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Prokaryotic Life in the Deep Ocean's Water Column

Gerhard J. Herndl,[1,2,*] Barbara Bayer,[3] Federico Baltar,[1] and Thomas Reinthaler[1,*]

[1]Department of Functional and Evolutionary Ecology, University of Vienna, Vienna, Austria; email: gerhard.herndl@univie.ac.at

[2]Department of Marine Microbiology and Biogeochemistry, Royal Netherlands Institute for Sea Research (NIOZ), Utrecht University, Den Burg, The Netherlands

[3]Department of Microbiology and Ecosystem Science, Centre for Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria

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*These authors contributed equally to this article

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Abstract

The oceanic waters below a depth of 200 m represent, in terms of volume, the largest habitat of the biosphere, harboring approximately 70% of the prokaryotic biomass in the oceanic water column. These waters are characterized by low temperature, increasing hydrostatic pressure, and decreasing organic matter supply with depth. Recent methodological advances in microbial oceanography have refined our view of the ecology of prokaryotes in the dark ocean. Here, we review the ecology of prokaryotes of the dark ocean, present data on the biomass distribution and heterotrophic and chemolithoautotrophic prokaryotic production in the major oceanic basins, and highlight the phylogenetic and functional diversity of this part of the ocean. We describe the connectivity of surface and deep-water prokaryotes and the molecular adaptations of piezophilic prokaryotes to high hydrostatic pressure. We also highlight knowledge gaps in the ecology of the dark ocean's prokaryotes and their role in the biogeochemical cycles in the largest habitat of the biosphere.

1. INTRODUCTION

The dark ocean (defined here as depths below 200 m) is characterized by low temperature, high hydrostatic pressure, and high inorganic nutrient concentrations. Comprising the mesopelagic (200–1,000 m), bathypelagic (1,000–4,000 m), abyssopelagic (4,000–6,000 m), and hadopelagic (>6,000 m) zones, this ecosystem of roughly 1.3×10^9 km^3 represents the largest habitat in the biosphere (Bar-On et al. 2018). Compared with the sunlit epipelagic waters, relatively little is known about dark-ocean biological processes. However, over the last two decades, research on dark-ocean microbes (the most important biological entities, particularly in the deep water column) has received considerable attention. Global ocean expeditions such as *Tara* Oceans (Bork et al. 2015) and the Malaspina Circumnavigation Expedition (Duarte 2015), as well as other major deep-sea expeditions for individual research projects, have advanced our knowledge of the functioning of the dark-ocean microbiome. The previous assumption that the deep-ocean water column is characterized by low microbial abundance and metabolic activity has changed with the discovery of novel microbial metabolic pathways, refining our view of the biogeochemical cycling of organic matter by deep-sea microbes.

Here, we review and focus on the current status of knowledge of the dark ocean's prokaryotic abundance and biomass production in the meso- and bathypelagic water column, prokaryotic phylogenetic and functional diversity, vertical connectivity, and the role of hydrostatic pressure. We also highlight knowledge gaps and challenges in our understanding of the functioning of deep-sea microbes and their role in the carbon cycling of the global ocean.

2. PROKARYOTIC ABUNDANCE AND HETEROTROPHIC BIOMASS PRODUCTION THROUGHOUT THE WATER COLUMN

Despite the major advances in omics approaches and the resulting refined knowledge of prokaryotic metabolic capabilities, the relatively simple metrics of biomass and biomass production still provide the base to evaluate the ecological role of microbes in the aquatic environment. Since the reviews by Arístegui et al. (2009) and Robinson et al. (2010), however, advances in the classical methodologies to determine these two basic parameters have not kept pace with those based on omics approaches.

Prokaryotes in the dark ocean are fundamental for the cycling of carbon, nitrogen, and phosphorus, and it is now unequivocally clear that they are metabolically active from the surface ocean to the seafloor, remineralizing complex organic matter to inorganic nutrients and CO_2. Inverse modeling of physical and geochemical measurements has helped us understand the cycling of elements in the global ocean; however, these models generally do not include parameterizations of the variability and magnitude of microbial heterotrophic activity that would provide a high-resolution view of biological processes in the dark ocean, which decreases the predictive power of the model outcomes. Heterotrophic activity is often used as a loose term to describe the assimilation rate of radiolabeled organic substrates by microbes, although microbial activity should encompass assessments of both production and respiration. A simple reason for this bias is the sensitivity and ease of measuring prokaryotic heterotrophic biomass production (PHP) throughout the water column, whereas the current methodology limits high-throughput measurements of respiration rates (Robinson 2019). Yet there is a limited understanding of the global variability of prokaryotic abundance and biomass production in the water columns of major oceanic basins.

We collated a dark-ocean data set on prokaryotic abundance and heterotrophic production, including metadata spanning all major open-ocean regions. All data sources are listed in **Supplemental Table 1**. Only prokaryotic data with unambiguous metadata (cruise information,

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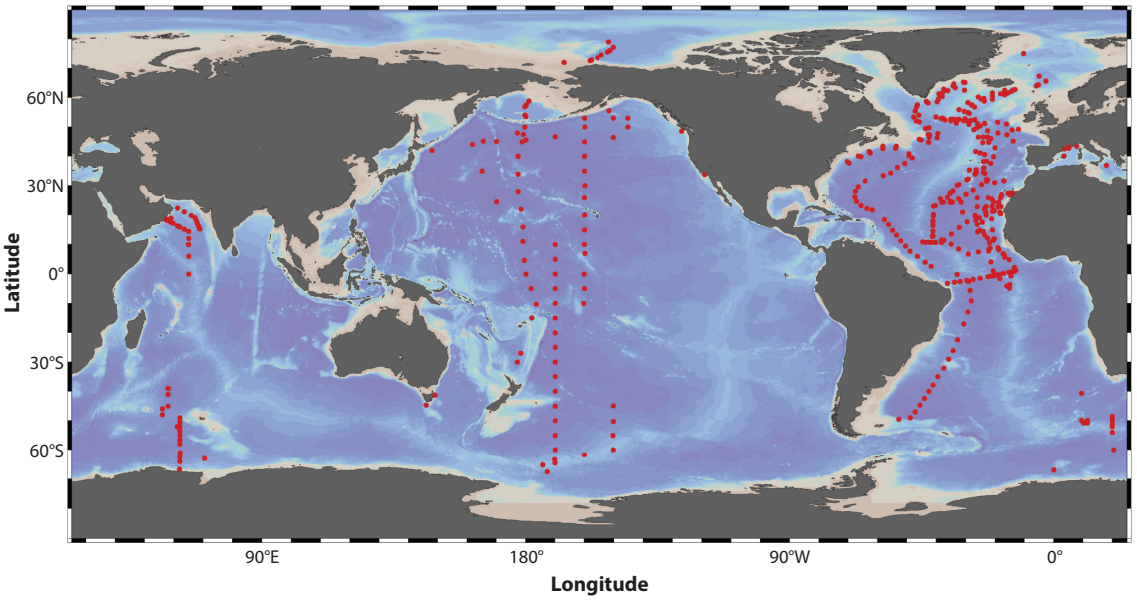

Figure 1

Map of stations included in the data set. Only stations off the continental shelf have been considered. The Mediterranean Sea was excluded from the analysis.

station, and longitude and latitude) were chosen for the analysis (**Figure 1**). Fewer data are available from the stormy seasons (depending on the hemisphere) and from the Southern Hemisphere. The Indian Ocean in particular is undersampled, with essentially no data available between 0.8°N and 50°S. Except for the upper mesopelagic (to ~500-m depth), where the data density is higher, greater depths have been sampled with roughly similar intensity (**Figure 2**).

