LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 67, 2022, 680–683

doi: 10.1002/lno.12026

Reply to comment: Controls on turnover of marine dissolved organic matter—testing the null hypothesis of purely concentration-driven uptake

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Abstract

Our recent bioassay experiments indicate that molecular properties are a primary control on the microbial utilization of dissolved organic matter in the ocean. This finding is questioned by Lennartz and Dittmar who modeled our experiments and concluded that our observations could be largely explained by concentration-driven uptake independent from molecular properties. We suggest the authors' models are deficient for establishing the relative roles of molecular properties and concentration-driven uptake. Our conclusion is consistent with earlier and recent experimental results and biogeochemical observations, supporting a unified theory with molecular properties as a more prominent control than concentration-driven uptake on marine organic carbon accumulation.

Earth's oceans hold a vast quantity of refractory dissolved organic carbon (RDOC) that plays an important role in carbon sequestration and regulation of global climate. Mechanisms behind the long-term persistence of RDOC remain elusive today (Baltar et al. 2021). Over the past five decades, there has been growing interest in determining the factors controlling the utilization and accumulation of RDOC in the ocean (Barber 1968; Williams and Druffel 1987; Jannasch 1995; Ogawa et al. 2001; Jiao et al. 2014; Arrieta et al. 2015; Benner and Amon 2015; Walker et al. 2016; Shen and Benner 2018, 2020; Bercovici et al. 2021; Zakem et al. 2021). The relative significance of two long-debated mechanisms, stable molecular properties (e.g., Barber 1968; Shen and Benner 2020) and low molecular concentrations (e.g., Jannasch 1995; Arrieta et al. 2015), is the focus of our recent article (Shen and Benner 2020) and the Comment by (Lennartz and Dittmar 2022).

In our study, we evaluated the significance of these two mechanisms using bioassay experiments with unfiltered seawater collected at five depths (50–1500 m) at the Bermuda Atlantic Time-Series Study site (BATS) (Shen and Benner 2020). The incubations were separately amended with two seawater-derived substrates with different molecular compositions: plankton-derived dissolved organic matter from surface water (pDOM; enriched in amino acids and carbohydrates) and C-18 extracted DOM from deep water (C-18 DOM; depleted in labile components). Our results showed that microbial communities from all sampling depths rapidly utilized additions of pDOM but failed to utilize additions of C-18 DOM, suggesting molecular properties are a primary control on the microbial utilization of DOM in the ocean. In their comment, Lennartz and Dittmar simulated our experimental results using numerical models that tested the null hypothesis of purely concentration-driven microbial uptake. Their models produced results comparable to those of our bioassay experiments, leading the authors to conclude that most of our experimental observations could be explained by concentration-driven uptake and were not necessarily reflective of molecular properties.

The models used by Lennartz and Dittmar involved few parameters and many assumptions. Their network models assumed that all DOC compounds were on average equally available to a part of the microbial community. The authors justified this assumption by simply citing previous genomic studies (e.g., Sogin et al. 2006; Landry et al. 2017; Salazar and Sunagawa 2017). However, those previous studies showing broad metabolic potential of microbes did not conclude the omnipotence of microbes in utilizing all DOC compounds in the ocean. The diversity of microbes in the ocean is amazing, but still relatively little is known about their metabolic capabilities and growth (Arístegui et al. 2009; Kujawinski 2011; Sunagawa et al. 2015). The longevity of DOC in the model

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was shaped by the limitation of bacterial growth on low DOC concentrations, and a half-saturation constant acted as an important control on the size of the DOC reservoir. The model also set a theoretical minimum DOC concentration that could be utilized by microbes. Given the complexity and heterogeneity of the global ocean, it is unlikely that these simple growth and substrate utilization models are adequate for representing DOC utilization by all microbes.

Leaving the validity of model assumptions aside, we found that Lennartz and Dittmar's models were somewhat circular. The models were spun-up to a preset condition that would react differently to supplied compounds via distance from supply. By knowing in advance the distinct bioreactivity of the two substrates used in our experiments, the authors assigned the fast-degrading pDOM the shortest pathlength (Position 0 in the network) where a very high abundance of pDOM degraders is present. In contrast, the slow-degrading C-18 DOM was assigned to the longest pathlength associated with a low abundance of C-18 DOM degraders. As such, the degradation rates of substrates were not related to the difference in their molecular concentrations but instead dependent on their network positions and degrader abundances. While their models tested concentration-driven uptake, there were not any comparisons of compound concentrations between the two substrates used in our experiments. The different assignments for substrate positions and for abundances of substrate-specific consumers are indicative of the importance of molecular properties, which is in support of our original conclusion. The authors further explored a modified model, where the inclusion of varying uptake rates led to much better results than their basic model with equal compound reactivity. Likewise, the varying uptake rates were set as a function of pathlength and were intimately linked with the molecular properties of substrates.

