FM, et al. (2009) The genomic basis of trophic strategy in marine bacteria. *Proc Natl Acad Sci USA* 106(37):15527–15533.

16 Giovannoni SJ, Vergin KL (2012) Seasonality in ocean microbial communities. *Science* 335(6069):671–676.

17 Morris RM, et al. (2005) Temporal and spatial response of bacterioplankton lineages to annual convective overturn at the Bermuda Atlantic time-series study site. *Limnol Oceanogr* 50(5): 1687–1696.

18 DeLong EF, et al. (2006) Community genomics among stratified microbial assemblages in the ocean's interior. *Science* 311(5760): 496–503.

19 Hutchinson GE (1961) The paradox of the plankton. *Am Nat* 95(882):137–145.

20 Bushdid C, Magnasco MO, Vosshall LB, Keller A (2014) Humans can discriminate more than 1 trillion olfactory stimuli. *Science* 343(6177):1370–1372.