Prokaryotic cell abundance was converted to prokaryotic biomass using a conversion factor (CF) of 10 fg C per cell, a CF typical for open-ocean prokaryotes (Herndl et al. 2005). We

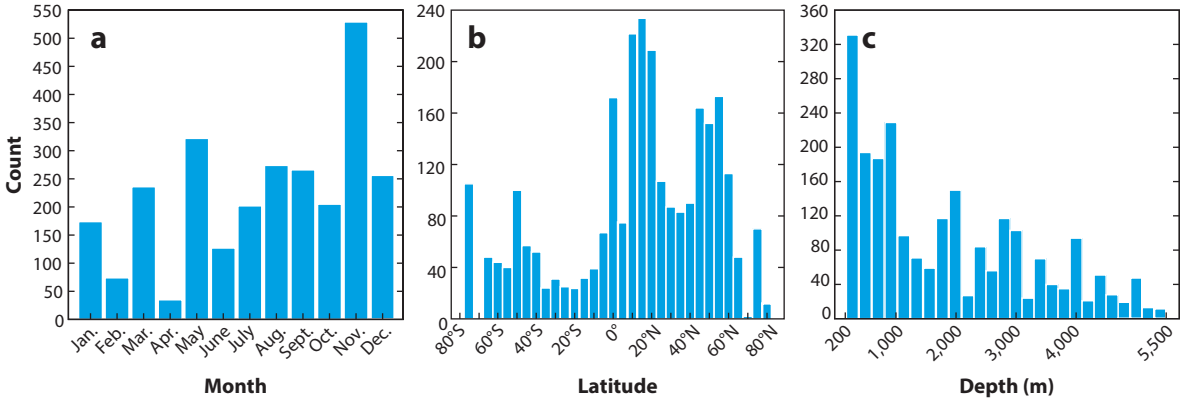

Figure 2

Histograms of the distributions and numbers of samples (*a*) in different months of the year, (*b*) at different latitudes, and (*c*) at different depth levels.

Table 1 Bacterial biomass and production in the major ocean basins

Ocean	Depth[a]	Biomass[b] (μmol C m^{-3})	Production[b] (μmol C m^{-3} d^{-1})	CSP[c] (amol C $cell^{-1}$ d^{-1})	n[d]	Biomass[e] (mmol C m^{-2})	Production[e] (mmol C m^{-2})	n[d]
Arctic	Epipelagic	335.6 ± 28.2	19.05 ± 5.34	37.5 ± 11.6	83	44.7 ± 0.2	2.09 ± 0.3	NA
	Mesopelagic	64.6 ± 4.3	1.55 ± 1.00	13.8 ± 9.0	43	37.0 ± 2.5	0.07 ± 0.0	7
	Bathypelagic	15.7 ± 1.0	0.01 ± 0.00	0.5 ± 0.1	41	42.0 ± 0.7	0.02 ± 0.0	6
Atlantic	Epipelagic	264.6 ± 4.9	16.7 ± 0.89	54.0 ± 3.1	348	69.2 ± 0.2	9.49 ± 0.2	NA
	Mesopelagic	87.8 ± 2.2	1.42 ± 0.11	14.8 ± 1.7	507	88.0 ± 1.9	1.83 ± 0.1	211
	Bathypelagic	27.4 ± 0.7	0.22 ± 0.02	5.6 ± 0.5	869	76.0 ± 3.4	0.48 ± 0.1	95
Pacific	Epipelagic	497.0 ± 19.3	40.93 ± 2.33	82.5 ± 6.0	532	99.1 ± 0.2	10.72 ± 0.3	NA
	Mesopelagic	105.7 ± 3.0	1.49 ± 0.20	11.6 ± 1.7	459	73.0 ± 3.4	0.44 ± 0.1	64
	Bathypelagic	21.7 ± 0.8	0.05 ± 0.01	1.9 ± 0.1	375	74.0 ± 6.2	0.11 ± 0.0	41
Indian	Epipelagic	589.2 ± 13.1	96.97 ± 3.91	121.6 ± 4.4	657	69.1 ± 0.1	6.52 ± 0.2	NA
	Mesopelagic	195.0 ± 10.1	9.25 ± 0.94	58.3 ± 7.3	222	112.0 ± 5.8	6.78 ± 1.0	41
	Bathypelagic	41.2 ± 1.5	8.95 ± 0.89	226.4 ± 25.9	142	94.0 ± 6.2	16.28 ± 6.4	7
All oceans	Epipelagic	476.2 ± 9.0	57.33 ± 1.98	90.0 ± 2.9	1,620	82.3 ± 0.2	10.41 ± 0.2	NA
	Mesopelagic	113.0 ± 2.6	2.86 ± 0.21	21.4 ± 1.7	1,231	87.0 ± 1.8	2.10 ± 0.2	323
	Bathypelagic	27.0 ± 0.5	1.04 ± 0.11	26.4 ± 3.1	1,427	75.0 ± 2.8	1.10 ± 0.4	149

[a]Epipelagic, 5–200-m depth; mesopelagic, 200–1,000-m depth; or bathypelagic, 1,000–4,000-m depth.
[b]Mean ± standard error of volumetric data averaged over the different depth levels.
[c]Cell-specific bacterial production ± standard error.
[d]Number of volumetric measurements. Abbreviation: NA, not applicable.
[e]Depth-integrated values.

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exclusively considered leucine incorporation (either ^{3}H or ^{14}C labeled) as a proxy for PHP and excluded ^{3}H-thymidine incorporation, a proxy for DNA synthesis and hence growth. Recently, it has been argued that ^{14}C-labeled leucine should be the preferred tracer for estimating PHP because leucine is prone to cleavage of methyl groups, and thus, a variable fraction of ^{3}H-labeled leucine will not be detected in assays; consequently, PHP might be underestimated when using ^{3}H-leucine (Hill et al. 2013, Giering & Evans 2022). Most of the PHP measurements, however, were performed using ^{3}H-leucine. Thus, based on the above argument, there is likely some bias in the data. PHP was measured with the filter method (Kirchman et al. 1985) and the microfuge method (Smith & Azam 1992), with slight variations among studies. In a selected data set of the open western Atlantic, both methods correlated well over the whole water column ($r^2 = 0.98$, $p < 0.0001$); however, on average, data from the filtration method are higher by 20–30% compared with parallel samples measured with the microfuge method (**Supplemental Figure 1**).

Most studies used a leucine-to-carbon conversion of 1.5 kg C mol^{-1} leucine incorporated. There is considerable debate on the possible ranges of leucine CFs for PHP (Baltar et al. 2010a, Teira et al. 2015), varying between 0.02 and 36.4 kg C mol^{-1} leucine (Giering & Evans 2022). Due to the lack of a universally applicable and consistent CF, we recalculated all data to the theoretical CF of 1.5 kg C mol^{-1} leucine (Simon & Azam 1989). This CF might also vary with water column depth; however, experiments trying to establish CFs that take in situ pressure conditions into account have not yet been reported.

