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

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Abstract
Our recent finding that dilution limits dissolved organic carbon (DOC) utilization in the deep ocean (*1*) **has been criticized** (*2*) **based on the common misconception that lability equates to rapid and complete utilization. Even when considering the redefinition of recalcitrant DOC recently proposed by Jiao et al.**(*3*) **the dilution hypothesis best explains our experimental observations.**

A large reservoir of DOC preserved within the deep oceanic water masses has been termed "refractory" or "recalcitrant" in the bulk of the scientific literature. Recalcitrant DOC is defined as "resistant to rapid microbial degradation"(*4*) or "resistant to microbial utilization"(*3*) implying that the intrinsic properties of these substrates make them unavailable to oceanic microbes. In a recent study(*1*), we tested the alternative hypothesis that the dilution of the different constituents of DOC rather than their chemical properties limits DOC utilization in the deep ocean (*5*). Our results show that a substantial part of the deep-sea DOC pool is labile (i.e., available to the in situ microbial community) but the low individual concentrations of its many constituents slow down the overall rates of utilization. Jiao et al.(*6*) recently redefined recalcitrant DOC (RDOC) as consisting of two pools: $RDOC_c$ comprising labile compounds that are too diluted to be efficiently utilized by bacteria (the same mechanism that we demonstrate) and $RDOC_t$ consisting of labile compounds that cannot be used by the actual microbial community in their environmental context. Our study does not rule out the existence of recalcitrant molecules, which would be impossible to test experimentally for each of the thousands of different compounds present. Yet, Jiao et al. (*6*) implicitly deny the existence of structurally recalcitrant molecules in the DOC pool as none of the RDOC fractions in their new definition is inherently "recalcitrant". Thus, Jiao et al.(*6*) concur with our assertion that much of the deep oceanic DOC is not intrinsically recalcitrant as defined in the bulk of existing literature (*3*, *4*, *7*, *8*).

In their comment, Jiao et al. (*2*) argue that our data do not support the dilution hypothesis because increasing the DOC concentrations in our experiments did not result in a much higher percentage of DOC utilization. They also model the DOC pool as an even mixture of compounds present at three concentration levels. Based on this crude model, they estimate that DOC availability should increase on average from 6 to 90% when increasing the bulk DOC concentration by a factor of 10 if all of the DOC were labile. Consequently, and based on the fact that the fraction of carbon utilized at the end of our experiments did not exceed 6% in most cases, Jiao et al. (*2*) estimate that labile carbon represents at most 6% of the total DOC and conclude that the remaining 94% must be recalcitrant or, according to their new definition of recalcitrant DOC, "context-recalcitrant" DOC.

Unfortunately, their model is flawed by the underlying misconception that lability implies immediate and complete utilization regardless of concentration. Thus, they assume that the amount of DOC consumed at the end of the experiments represents

the total amount of labile DOC present over threshold concentrations. This naïve expectation assumes that 40 days suffice to utilize all the labile DOC in the samples and that the utilization of a given substrate is either inhibited or proceeds at maximum speed in contrast with the well-established concentration-dependent model of microbial substrate utilization (*9*). A more likely scenario, consistent with microbial substrate uptake kinetics, is that in the deep ocean DOC utilization will be relatively slow, limited by molecular diffusion, with uptake systems operating well below their maximum velocity at the low end of the Monod curve. Our experimental data indicate that labile DOC was not used completely at the end of the experiment and that substantial DOC consumption occurred after no apparent increase in cell abundance was detectable (*1*). Although our experiment was designed to test the growth response of bacteria and not to estimate the total amount of labile DOC a more realistic estimation of what happened in our experiment can be obtained by using rates of utilization rather than final concentrations.

To better illustrate these processes, we performed a simulation in which utilization was solely limited by concentration (Figure 1A). Total consumption rates were higher with increasing concentrations; however, substrate consumption did not decrease down to the control levels after one year. The % of DOC utilized did increase with increasing concentrations but very slowly. The total DOC utilized after 40 d equaled 2.1, 2.6, 3.7 and 5.3% of the initial concentration for controls, two-, five- and ten-fold concentrated treatments (Figure 1B), well within the range of our observations but probably not enough to allow significant statistical comparisons given the scatter associated to a 40-day incubation experiment. Hence, this simulation demonstrates that the small proportion of DOC used in our experiments is consistent with the dilution hypothesis although complete consumption of labile substrates would take much longer as it does in the deep ocean. Thus, the use of short-term incubation experiments to quantify the amount of labile DOC present in natural samples proposed by Jiao and colleagues leads to wrong conclusions (2).