Lennartz and Dittmar attributed the low degradation rate of C-18 DOM to the low abundance of C-18 DOM degraders and the substantial time needed for these specific consumers to grow. Their interpretation suggests the existence of specialized microorganisms, which contradicts their initial assumptions, i.e., all DOC compounds are equally available to microbes. More importantly, the occurrence of substrate-specific consumers in their models points to the varying responses of microbial communities to different organic substrates. This also supports our original conclusion.

Our experimental observations are consistent with previous incubation experiments, composition and age characterizations, and recent modeling results. Previous bioassay experiments with enriched marine DOC all presented similar results, including the study conducted by Arrieta et al. (2015). It is only the interpretation of those experiments that has varied (e.g., Arrieta et al. 2015; Jiao et al. 2015). Bioassay experiments in our and previous studies have repeatedly shown that the bioavailability of DOC from various oceanic regions increased only marginally when concentrated (Barber 1968; Arrieta et al. 2015; Sosa et al. 2015; Shen and Benner 2018). This was seen regardless of enrichment factor or sampling depth (Bercovici et al. 2021). Further, recent bioassay experiments separately feeding bacteria free and combined forms of amino acid have shown contrasting bacterial responses (Paerl et al. 2020). They found that marine bacteria rapidly utilized free tyrosine but failed to grow on dityrosine, an oxidative dimer of tyrosine, even at high concentrations (60 μmol C L^{-1}) (Paerl et al. 2020). These experimental results consistently suggest a secondary role of molecular concentration in controlling the microbial utilization of marine DOC.

A rationale and support for the concentration-driven persistence of DOC is in part derived from a perception of high DOM molecular diversity as suggested by ultra-high-resolution mass spectrometry (e.g., Arrieta et al. 2015; Dittmar et al. 2021). A recent study using ion mobility mass spectrometry to explore isomer diversity of natural DOM found that structurally distinct isomers only occurred in a small fraction of the detected DOM formulas (Lu et al. 2021). Based on field measurements and bioassay experiments, Lu et al. (2021) further demonstrated that the isomers in natural DOM became less diverse with advanced degradation. These new observations suggest that the molecular diversity of DOM in the ocean, particularly in the deep ocean, is likely lower than what has been speculated (e.g., > 600,000 DOM molecules per liter of deep water; Mentges et al. 2017; Dittmar et al. 2021). Therefore, a greater number of DOM molecules in the ocean likely persist at higher concentrations than previously thought (e.g., < 3 pmol C L^{-1}; Dittmar et al. 2021). Previous studies indicated that more than half of the solid-phase extracted DOM from the surface and deep oceans was composed of potentially refractory carboxyl-rich alicyclic molecules (CRAM) (Hertkorn et al. 2006, 2013; Lian et al. 2021). The high relative concentrations of CRAM do not lead to their rapid degradation in the ocean, suggesting a minor influence of molecular concentration on DOM residence time.

In further support, the first atomic-resolution images of marine low-molecular-weight (LMW) DOM shown by Fatayer et al. (2018) also pointed to a primary control of molecular structure on the persistence of DOM. Using atomic force microscopy, these authors examined molecular-level architectures on LMW DOM extracted in similar concentrations from the surface (7.5 m; 15 ± 1 μmol C L^{-1}) and deep waters (2500 m; 12 ± 1 μmol C L^{-1}) of the Hawaii Ocean Time Series station ALOHA. They found that approximately 20% of the molecules measured in the deep sample possessed planar moieties, a value almost tripling that (8%) for surveyed molecules from the surface. Further, planar structures identified in the deep sample were found to contain a higher relative content of aromatic sp^2 hybridized carbon indicating greater aromaticity of DOM in the deep ocean than in the surface ocean. Their findings suggest a buildup of planar aromatic molecules at depth. The buildup cannot be explained by the concentration-dependent uptake, because this mechanism would predict higher rates of degradation for the more abundant planar

aromatic molecules in the deep ocean than in the surface ocean. Such a prediction is inconsistent with the more negative DOC $\Delta^{14}C$ values (i.e., older ages) observed in the deeper ocean (Fatayer et al. 2018).

Our findings are consistent with a recent modeling study that investigated the persistence of DOC in the ocean and estimated the relative contribution of functionally labile vs. refractory molecules to the total organic carbon pool (Zakem et al. 2021). These authors developed a mechanistic model that included a more complete set of biological parameters and substrate-microbe interactions than that by Lennartz and Dittmar. The mechanistic model revealed a bimodal distribution of labile and refractory molecule concentrations suggestive of distinct controls on organic matter persistence. It was found that functionally labile molecules persist at low concentrations (10^{-4} to 1 μmol C L^{-1}) and together make up a relatively small fraction of the total organic carbon pool. In contrast, the majority of the organic carbon pool was found to be composed of refractory molecules that persist at relatively high concentrations (0.1–10 μmol C L^{-1}). The modeling results of Zakem et al. (2021) are more consistent with our experimental observations, and they suggest that molecular properties play a more prominent role in controlling the persistence of marine DOC than does molecular concentration.