The averaged rates and stocks of prokaryotic biomass and production in the epipelagic, mesopelagic, and bathypelagic (**Table 1**) are lower by at least 50% than those published in an earlier review (Arístegui et al. 2009). This is due to the vastly increased data set reported here ($n = 4{,}300$ data points), including new data from major regions of the oligotrophic subtropical gyres, the Arctic Ocean, and the Southern Ocean. We also excluded the Mediterranean Sea and

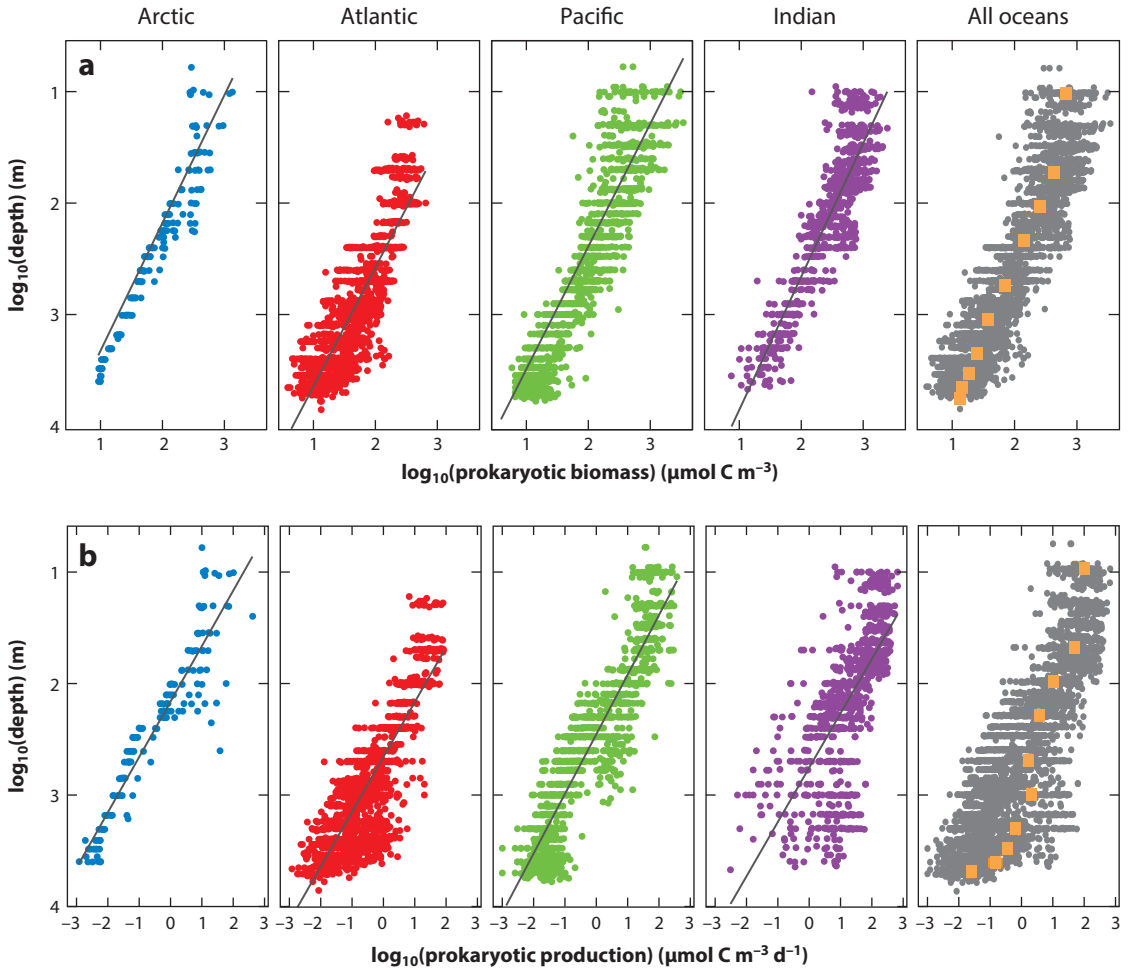

Figure 3

(*a*) Prokaryotic biomass versus depth and (*b*) prokaryotic production versus depth using $\log_{10}$ transformed data in the Arctic Ocean, the Atlantic Ocean, the Pacific Ocean, the Indian Ocean, and all oceanic regions combined. Linear regressions on transformed data (for intercepts, slopes, and statistics, see **Supplemental Table 3**) are shown as solid gray lines. The orange squares in the far-right subpanels indicate averages of depth bins with range limits of 5, 50, 100, 200, 500, 1,000, 2,000, 3,000, 4,000, and 5,000 m (see **Supplemental Table 2**). The standard errors are smaller than the squares.

considered only data where both prokaryotic abundances and PHP measurements were available, yielding a congruent data set (compare **Table 1** with table 1 in Arístegui et al. 2009).

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In all oceanic basins, prokaryotic biomass decreases exponentially between 10- and 5,000-m depth, with a biomass of 676 ± 18 µmol C m^{-3} (mean ± standard error) at the surface and 13 ± 1 µmol C m^{-3} (mean ± standard error) at 5,000-m depth, averaged over all ocean basins (**Figure 3*a***; **Supplemental Table 2**). The regression slopes of log-log transformed data of biomass versus depth are similar among the different ocean basins, averaging −0.73 (**Figure 3*a***; **Supplemental Table 3**). This decrease in biomass with depth is steeper than previously reported (Arístegui et al. 2009) and is probably due to the low quality of organic substrate required to support PHP in large parts of the open ocean's interior in the revised data set. With the exception

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of the Atlantic compared with the Pacific, however, significant differences in intercepts (range of 3.6–3.9; **Supplemental Table 3**) indicate that the lowest prokaryotic carbon biomass is in the Arctic Ocean and the highest is in the Indian Ocean (**Figure 3*a***).

Prokaryotic production also decreases exponentially (99.56 to 0.02 μmol C m^{-3} d^{-1}) from the euphotic zone to the bathypelagic. Approximately 98% of prokaryotic production is attenuated from the surface to the base of the mesopelagic (**Figure 3*b***; **Supplemental Table 2**). With the exception of the Indian Ocean, all ocean basins showed similar slopes of decrease of approximately −1.57 (**Supplemental Table 3**). Most of the Indian Ocean data are from the Arabian Sea, a rather special region with restricted circulation and extensive hypoxic conditions beneath the seasonal pycnocline (Ducklow 2000). The Atlantic and the Pacific have similar regression intercepts and slopes, but covariance models indicate differences in the Indian Ocean and the Arctic Ocean. This might be due to a smaller variability in environmental parameters such as temperature and nutrient availability in these basins or, alternatively, to the current database focusing on specific regions in these areas.