The corollary of the dilution hypothesis that “prokaryotic consumption is related to DOC concentration” still holds since both microbial growth and total substrate consumption markedly increased in concentrated samples as compared to controls clearly demonstrating that concentration limited prokaryotic growth. Jiao et al. (*2*) argue that the growth observed in our experiments could be due to a small amount of labile DOC already detectable in the unamended controls, implicitly admitting that growth (and therefore DOC consumption) was limited by concentration at least for this small fraction of DOC that they estimate to be 6% of the bulk concentration. Yet, consumption was detected across thousands of different compounds in both controls and concentrated samples (*1*) which probably comprise a much higher proportion of the total DOC. Thus, a large fraction of the DOC compounds (and of the bulk DOC) in our samples was neither intrinsically- nor context-recalcitrant (there was no difference in “biogeochemical context” between treatment and controls apart from DOC concentration) and dilution remains the mechanism that best explains our results. The fact that we detected utilization of fewer compounds in the concentrated treatment in one of the experiments does not make these molecules less labile.

Jiao et al. also argue that other sources of labile materials like chemolithoautotrophy, grazing and viral lysis may have played a role, however it is unclear how chemolithoautotrophy would be enhanced by increasing DOC concentrations. Increases in grazing and viral lysis are incidentally derived from enhanced bacterial growth caused by higher DOC concentrations but not the primary factor fueling bacterial growth.

Our results indicate that apparent recalcitrance is largely related to low substrate concentrations. Thus, deep oceanic microbes may not be functioning as a "carbon pump" (*2*, *3*), transforming labile DOC into recalcitrant compounds prone to accumulate in the ocean but rather as efficient degraders of a vast array of compounds even at very low, growth-limiting concentrations. Microbial production of persistent DOC by mechanisms other than substrate dilution remains to be demonstrated although recent reports suggest that this contribution may be minor (*10*).