While we suggest molecular properties as a primary control on the utilization of marine DOC, we do not exclude other influencing factors. In addition to molecular properties and concentrations, microbial community structure and environmental conditions (together referred to as "ecosystem properties") also play important roles in the utilization and accumulation of DOC. The above-mentioned study by Zakem et al. (2021) provides a framework for organic carbon accumulation that unifies molecular properties, dilution, and ecosystem factors. They pointed to ecosystem properties as important factors for organic carbon consumption and accumulation. In a previous study, we conducted a series of consecutive bioassay experiments investigating the removal of refractory DOC under different ecosystem conditions, including additions of distinct microbial communities, labile substrates and exposure to solar radiation (Shen and Benner 2018). We observed that a significant fraction of DOC initially resistant to native microbes (even when concentrated 10-fold) could be rapidly removed when exposed to novel ecosystem conditions. Such rapid removal of refractory DOC was invoked by changes in ecosystem properties rather than molecular concentration.

Understanding the controls on the removal of DOC is relevant for predicting the response of the DOC reservoir to changing climate. The models testing concentration-driven uptake led Lennartz and Dittmar to conclude that the size of the marine DOC reservoir is strongly affected by microbial physiology. In contrast, our previous experiments pointed to a different control that ties the fate of marine DOC to the physical state of the ocean. We found that DOC resistant to utilization by native microbes due to molecular properties could in part be removed when the ambient microbial and environmental conditions change (Shen and Benner 2018). Considering that molecular and ecosystem properties in the global ocean can vary considerably in space and time, we proposed ocean circulation as a key driver regulating the size of the ocean DOC reservoir (Shen and Benner 2018). Ocean mixing can change ecosystem properties and facilitate the removal of refractory molecules in the ocean. The projected slowing of overturning circulation would favor the sequestration of carbon in refractory DOC in the ocean.

References

Arístegui, J., J. M. Gasol, C. M. Duarte, and G. J. Herndl. 2009. Microbial oceanography of the dark ocean's pelagic realm. Limnol. Oceanogr. **54**: 1501–1529.

Arrieta, J. M., E. Mayol, R. L. Hansman, G. J. Herndl, T. Dittmar, and C. M. Duarte. 2015. Dilution limits dissolved organic carbon utilization in the deep ocean. Science **348**: 331–333. doi:10.1126/science.1258955

Baltar, F., and others. 2021. What is refractory organic matter in the ocean? Front. Mar. Sci. **8**: 642637. doi:10.3389/fmars.2021.642637

Barber, R. T. 1968. Dissolved organic carbon from deep waters resists microbial oxidation. Nature **220**: 274–275. doi:10.1038/220274a0

Benner, R., and R. M. Amon. 2015. The size-reactivity continuum of major bioelements in the ocean. Ann. Rev. Mar. Sci. **7**: 185–205. doi:10.1146/annurev-marine-010213-135126

Bercovici, S. K., M. C. Arroyo, D. De Corte, T. Yokokawa, and D. A. Hansell. 2021. Limited utilization of extracted dissolved organic matter by prokaryotic communities from the subtropical North Atlantic. Limnol. Oceanogr. **66**: 2509–2520.

Dittmar, T., and others. 2021. Enigmatic persistence of dissolved organic matter in the ocean. Nature Reviews Earth & Environment **2**: 570–583. doi:10.1038/s43017-021-00183-7

Fatayer, S., and others. 2018. Direct visualization of individual aromatic compound structures in low molecular weight marine dissolved organic carbon. Geophys. Res. Lett. **45**: 5590–5598. doi:10.1029/2018GL077457

Hertkorn, N., and others. 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochim. Cosmochim. Ac. **70**: 2990–3010. doi:10.1016/j.gca.2006.03.021

Hertkorn, N., M. Harir, B. Koch, B. Michalke, and P. Schmitt-Kopplin. 2013. High-field NMR spectroscopy and FTICR mass spectrometry: Powerful discovery tools for the molecular level characterization of marine dissolved organic matter. Biogeosciences **10**: 1583–1624. doi:10.5194/bg-10-1583-2013

Jannasch, H. W. 1995. The microbial turnover of carbon in the deep-sea environment, p. 1–11. *In* N. Handa and T. Ohsumi [eds.], Direct Ocean disposal of carbon dioxide. Terra Scientific Publishing Company (TERRAPUB).