Describing the log-log decrease in cell-specific heterotrophic activity (i.e., leucine uptake rate divided by cell abundance) with depth in the world's oceans yields a slope of −0.80 ($r^2 = 0.49$, $p < 0.0001$), indicating a reduction in rates of >99% from the euphotic zone to the base of the bathypelagic (data not shown). Cell-specific leucine uptake rates vary from 121 amol C $cell^{-1}$ d^{-1} in the epipelagic to 0.5 amol C $cell^{-1}$ d^{-1} in the bathypelagic (**Table 1**). In the Arabian Sea of the Indian Ocean, cell-specific rates are significantly higher in the meso- and bathypelagic than they are in the other oceanic regions. Excluding the Arabian Sea from the global data set decreases the overall cell-specific activity by approximately 18 amol C $cell^{-1}$ d^{-1}. Thus, while heterotrophic prokaryotes are generally active throughout the water column, particularly in the Arctic, Atlantic, and Pacific Oceans, cell-specific production is significantly reduced in the bathypelagic and is on average between 1% and 10% of the activity in the epipelagic realm.

3. PATTERNS OF PROKARYOTIC BIOMASS AND HETEROTROPHIC PRODUCTION IN THE ATLANTIC AND PACIFIC

To compare the variability of prokaryotic biomass and PHP of the Atlantic and Pacific, we binned the depth-integrated data from the compiled cruises into latitudinal bands of 10° (a distance of approximately 1,100 km). The large gap in data coverage of the Indian Ocean did not allow such an analysis in that ocean. In the mesopelagic and bathypelagic waters, the amount and pattern of integrated prokaryotic biomass are similar between the two oceans, with generally higher biomass in the subpolar gyres and the equatorial region (**Figure 4*a–d***). Depth-integrated PHP is generally more complex than the latitudinal biomass distribution (**Figure 4*e–h***). In the Atlantic, decreasing rates of PHP are found from the Greenland–Iceland–Norwegian Sea toward the equator, indicating the evolution of initially young water masses formed in the northern North Atlantic and decreasing bioavailability of dissolved organic matter (DOM) toward the lower latitudes (see also Reinthaler et al. 2010).

Increased PHP at higher latitudes is also apparent for the mesopelagic waters in the Pacific, whereas bathypelagic production shows a high latitudinal variability. The pattern in mesopelagic PHP in the Pacific has recently been interpreted to reflect the influence of sinking particulate organic carbon (POC) flux, while the pattern in PHP in the bathypelagic zone could be due to regional hydrographic features and organic carbon delivery to deep waters (Yokokawa et al. 2013, Boeuf et al. 2019, Luo et al. 2022). Net primary production is related to depth-integrated prokaryotic biomass or PHP in the Atlantic and Pacific data sets (**Supplemental Figures 2 and 3**), supporting the finding that heterotrophic prokaryotic biomass reflects the long-term POC export

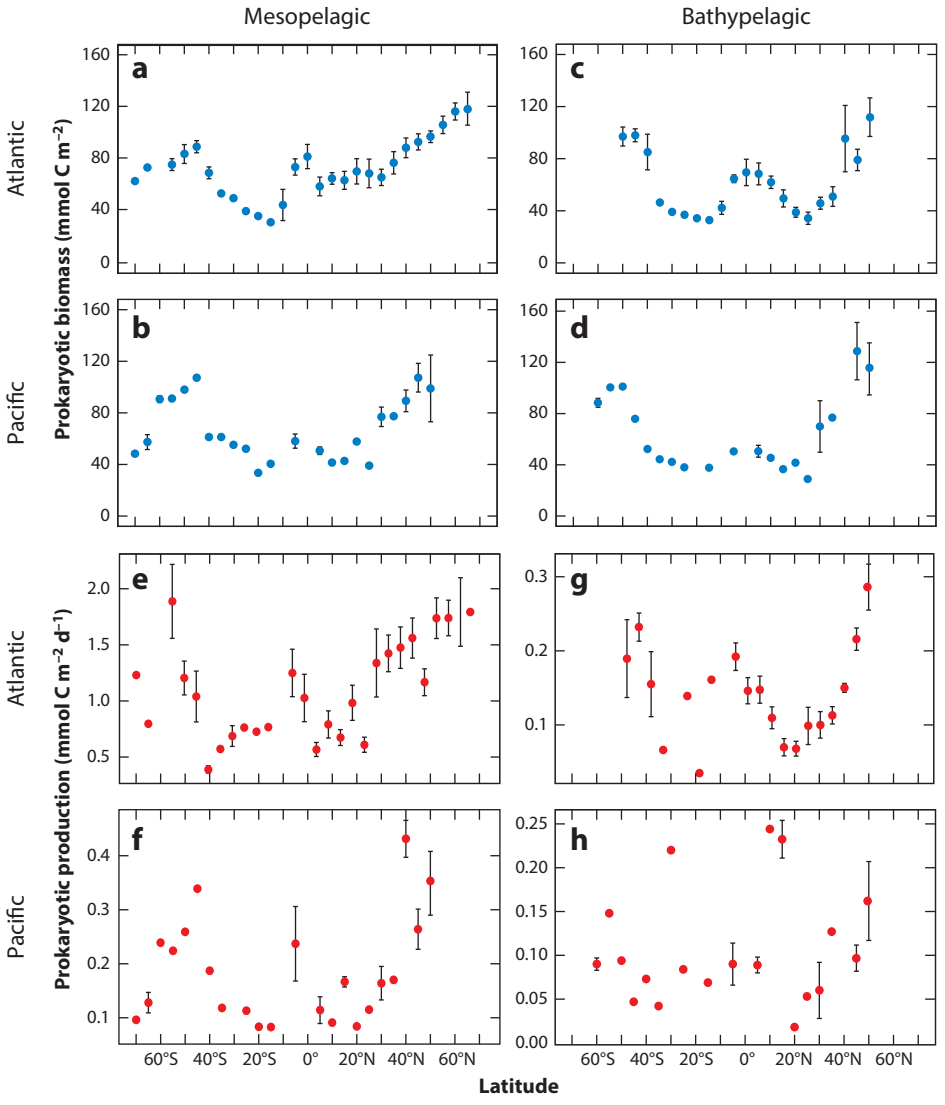

Figure 4

(*a–d*) Depth-integrated prokaryotic biomass and (*e–h*) depth-integrated prokaryotic production in the mesopelagic (200–1,000-m depth) and bathypelagic (1,000–4,000-m depth) water column of the Atlantic and Pacific. Depth-integrated values were binned into 5° latitudinal bins from 70°N to 70°S. Error bars indicate standard errors. For data points without error bars, only one or two complete depth profiles per latitudinal bin were available. For calculations, see the **Supplemental Methods**.

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(Hansell & Ducklow 2003), whereas PHP is indicative of the short-term input of labile and/or semilabile material (Nagata et al. 2000).

