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OPINION
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The Enduring Questions: What's for Dinner? Where's My Knife? …and Can I Use My Fingers? (Unanswered) Questions Related to Organic Matter and Microbes in Marine Sediments

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Heterotrophic microbial communities play key roles in processing and remineralizing organic matter in marine sediments: they are the "final gatekeepers" that determine the types and quantity of organic matter that is ultimately buried in sediments—processes important to our understanding of past global environments, as well as to the production of petroleum products that still fuel much of modern society. These communities' capabilities also help us understand the energetic and metabolic boundaries of life. Work over the past decades has revealed much information about sedimentary microbial communities: their overall composition (Bacteria and Archaea), the sequence of terminal respiration processes occurring with progressive burial depth in sediments, and the depth to which they can be detected; research on the members and metabolism in the "deep biosphere" has assumed a central position in organic geochemistry, environmental microbiology, and molecular ecology (Orcutt et al., 2013; D'Hondt et al., 2019).

Despite this gain in knowledge, we are left with important questions about heterotrophic microbial communities in sediments, some of which can be summarized simply: What do they eat? How do they gain access to their substrates? How do they sense that specific substrates are out there? Our understanding is particularly incomplete with regard to the types of complex organic matter accessed by these communities. At a recent workshop[1], we discussed a number of key questions with respect to these issues, and here we focus on some of the questions raised in our discussion, in the hope that they will spur further thought and research.

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[1] "Future directions in marine organic biogeochemistry," held at the Hanse Institute for Advanced Study, Delmenhorst, Germany; April 27-30 2019.

SEDIMENTARY ORGANIC MATTER

What does sedimentary organic matter look like, how do organisms see It? What are key characteristics of organic matter at different geographic locations and of different ages that sustain microbial life? How could we measure the impact of microbial processing on the structure of organic matter? How can we constrain the selectivity of microbial utilization of organic matter in sedimentary contexts?

From an analytical perspective, the fraction of "uncharacterized" organic matter is already high in surficial sediments (e.g., Hedges et al., 2000), although in such sediments, microbial consumption of this uncharacterized material can be rapid (Arnosti and Holmer, 2003). Deeper in sediments, with fewer cells and lower activities, much of the organic matter is also uncharacterized. How does this uncharacterized material change with depth, such that a fraction is bioavailable in surficial sediments, but much of the rest apparently is unavailable at deeper depths? Our measurements are not yet capturing what is important for microbial communities in terms of their activity and the reactivity of organic matter.

Bulk measurements of organic matter are often not particularly enlightening in this respect; we do not understand the constraints on the microbial populations found in sediments with high dissolved organic carbon (DOC) or high sedimentary organic matter concentrations, where activity apparently is low. For example, in Mediterranean sediments, differences in cell abundances between organic-rich sapropels and the adjacent non-sapropels are not proportional to the vast differences in organic matter concentration (Coolen et al., 2002). Marine-derived organic matter with unusually high C/N ratios in Mediterranean sediments (e.g., Calvert and Fontugne, 2001) and in Cretaceous Black Shales (e.g., Meyers et al., 2006) suggests particularly efficient degradation of the N-rich organic matter fraction in these settings. Is N removal occurring at burial, rather than at depth? How could we measure these processes, how could we determine what is a suitable structural feature for a microbe to metabolize?

The focus on characterizing bulk structures or specific components of larger macromolecules might miss important features if microbial communities are "nibbling around the edges" of macromolecules—selectively utilizing certain structural moieties (such as methoxy groups, for example). The number and types of reactive sites present in sedimentary organic matter must decrease in deeper sediments, but we don't capture this feature with our current measurements, nor do we know the types of reactive sites/functional groups that are important.

In terms of overall controlling factors on organic matter processing in sediments, to what extent do abiotic reactions also affect organic matter availability? Are physical factors that we do not capture with adequate resolution important in determining organic matter availability? What role does upward migration of smaller organic molecules from geothermally heated sediments play in feeding heterotrophic metabolism? Do small molecules in such systems trigger microbial activities, and lead to degradation of other types of organic matter?

ORGANISMS OF THE DEEP

How are communities of organisms organized in the deep? What are the characteristics of these organisms? Are there important properties of sedimentary microbial communities that we don't know about, such as metabolic networks?

