TECHNICAL COMMENT

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Comment on "Dilution limits dissolved organic carbon utilization in the deep ocean"

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Arrieta *et al*. (Reports, 17 April 2015, p. 331) propose that low concentrations of labile dissolved organic carbon (DOC) preclude prokaryotic consumption of a substantial fraction of DOC in the deep ocean and that this dilution acts as an alternative mechanism to recalcitrance for long-term DOC storage. Here, we show that the authors' data do not support their claims.

The mechanisms controlling the reactivity/recalcitrance continuum of dissolved organic carbon (DOC) in the nutrient-rich deep ocean remain a "recalcitrant" problem due to the complexity of the underlying biogeochemical interactions and the limitations of current methods (*1*). The experimental study by Arrieta *et al.* (*2*) claims to provide new evidence for the existence of labile DOC that behaves as recalcitrant DOC (RDOC) when its concentration is below the threshold for prokaryotic utilization. The publication of Arrieta *et al.*'s data (*2*) is timely given that a recent review article (*3*) proposed that there are two categories of RDOC in the ocean: RDOC that consists of compounds occurring at concentrations below the uptake thresholds of prokaryotes [concentration-constrained ($RDOC_c$)] and RDOC that is recalcitrant in a given biogeochemical context [environmental context-dependent ($RDOC_t$)]. Dilute labile DOC *sensu* Arrieta *et al.* (*2*) is the same as $RDOC_c$ (*3*).

Arrieta *et al.* (*2*) tested experimentally the null hypothesis that "no significant increase in prokaryotic growth should be detectable when increasing DOC concentrations." Their enrichment experiments showed increased prokaryotic growth, consistent with the dilution hypothesis. However, if low concentrations did limit prokaryotic growth in Arrieta *et al.*'s (*2*) incubations, then the corollary of their hypothesis should also hold—i.e., the prokaryotic consumption of DOC should be related to the DOC concentration. We therefore reanalyzed the data of Arrieta *et al.* (*2*) to test the null hypothesis that no significant increase in DOC consumption by prokaryotes should be detectable when increasing DOC concentrations. Plotting the authors' incubation measurements from Stations K, L, and N (data from Station M were anomalous due to a second phase of intense growth) shows, first, that the relative fraction of DOC consumed by prokaryotes is below 6% in each of the assays and, second, that this fraction is independent of the DOC enrichment (2×, 5×, or 10×) (Fig. 1). The lack of rejection of our null hypothesis indicates the inconsistency of Arrieta *et al.*'s data with the dilution hypothesis.

Arrieta *et al.* (*2*) also based their conclusion that dilute labile DOC is a substantial fraction of DOC in the deep ocean on the results of specific growth rate experiments, in which they used population growth kinetics to estimate the minimum concentration of DOC required for growth of deepwater prokaryotic communities [figure 2, figure S3, and table S2 in (*2*)]. However, the minimum concentration of bulk DOC required for growth of a prokaryotic community cannot be equated with the threshold values of availability of single DOC compounds. Instead, the dilution hypothesis as formulated by Arrieta *et al.* (i.e., most organic substrates in the deep ocean are labile but cannot be used by prokaryotes at concentrations below the levels matching the energetic investment required for their uptake and degradation) suggests that at steady state, and neglecting abiotic processes that could transform DOC, the supply of individual dilute labile DOC compounds is balanced by prokaryotic uptake to just below their respective uptake thresholds. This is illustrated in Fig. 2, which shows that, if low concentrations of individual labile DOC compounds had indeed prevented prokaryotic utilization in the deep-water samples, then the consumption of bulk DOC should have increased substantially with higher DOC enrichments, but it did not (Fig. 1). The majority (~94%) of bulk DOC remained at the end of the incubations (Fig. 1), which is not consistent with the dilution hypothesis and is thus in contradiction of Arrieta *et al.*'s (*2*) conclusion that their figures 1 and 2 "validated the dilution hypothesis