4. AUTOTROPHY VERSUS HETEROTROPHY OF PROKARYOTES

Microbial communities in the oxygenated water column of the dark ocean were initially considered to be mainly heterotrophic due to their wide capacity to incorporate amino acids into

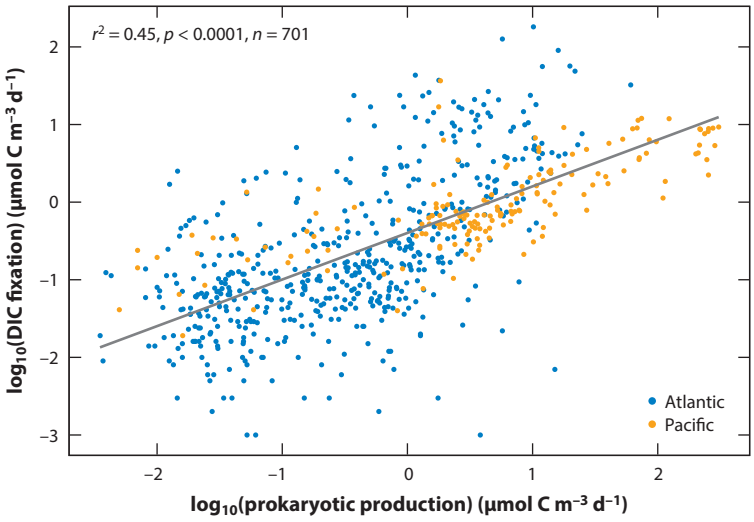

Figure 5

Prokaryotic production versus DIC fixation in the Atlantic (*blue dots*) and Pacific (*orange dots*). The line indicates a linear regression on $\log_{10}$ transformed data. The significant regression using 701 paired data points (*n*) explained 45% of the variability in both measurements. Abbreviation: DIC, dissolved inorganic carbon.

biomass (Ouverney & Fuhrman 2000, Teira et al. 2006). However, evidence provided by microbial radiocarbon signatures suggested a major role of chemoautotrophy in the mesopelagic (Hansman et al. 2009). Archaea in particular are thought to contribute to a large fraction of dissolved inorganic carbon (DIC) fixation in the deep ocean (Wuchter et al. 2003, Ingalls et al. 2006). In most North Atlantic water masses, dark DIC fixation is of the same order of magnitude as PHP (Reinthaler et al. 2010). A compilation of available PHP and dark DIC fixation rates from the Atlantic and Pacific deep waters indicates that PHP and DIC fixation rates are positively related (**Figure 5**).

Depth-integrated dark DIC fixation rates in North Atlantic deep waters range from 1.8 to 3.2 mmol C m^{-2} d^{-1}, corresponding to 15% to 53% of the phytoplankton export production (Reinthaler et al. 2010). This combined evidence has led to the conclusion that chemoautotrophic activity is substantial in the deep ocean and provides a fresh, non-sinking source of particulate organic matter (POM) to the deep ocean (Baltar et al. 2010c, Reinthaler et al. 2010). Additionally, recent culture experiments have shown that chemoautotrophs can release a considerable fraction (~5–15%) of their fixed DIC as dissolved organic carbon (DOC) (Bayer et al. 2022), which is partially composed of labile compounds that often limit heterotrophic production (Bayer et al. 2019a).

The main energy source fueling chemoautotrophy in the oxygenated ocean is ammonia, which is supplied via ammonification of particulate organic nitrogen (PON) (Middelburg 2011). However, measurements of DIC fixation in the deep ocean (Herndl et al. 2005, Reinthaler et al. 2010) are on average one order of magnitude higher than can be supported by ammonium supplied via the sinking flux of PON (Middelburg 2011, Zhang et al. 2020, Bayer et al. 2022). This discrepancy points toward unaccounted sources of ammonium in the deep ocean, alternative energy sources supporting chemoautotrophy, and/or a major contribution of heterotrophs to dark DIC fixation. Diel vertically migrating zooplankton and micronekton actively transport ammonium and organic nitrogen compounds to deeper water layers, averaging 8–45% of the sinking PON flux in

open-ocean environments (Steinberg et al. 2002). In addition to ammonia, potential alternative energy sources, including reduced sulfur compounds, hydrogen, and carbon monoxide, have been proposed to make up for some of the observed discrepancies (Reinthaler et al. 2010, Swan et al. 2011, Zhang et al. 2020) (discussed further in Section 5), but the concentrations and formation rates of these sources are most likely not sufficient in oxic deep water layers. Assimilation of inorganic carbon by heterotrophic organisms has been known for approximately 80 years (Krebs 1941), and its potential importance in various environments has recently been reviewed (Braun et al. 2021). Heterotrophic microbes fix CO_2 via a variety of carboxylation reactions as part of their central metabolism (Erb 2011), including fatty acid biosynthesis, leucine catabolism, purine biosynthesis, and anaplerotic pathways. The few available data on heterotrophic CO_2 fixation suggest that 1–10% of carbon in prokaryotic biomass is derived from assimilation of CO_2 (Sorokin 1966, Roslev et al. 2004), while photoheterotrophs and methanogens encoding the serine cycle can derive up to 30% and 50%, respectively, from CO_2 (Crowther et al. 2008, Yang et al. 2013, Palovaara et al. 2014). Thus, in epipelagic waters, heterotrophic bacterial groups likely contribute significantly to dark DIC fixation (Alonso-Sáez et al. 2010). In the dark ocean, however, heterotrophic CO_2 fixation pathways, such as anaplerosis, might be less important due to the limited availability of organic carbon.

Because of the limited input of fresh DOM into the dark ocean by convection, advection, and subduction, heterotrophic microbes rely on the flux of POM rather than on DOM (Arístegui et al. 2002). However, the measured prokaryotic carbon demand in deep waters has repeatedly been reported to be significantly higher than the supply of organic carbon to the dark ocean via the sinking particle flux (Reinthaler et al. 2006, Steinberg et al. 2008, Baltar et al. 2009). Adding the measured dark DIC fixation, representing new organic carbon in the dark ocean, to the POC flux is also insufficient to close the discrepancy between the prokaryotic heterotrophic carbon demand and POC supply (Herndl & Reinthaler 2013). By constraining estimated dark-ocean respiration and modeling, Giering et al. (2014) were able to reconcile the mesopelagic carbon budget at a site in the North Atlantic. However, sensitivity analysis on a published data set indicates that bacterial growth efficiencies and assumed cell carbon contents have a large effect on the magnitude of the carbon imbalance (Burd et al. 2010) (see the sidebar titled Mismatch of Particulate Organic Carbon Flux and Prokaryotic Carbon Demand). Heterotrophic production rate measurements are significantly overestimated when measured under atmospheric pressure conditions. At 1,000- and 4,000-m depths, in situ leucine incorporation amounts to only 50–60% and 30–40%, respectively, of that measured at atmospheric pressure conditions (Amano et al. 2022). Additionally, simulated particle flux into the ocean's interior and measured respiration under increasing hydrostatic pressure showed a substantial decline in respiration rates with increasing hydrostatic pressure (Stief et al. 2021). This suggests that the metabolic activity of microbes, at least on sinking particles, is greatly reduced in the deeper meso- and bathypelagic waters as compared with rates measured under atmospheric pressure conditions (Stief et al. 2021). Including in situ pressure effects, sinking POC contributed 60% and 100% in the Pacific and the Atlantic, respectively (Amano et al. 2022). Thus, accounting for the effect of hydrostatic pressure and the temporal uncoupling between supply and consumption of POC (Uchimiya et al. 2018), the imbalance in the deep-ocean carbon budget might be partially resolved.