How important is cell-cell communication in the deep biosphere? At cell densities of ca. 10^2 to 10^6 cm^{-3} at burial depth in excess of 1 km (Parkes et al., 2014; Inagaki et al., 2015), perhaps there is no communication among cells, as from a microbial perspective, cells are separated by astronomically long distances. But perhaps these organisms are clustered together in small communities ("frontier towns," so to speak), and the longest distances are between communities, rather than individual organisms.

Especially in this respect, are fungi important? Marine fungi with the capabilities for extracellular hydrolysis of model macromolecules are being detected more frequently in the marine environment (Gutiérrez et al., 2011). Fungi have been detected in sediments deep in the ocean (Le Calvez et al., 2009) as well as in the deep biosphere (Edgcomb et al., 2011; Liu et al., 2017; Ivarsson et al., 2018), which brings the possibility of exploring metabolic networks of bacteria and fungi in the marine sedimentary environment, as previously found above sea level. An alternative to overcome astronomically long distances, from a microbial perspective, is the possibility of bacterial mobilization through fungal hyphae (a "fungal highway"), as documented in soil (Kohlmeier et al., 2005), and food chemistry (Zhang et al., 2018) literature. There is already evidence for potential consortia of fungi and sulfate-reducing bacteria in deep granite fractures (Drake et al., 2017). Something is going on. Deeper insight into metabolic networks of bacteria, archaea, and fungi may shed light on microbial organization in the deep biosphere.

In this respect, better knowledge of the means by which electrons are transferred between partners would be helpful. For example, are quinones important? These compounds engage in facile and reversible redox reactions and are known moieties of complex natural organic matter. In soil and river sediments, they can catalyze redox reactions by an electron shuttling mechanism (Uchimiya and Stone, 2009). To date, little is known about such mechanisms in marine sediments. Perhaps this is an area in which work with pure cultures, as well as experiments with defined members, would be useful in order to better understand interactions in the environment. Isolating bacteria in the lab tears apart degradative networks, so we have a very incomplete picture of microbial interactions, but such isolates provide the opportunity for detailed physiological investigations that cannot be carried out in the environment.

SOME THOUGHTS, MOVING FORWARD

- *A renewed focus on process-based studies* would be helpful, given that in surficial sediments (where organic matter transformations are sufficiently rapid), we can currently measure the "bookends" of organic matter transformations: initial enzymatic hydrolysis of macromolecules, and terminal respiration rates (e.g., Arnosti and Jørgensen, 2006). The sequential transformations of complex macromolecules that occur between these steps are largely unknown, however.

- *Better identification of proteins produced by sedimentary communities* would provide much-needed insight into their metabolism. Since current protein databases overwhelmingly do not represent organisms from marine sediments (much less the deep biosphere), and organisms in these environments probably have metabolic solutions to physiological/geochemical "problems" that are not found in more-typically investigated environments, we end up with a substantial fraction of unclassified proteins. Focused culture work might provide some of the missing answers.
- *Alternative methods to determine microbial activity and growth* are needed. Measurement of protein production (leucine incorporation) seems problematic, given the very slow growth in the deep biosphere, where most carbon may go to respiration rather than to an increase in cellular biomass. Although high concentrations of isotopically-labeled substrates have been used in the lab to demonstrate viability of cells from the deep biosphere (e.g., Morono et al., 2011), what other approaches could be used to get at growth rates under quasi-*in-situ* conditions? Optimization of the lipid-based dual stable isotope approach introduced by Wegener et al. (2012) could be a good start, as exemplified by increased sensitivity recently achieved using radioisotopic labeling (Evans et al., 2019).
- *New methods to analyze macromolecular organic matter* will be required. Some techniques (such as thermochemical analyses of organic matter) may be informative in cases where there is a thermal gradient in deeply buried sediments. Significant progress has been made in characterizing lipids in sediments (e.g., Wörmer et al., 2015), but lipids do not constitute the bulk of sedimentary organic matter. Other techniques that might provide information about bioavailable functional groups, or provide better characterization of complex macromolecules under conditions more reminiscent of sedimentary matrices, would be extremely helpful.

Considering the initial question—what's for dinner?—we are left with the image of hungry cells in the dark, sensing their environment, trying to determine whether a knife or fingers would be more helpful in acquiring food. Filling in this picture with a more realistic view of key unknowns—how organisms sense their substrates, what characteristics make these substrates suitable, and how organismal requirements and substrate characteristics change with depth and location in marine sediments—will doubtless keep the research community occupied for the next decades.

AUTHOR CONTRIBUTIONS

The manuscript was written by CA, based on notes taken during the workshop breakout group discussion, with input by all co-authors.

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