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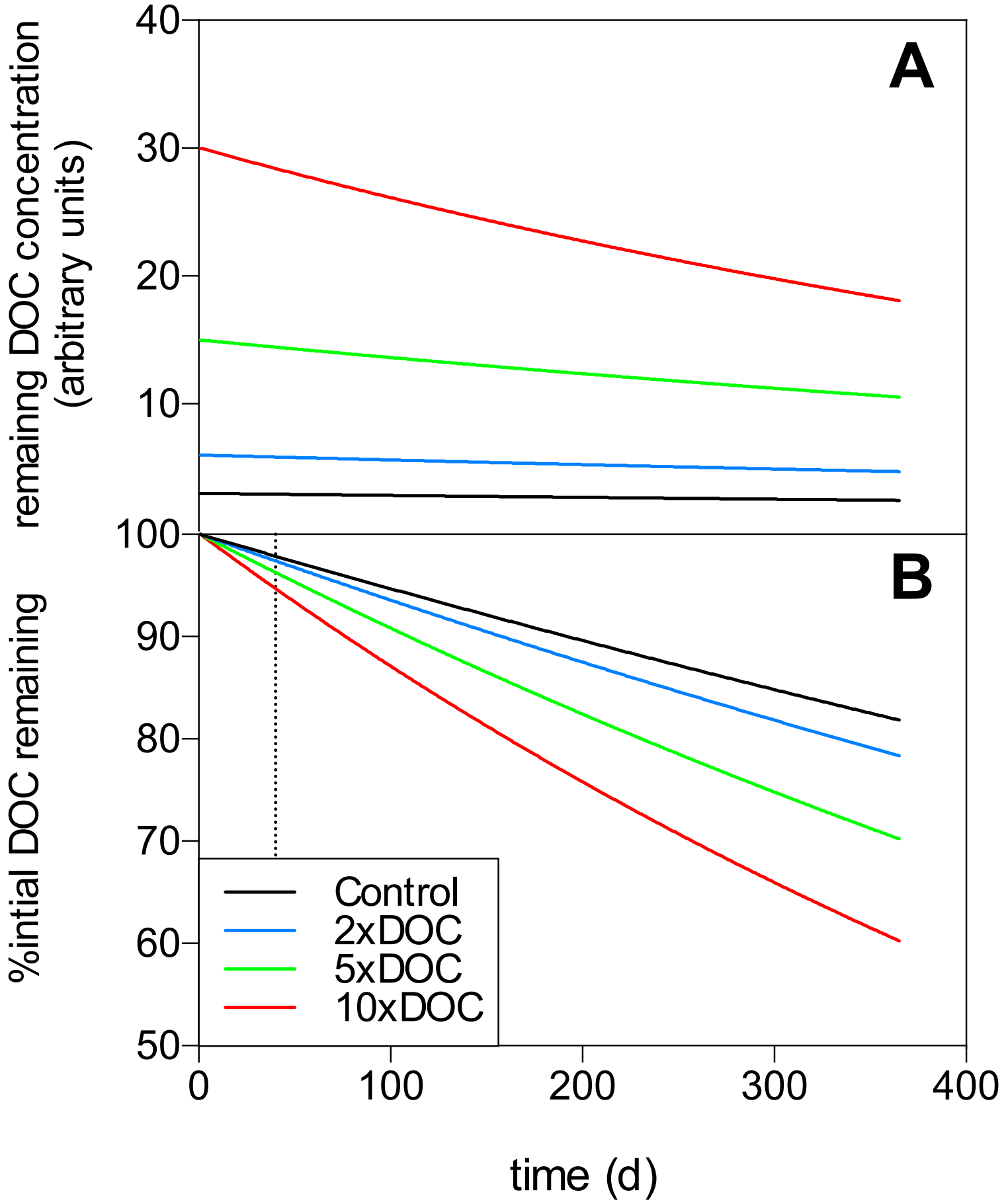
Figure legends

Figure 1. Expected DOC utilization as a function of DOC enrichment in a scenario where utilization is limited by dilution. We started our simulation with three different DOC concentration levels in each sample (0.8, 1 and 1.2 arbitrary units in the control and the corresponding two-, five- and ten-fold initial concentrations) for consistency with the model of Jiao et al.(*2*). The rate of utilization for each DOC pool was calculated as a function of substrate concentration and cell abundance, assuming a specific substrate affinity of $1x10^{-11}$ (l $cell^{-1}$ d^{-1}) and using the maximum cell abundances observed in experiment N for each treatment (55, 67, 97 and 139 $x10^6$ prokaryotes L^{-1} for the controls, two-, five- and ten-fold DOC treatments, respectively). The average specific substrate affinity was estimated from the 2% decrease in DOC concentration observed in control treatments of experiments K-N after 40 days. Our calculated utilization rates are proportional to substrate concentration and we have assumed one average affinity for all the substrates, thus our model does not change if we use one or many substrates and therefore only bulk DOC concentrations are presented. This simplified model also assumes that all of the bacteria present were able to utilize all of the substrates present with the same average affinity and is likely to result in an overestimation of utilization rates as compared to the real world scenario involving a diverse microbial community feeding on a very diverse molecular mixture of DOC including a large range of concentrations and affinities. Despite these limitations this is a more realistic approach that more closely resembles the processes measured in our experiments as well as those occurring in the deep ocean.

A. Bulk consumption of DOC over time. Increasing substrate concentration ten-fold results in a much higher bulk consumption rate, however labile substrates are not brought down to the initial levels after one year of incubation.

B. Percentage of substrate remaining over one year of incubation. At least 95% of the initial substrate remains unused after 40 days (dotted line) of microbial consumption even in the most concentrated treatments. Note that we assumed that utilization rates increase constantly with concentration and this is only valid at the low end of the Monod model. If substrate concentrations reached saturation levels in the more concentrated treatments as observed in our experiments, the differences between the percentage of utilization in concentrated versus control treatments would be even smaller.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