5. PHYLOGENETIC AND FUNCTIONAL DIVERSITY

The microbial communities in the deep ocean differ from those inhabiting surface waters. A recurring feature in deep waters is the high proportion of archaea, driven mainly by the increase in the abundance of marine group I archaea, currently known as thaumarchaea, which constitute

MISMATCH OF PARTICULATE ORGANIC CARBON FLUX AND PROKARYOTIC CARBON DEMAND

Prokaryotic organic carbon turnover is not implemented in current ocean models despite climate change–induced temperature increases that likely have a significant impact on the metabolic activity of prokaryotes in the dark ocean. To estimate the organic carbon that is needed for prokaryotes to grow in the dark ocean, the particle flux based on sediment trap data (Antia et al. 2001, Honjo et al. 2008) has been compared with the prokaryotic carbon demand [PCD, equal to PHP plus respiration (R)] (Herndl & Reinthaler 2013, Giering et al. 2014). While prokaryotic production is easy to measure, respiration is generally estimated from assumed prokaryotic growth efficiency (PGE) [PGE = PHP/(PHP + R), and R = (PHP/PGE) − PHP] because obtaining precise oxygen consumption measurements as a surrogate for respiration is challenging (Reinthaler et al. 2006). Thus, apart from missing respiration measurements, an important source of uncertainty in this simple carbon matching is the choice of CF needed to recalculate from the original measurement units (assimilation of radiolabeled substrate and oxygen) to units of carbon (Romero-Kutzner et al. 2015, Giering & Evans 2022). Sensitivity analyses using a range of CFs together with statistical bootstrapping techniques allow the estimates to be constrained.

up to 40% of total cell abundance (Karner et al. 2001, Teira et al. 2006). While recent genome analyses indicate that heterotrophic thaumarchaea are widespread, albeit low in abundance in the mesopelagic (Aylward & Santoro 2020), all cultivated representatives thus far are obligate chemoautotrophic ammonia oxidizers (Könneke et al. 2005, Qin et al. 2017, Bayer et al. 2019b), carrying out the first and rate-limiting step of nitrification (Ward 2011). As such, their abundances and activities are tightly bound to the flux of POM from the surface waters (Santoro et al. 2019). In the open ocean, the highest absolute abundances of thaumarchaea are found just below the photic zone and often correlate with maximum nitrification rates (Beman et al. 2008, Shiozaki et al. 2016). Environmental and culture studies revealed that some thaumarchaea have the metabolic flexibility to use urea and/or cyanate for ammonia oxidation (Bayer et al. 2016, Santoro et al. 2017, Carini et al. 2018, Kitzinger et al. 2019), while the accessibility of other dissolved organic nitrogen sources, such as amino acids, amides, and amines, is limited and requires initial heterotrophic remineralization (Damashek et al. 2021). Other archaeal groups, including marine group II and III euryarchaea, make up only a small fraction of cells in the deep ocean, and most genomic evidence so far suggests heterotrophic lifestyles, with a focus on high-molecular-weight organic matter degradation (see Santoro et al. 2019 and references therein).

Major bacterial groups of the dark ocean include diverse Alpha- and Gammaproteobacteria, SAR406/marine group A, SAR324, SAR202, Bacteroidetes, Verrucomicrobia, Actinobacteria, and Planctomycetes (Sunagawa et al. 2015, Salazar et al. 2016, Acinas et al. 2021). Only a few of these groups have cultured representatives, and our knowledge of the functional diversity of these groups relies greatly on metagenomic analyses. One of the well-studied bacterial groups is the alphaproteobacterial SAR11 clade (Pelagibacterales), which constitutes approximately 20% of prokaryotic cells in the deep ocean (Morris et al. 2002, Eiler et al. 2009). While most subclades predominantly inhabit the surface ocean, subclade IIb is found mainly in the upper mesopelagic, and there is evidence for a piezotolerant subclade Ic present in meso- and bathypelagic waters (Thrash et al. 2014, Giovannoni 2017). The SAR11 clade is overall relatively conserved and evolved into niches of harvesting labile, low-molecular-weight DOM, including C1 and methylated compounds (Giovannoni 2017 and references therein). By contrast, members of the SAR202 cluster have evolved a different metabolic strategy to adapt to the low reactivity of deep-ocean DOM by specializing in the oxidation of relatively recalcitrant to refractory compounds (Landry

SUBSTRATE UPTAKE STRATEGIES OF MARINE PROKARYOTES: IS MORE REALLY MORE?

Bacteria and archaea use various strategies to consume organic carbon. In the oligotrophic ocean, prokaryotes require active uptake systems that are able to scavenge low concentrations of available DOC efficiently. Such high-affinity transporters share a common feature: the presence of substrate-binding proteins (SBPs) that trap substrate in the periplasm. ABC transporter systems represent the most abundant high-affinity transporters at all depths, and their relative contribution to metaproteomes increases from the surface to the deep ocean (Bergauer et al. 2018). Recent models suggest that the high affinity of ABC transport is a function of SBP abundance (Bosdriesz et al. 2015, Norris et al. 2021), leading to half-saturation concentrations (K_M) that are more than a thousandfold smaller than its binding protein's dissociation constant (K_D) (Norris et al. 2021). Consequently, prokaryotes adapted to oligotrophic conditions, such as SAR11, devote much of their energy to synthesizing SBPs (Sowell et al. 2009) and have extraordinarily high uptake affinities (Noell & Giovannoni 2019). However, the reliance on binding proteins to achieve high affinities comes at a high cost and precludes high growth rates (Norris et al. 2021), which likely represents a beneficial strategy for free-living, nonmotile prokaryotes that are confined to environments with low inputs of fresh organic matter. Hence, high abundances of transporter proteins in the deep ocean are likely the result of an adaptive response to oligotrophic conditions and do not necessarily imply high uptake rates.

et al. 2017, Liu et al. 2020, Saw et al. 2020). SAR202 bacteria are abundant members of microbial communities, increasing from an approximately 5% relative abundance in the mesopelagic to up to 30% in the bathypelagic (Morris et al. 2004, Varela et al. 2008), where they occupy specialized niches within deep-ocean ecosystems (Saw et al. 2020).

Heterotrophic production in the deep ocean is generally limited by the availability of organic carbon. In a metaproteomic study, the fraction of transporters responsible for organic matter uptake increased from approximately 23% in the euphotic layers to 39% in bathypelagic waters, suggesting an adaptation of microbial communities to the changes in the quality and quantity of organic matter in the deep ocean (Bergauer et al. 2018). Substrate-binding proteins of ATP-binding cassette (ABC) transporter complexes make up a major fraction of deep-ocean metaproteomes (Bergauer et al. 2018), potentially implying high specific affinities of these transporters by maintaining a high ratio of binding proteins to transport (Norris et al. 2021) (see the sidebar titled Substrate Uptake Strategies of Marine Prokaryotes: Is More Really More?). Deep-sea microbes are thought to be preferentially associated with particles (Baltar et al. 2009), which is supported by an increase with depth in the abundances and activities of secretory enzymes catalyzing the breakdown of carbohydrates and proteins (Baltar et al. 2010b, Zhao et al. 2020). The higher proportion of secretory to total enzymes in the deep ocean is driven mainly by members of the Alphaproteobacteria (Rhodobacterales and Sphingomonadales) and Gammaproteobacteria (Alteromonadales and Oceanospirillales), indicating a preferential utilization of POM by these groups (Zhao et al. 2020).