Jiao, N., and others. 2014. Mechanisms of microbial carbon sequestration in the ocean–future research directions. Biogeosciences **11**: 5285–5306. doi:10.5194/bg-11-5285-2014

Jiao, N., and others. 2015. Comment on "dilution limits dissolved organic carbon utilization in the deep ocean". Science **350**: 1483–1483. doi:10.1126/science.aab2713

Kujawinski, E. B. 2011. The impact of microbial metabolism on marine dissolved organic matter. Ann. Rev. Mar. Sci. **3**: 567–599.

Landry, Z., B. K. Swan, G. J. Herndl, R. Stepanauskas, and S. J. Giovannoni. 2017. SAR202 genomes from the dark ocean predict pathways for the oxidation of recalcitrant dissolved organic matter. mBio **8**: e00413–e00417.

Lennartz, S. T., and T. Dittmar. 2022. Controls on turnover of marine dissolved organic matter – Testing the null hypothesis of purely concentration-driven uptake: Comment on Shen and Benner, "Molecular properties are a primary control on the microbial utilization of dissolved organic matter in the ocean.". Limnol. Oceanogr. **67**: 673–679. doi:10.1002/lno.12028

Lian, J., and others. 2021. Microbial transformation of distinct exogenous substrates into analogous composition of recalcitrant dissolved organic matter. Environ. Microbiol. **23**: 2389–2403.

Lu, K., X. Li, H. Chen, and Z. Liu. 2021. Constraints on isomers of dissolved organic matter in aquatic environments: Insights from ion mobility mass spectrometry. Geochim. Cosmochim. Ac. **308**: 353–372. doi:10.1016/j.gca.2021.05.007

Mentges, A., C. Feenders, M. Seibt, B. Blasius, and T. Dittmar. 2017. Functional molecular diversity of marine dissolved organic matter is reduced during degradation. Front. Mar. Sci. **4**: 194. doi:10.3389/fmars.2017.00194

Ogawa, H., Y. Amagai, I. Koike, K. Kaiser, and R. Benner. 2001. Production of refractory dissolved organic matter by bacteria. Science **292**: 917–920. doi:10.1126/science.1057627

Paerl, R. W., I. M. Claudio, M. R. Shields, T. S. Bianchi, and C. L. Osburn. 2020. Dityrosine formation via reactive oxygen consumption yields increasingly recalcitrant humic-like fluorescent organic matter in the ocean. Limnol. Oceanogr. Lett **5**: 337–345.

Salazar, G., and S. Sunagawa. 2017. Marine microbial diversity. Curr. Biol. **27**: R489–R494.

Shen, Y., and R. Benner. 2018. Mixing it up in the ocean carbon cycle and the removal of refractory dissolved organic carbon. Sci. Rep. **8**: 2542. doi:10.1038/s41598-018-20857-5

Shen, Y., and R. Benner. 2020. Molecular properties are a primary control on the microbial utilization of dissolved organic matter in the ocean. Limnol. Oceanogr. **65**: 1061–1071. doi:10.1002/lno.11369

Sogin, M. L., and others. 2006. Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc. Natl. Acad. Sci. **103**: 12115–12120. doi:10.1073/pnas.0605127103

Sosa, O. A., S. M. Gifford, D. J. Repeta, and E. F. DeLong. 2015. High molecular weight dissolved organic matter enrichment selects for methylotrophs in dilution to extinction cultures. ISME J. **9**: 2725–2739. doi:10.1038/ismej.2015.68

Sunagawa, S., and others. 2015. Structure and function of the global ocean microbiome. Science **348**: 1261359.

Walker, B. D., S. R. Beaupré, T. P. Guilderson, M. D. McCarthy, and E. R. Druffel. 2016. Pacific carbon cycling constrained by organic matter size, age and composition relationships. Nat. Geosci. **9**: 888–891. doi:10.1038/NGEO2830

Williams, P. M., and E. R. M. Druffel. 1987. Radiocarbon in dissolved organic matter in the central North Pacific Ocean. Nature **330**: 246–248. doi:10.1038/330246a0

Zakem, E. J., B. B. Cael, and N. M. Levine. 2021. A unified theory for organic matter accumulation. Proc. Natl. Acad. Sci. **118**: e2016896118. doi:10.1073/pnas.2016896118

Acknowledgments

This work was supported by National Natural Science Foundation of China (NSFC) (42106040 to Yuan Shen), the Fundamental Research Funds for the Central Universities of China (20720210076 to Yuan Shen), and by the National Science Foundation of the USA (PLR-1504137 to Ronald Benner).

Conflict of Interests

None declared.

Submitted 13 September 2021
Revised 29 December 2021
Accepted 07 January 2022

Associate editor: Florence Schubotz