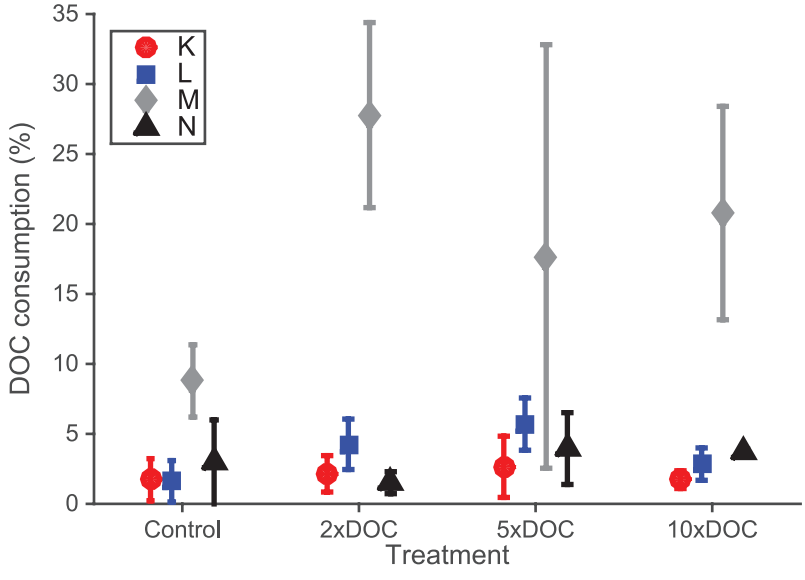

Fig. 1. DOC consumption as a function of DOC enrichment (data from Arrieta *et al.*). Apart from Station M, which is anomalous, all the data from Stations K, L, and N show that only <6% of the DOC is consumed regardless of enrichment (2×, 5×, and 10×), and the remaining >94% is not used.

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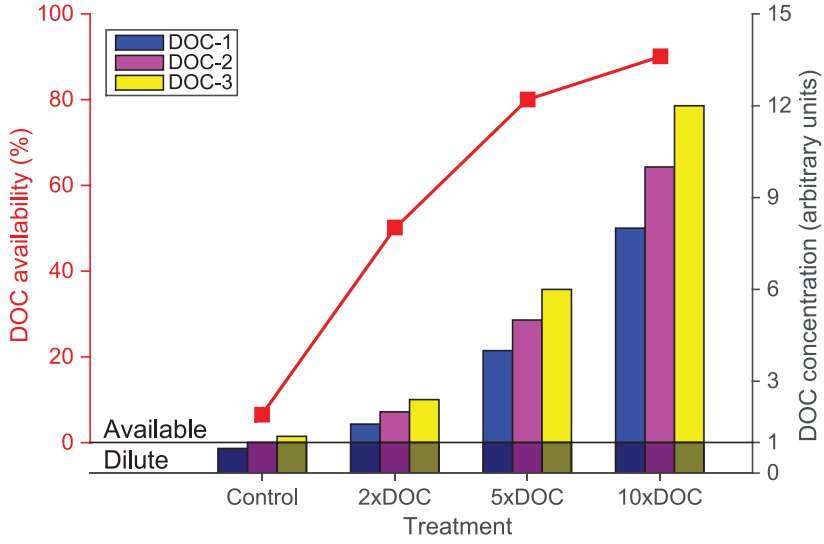

Fig. 2. The expected increase in DOC availability with increasing DOC enrichment in a scenario where the availability of labile DOC to prokaryotes is limited by dilution. It is assumed here that labile DOC compounds in the deep ocean are at three concentration levels (DOC-1, below uptake threshold; DOC-2 at threshold; and DOC-3, above threshold), and the threshold is set at a concentration of 1 (arbitrary units). This thought experiment reveals that the availability of labile DOC increases rapidly with increasing enrichment, so that an increasing proportion of labile DOC in the incubations should be taken up by the prokaryotes, which is contrary to what is observed in Fig. 1.

tested, showing that dilution limits C utilization in the deep ocean."

One possibility that could explain these contradictory interpretations of Arrieta *et al.*'s data is that the increased prokaryotic growth in the enrichment experiments did not reflect a release of dilution limitation by the enrichments. Some labile DOC compounds may have been in the original sample, and their concentrations accordingly increased with the bulk DOC enrichment. Hence, although the measured prokaryotic growth increased with DOC enrichment, the proportion of total DOC consumed (DOC consumption, %) did not (Fig. 1). This possibility is consistent with the fact that prokaryotes grew in most of the controls [in figure 1 and figure S2 in (*2*) , prokaryotic abundance typically doubled over 10 to 20 days in controls at 10 of the 14 stations]. Other potential labile DOC sources in the incubations include viral lysis (*4*), chemolithoautotrophic activities (*5*, *6*), and grazing by protists (*7*), which are independent of enrichment. This could explain Arrieta *et al.*'s Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) data, which showed generation of new DOC compounds during the incubations but no correlation between the numbers of compounds utilized and the enrichments. In fact, at one of the two stations sampled, Station P, more compounds were utilized in the controls than in the 5× enrichment (i.e., *1753* versus *936*, respectively) (*2*).

The use of novel technical and analytical approaches by Arrieta *et al.* (*2*) to experimentally study the prokaryotic uptake of deep-ocean DOC provides a renewed perspective on investigating the availability and recalcitrance of the huge inventory of DOC in the deep ocean, an enigma that was first highlighted in the seminal study of Barber in 1968 (*8*). However, as is often the case, probing the natural environment with advanced techniques (including here solid phase extraction of organic compounds and FT-ICR-MS) leads to new questions instead of simple testing of initial hypotheses. In the present case, although Arrieta *et al.*'s experiments (*2*) were inconclusive with regard to the dilution hypothesis, they lead the way to test this hypothesis in the future and provide new evidence for the microbial generation of $RDOC_t$ in deep oceanic waters (*1*, *9*).

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