One of the most abundant proteins found in the mesopelagic is nitrite oxidoreductase (Nxr)—a metalloenzyme encoded by chemoautotrophic nitrite-oxidizing Nitrospinae bacteria (Saito et al. 2020). Nitrospinae are low-abundance members of deep-ocean microbial communities (Pachiadaki et al. 2017), and the surprisingly high abundances of Nxr have been suggested to maximize contact efficiency with scarce nitrite (Saito et al. 2020). Cell-specific DIC fixation measurements of Nitrospinae suggest high metabolic activity (Pachiadaki et al. 2017), but fluxes of nitrite are unlikely to sustain the observed carbon assimilation (Zhang et al. 2020) even when accounting for higher DIC fixation yields, as recently reported (Bayer et al. 2022). While direct

evidence for the use of alternative energy sources by Nitrospinae in the deep ocean is lacking, nitrite oxidizers have been shown to be metabolically versatile in culture, being able to oxidize hydrogen (Koch et al. 2014) and sulfur (Füssel et al. 2017) or grow chemoorganoautotrophically on formate (Koch et al. 2015, Bayer et al. 2021).

Sulfur-driven chemolithoautotrophy might be more prevalent in the oxygenated deep ocean than was initially assumed (Swan et al. 2011). Detached from benthic processes, sulfur cycling is believed to be sustained mainly by lateral transport processes and particle microniches (Callbeck et al. 2021). Additionally, the degradation of dissolved organic sulfur compounds derived from sinking phytoplankton biomass (Landa et al. 2019) and local secretion by diel migrating zooplankton (Clifford et al. 2017, Tutasi & Escribano 2020) generate reduced and oxidized forms of inorganic sulfur species (e.g., sulfide, thiosulfate, and sulfite) in the oxic water column (Landa et al. 2019). In deep waters of the eastern tropical South Pacific, taurine uptake and desulfonation genes are dominated by SAR324 bacteria and the gammaproteobacterial ARCTIC96BD-19 clade (Landa et al. 2019 and references therein), suggesting that sulfite generation from taurine could be further oxidized to sulfate by these groups (Callbeck et al. 2021). SAR324 bacteria have recently been classified into multiple subclades inhabiting the entire water column (Boeuf et al. 2021, Malfertheiner et al. 2022), but the highest abundances are found in meso- and bathypelagic waters (Ghiglione et al. 2012, Malfertheiner et al. 2022). SAR324 bacteria are metabolically diverse, and subclades present in the deep ocean share genomic features of a mixotrophic lifestyle, including sulfur-based chemolithoautotrophy and C1 compound metabolism (Swan et al. 2011, Sheik et al. 2013, Boeuf et al. 2021).

Trace gases such as carbon monoxide (CO) and hydrogen (H_2) have been suggested as potential alternative energy sources fueling dark DIC fixation (Anantharaman et al. 2013, Zhang et al. 2020), and genes for trace gas oxidation are widespread and abundant in deep waters (Acinas et al. 2021, Lappan et al. 2022). The consumption of CO and H_2 in surface waters is significant (Xie et al. 2005, Lappan et al. 2022) and coincides with their production via photochemical oxidation and cyanobacterial N_2 fixation, respectively (Moore et al. 2009). High concentrations of H_2 are also found at hydrothermal vent sites (McCollom 2008) or during fermentation in anoxic environments (Kessler et al. 2019). Accordingly, high abundances of hydrogenases are found in hydrothermal plumes (Anantharaman et al. 2013) and on particles that might provide anoxic microniches (Acinas et al. 2021). However, CO and H_2 are probably not readily available to most free-living deep-ocean prokaryotes.

6. PARTICLES AS VEHICLE FOR MICROBES AND CONNECTIVITY OF PROKARYOTIC COMMUNITIES

There are two fractions of detrital POM in the oceanic water column, which are differentiated by their size and specific density: neutrally buoyant suspended particles and sinking particles (Bochdansky et al. 2010, Herndl & Reinthaler 2013). Generally, most of the detrital POM in the deep ocean consists of suspended particles (Kepkay 2000, Verdugo et al. 2004). The depth distributions of the suspended and sinking detrital POM in the open ocean differ. The concentration of suspended POC remains relatively constant with depth (Baltar et al. 2010c), whereas the concentration of sinking POC exponentially decreases with depth (Martin et al. 1987, Buesseler et al. 2007). While the origin of sinking POM is associated with phytoplankton blooms in euphotic layers, the source of suspended POM remains unclear (Herndl & Reinthaler 2013). Suspended POM could originate either from remnants of sinking particles fragmented by deep-ocean organisms or from autochthonous production by deep-water prokaryotes via chemolithoautotrophic activity (Baltar et al. 2010c).

The ubiquitous occurrence of different types of detrital particles in the oceanic water column plays a key role in the distribution and activities of marine microbes. Investigations performed in deep and surface waters at specific sites (Ghiglione et al. 2007, Eloe et al. 2011b, Crespo et al. 2013, Duret et al. 2019, Mestre et al. 2020) are in agreement with a global bathypelagic ocean analysis from the Malaspina Circumnavigation Expedition (Salazar et al. 2015). This analysis revealed a niche partitioning and differences in alpha and beta diversity between free-living and particle-associated microbial (nominal size cutoff 0.8 μm) communities at a global scale. The study's authors used the particle-association niche index (Stegen et al. 2012) to distinguish the preferences of microbes for a particle-associated or free-living lifestyle (Salazar et al. 2015). Applying this index to bathypelagic waters revealed that archaea (both euryarchaea and thaumarchaea) and the bacterial groups SAR86, SAR324, SAR406, and SAR202 exhibited a preference for a free-living lifestyle; Bacteroidetes, Firmicutes, Planctomycetes, Deltaproteobacteria clade OM27, and Desulfuromonadales were associated with particles (Salazar et al. 2015). Acinas et al. (2021) showed that these major differences in the community composition of deep-ocean particle-attached and free-living communities also translate into pronounced differences in functional diversity (**Figure 6**). These authors found that nitrification and CO oxidation genes were associated mostly with free-living assemblages, and H_2 oxidation genes were essentially linked to the particle-attached microbes. These pronounced differences in functional and phylogenetic diversity between free-living and particle-attached microbes provide even more evidence of the heterogeneous nature of the dark ocean and are a further indicator of the contrasting ecological niches that the deep ocean's particle-attached and free-living microbes occupy (**Figure 6**).

Contrasting factors are apparently controlling the free-living and particle-attached microbes in the deep ocean. Global circulation and water mass age are the parameters that more strongly affect the particle-attached microbes, whereas temperature and depth control the composition of free-living communities (Salazar et al. 2016). Surprisingly, the deep-ocean particle-attached microbiome but not the free-living microbiome showed indications of dispersal limitation and, consequently, basin specificity (Salazar et al. 2016). This is consistent with the notion that a significant portion of the deep-ocean particles colonized by microbes is not sinking POM but suspended particles (Baltar et al. 2009, 2010c; Herndl & Reinthaler 2013). Deep-ocean microbial respiration has been associated with suspended particles (Baltar et al. 2009, Bochdansky et al. 2010). A high proportion of dissolved versus total extracellular enzymatic activities has been reported in the deep sea measured with substrate analogs (Baltar 2018; Baltar et al. 2010a, 2013), which, based on the foraging theory (i.e., the theory that natural selection favors strategies of organisms to maximize net energy intake per unit time spent foraging; MacArthur & Pianka 1966) strongly suggests a preferential particle-attached lifestyle of dark-ocean microorganisms (Arístegui et al. 2009; Baltar et al. 2009, 2010b). A recent global survey on peptidases and carbohydrate-active enzymes—the two central enzyme groups targeting the two most abundant macromolecules in the ocean—revealed an increasing proportion of genes encoding secreted (i.e., dissolved) enzymes with depth (Zhao et al. 2020).

Deep-sea microbial communities are to a certain extent connected to surface assemblages (Cram et al. 2015a,b; Parada & Fuhrman 2017). Cram et al. (2015b) used a microbial association network to study the connection between community dynamics and environmental parameters within and between depth layers over a seasonal cycle. They found lagged and concurrent shifts in community composition between depths, indicating that deep-ocean free-living assemblages are linked to environmental conditions and/or communities in overlying waters. This connection between communities from different depth layers is probably due to migrating organisms transporting nutrients across otherwise stratified waters (Maas et al. 2020) and/or sinking particles transporting cells originating from overlying waters (Mestre et al. 2018). Thus,

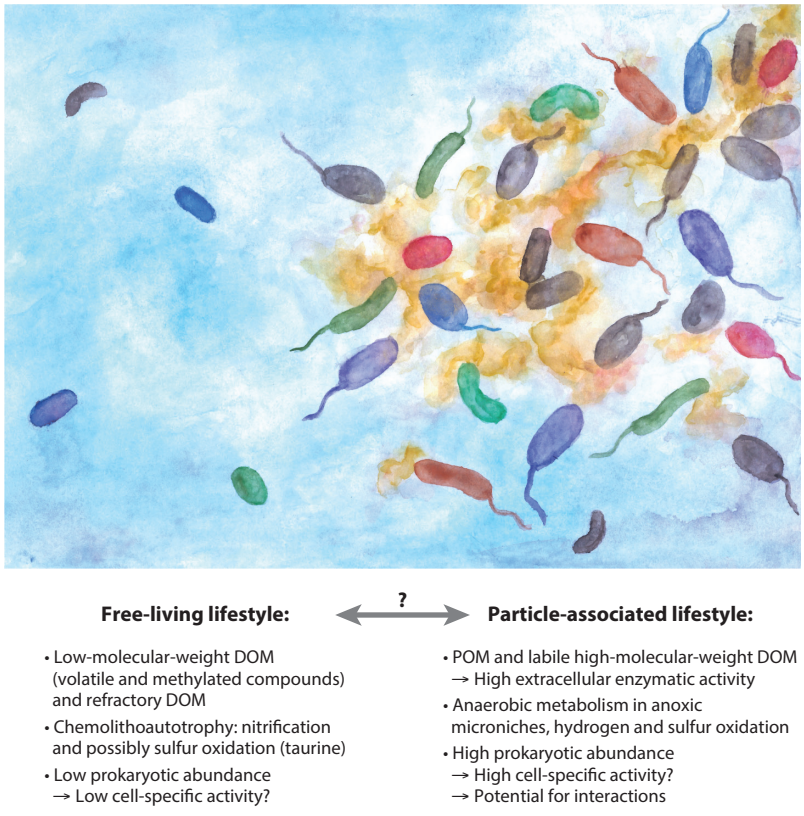

Figure 6

Illustration of free-living and particle-associated lifestyles of prokaryotes in the deep ocean. The different shapes and colors of the prokaryotic cells represent their phylogenetic and functional diversity. Prokaryotic abundances and distances between individual cells are not to scale but rather illustrate a hot spot of prokaryotic abundance and activity in the deep ocean. The abundances of particle-attached prokaryotes are on average three orders of magnitude higher than those of free-living prokaryotes. Assuming an even distribution of cells, the distance between free-living prokaryotes is 1,000 times larger than their size (equivalent to 1-cm-long cells that are 10 m apart from one another in every direction). Abbreviations: DOM, dissolved organic matter; POM, particulate organic matter.

the sunlit community composition and environmental conditions ultimately impact the microbial community on deep-water particles (Ruiz-González et al. 2020), which is consistent with the observed influence of phytoplankton community composition and grazing on the nutritional properties of sinking particles (Boyd & Newton 1995, Guidi et al. 2016, Bach et al. 2019). Consistently, the majority of the particle-associated prokaryotes detected in the bathypelagic are also found in epipelagic waters (Mestre et al. 2018).

Due to the fluctuating nature of the productivity of sunlit surface waters, it is expected that the connection between surface and deep-water prokaryotes via sinking particles varies seasonally (Poff et al. 2021). Moreover, the vertical structure of the water column also changes seasonally, which might also influence the connection between euphotic and deep-ocean microbial communities. A recent time-series study over a seasonal cycle of the microbial composition of surface and mesopelagic waters showed that the link between surface and deep-ocean microbial communities

is not just unidirectional (via sinking of particles) (Wenley et al. 2021). Based on their results, the study's authors concluded that the mechanisms connecting surface and mesopelagic microbial communities change seasonally, that is, via sinking particles during the productive season (spring and summer) and via deep mixing that brings deep-water taxa into the sunlit waters during winter overturning (Wenley et al. 2021). The influence of this mechanism is restricted to the depth of winter convective mixing.

7. ADAPTATIONS TO HIGH HYDROSTATIC PRESSURE AND LOW TEMPERATURE BY DEEP-SEA PROKARYOTES

The extent to which deep-sea prokaryotic communities are adapted to the hydrostatic pressure conditions remains enigmatic. While some studies report elevated activity of bathypelagic heterotrophic prokaryotic communities under in situ pressure conditions as compared with corresponding measurements under decompressed conditions, other studies report opposite findings (summarized in Tamburini et al. 2013). Since the pioneering work in deep-sea microbiology reviewed by Jannasch & Taylor (1984), it has been well known that there are piezophilic bacteria in the deep ocean (Lauro & Bartlett 2008). However, the fraction of piezophilic prokaryotes within the total abundance of deep-sea prokaryotes remains unknown.

On a community level, deep-sea prokaryotes are characterized by having larger genomes than their euphotic counterparts, which is indicative of a more opportunistic lifestyle prevailing in deep waters (DeLong et al. 2006). While only one piezophilic archaeon (*Pyrococcus yayanosii*) has been isolated thus far (Jun et al. 2011), a considerable number of piezophilic bacteria have been isolated and their genomes characterized (Eloe et al. 2011a, Jun et al. 2011, Kusube et al. 2017), allowing the identification of some specific features of piezophilic bacteria. The abovementioned archaeon *Pyrococcus yayanosii* is a thermopiezophilic representative, but most of the deep-sea piezophilic prokaryotes are psychropiezophiles (i.e., adapted to high hydrostatic pressure and low temperature). Most of the psychropiezophilic isolates belong to the Gammaproteobacteria, with representatives of *Colwellia*, *Shewanella*, *Photobacterium*, *Moritella*, and *Psychromonas* (Eloe et al. 2011a, Jebbar et al. 2015, Nogi 2017). Surprisingly, a large number of these piezophilic strains have been isolated from deep-sea amphipods (Kusube et al. 2017).

Comparison of genes and proteins of piezophilic versus piezosensitive *Colwellia* isolates revealed several features specific to the piezophilic lifestyle (Peoples et al. 2020). Evaluating the isoelectric point distributions of the *Colwellia* proteomes revealed a higher number of basic proteins in piezophilic compared with piezosensitive strains. Amino acid abundances in conserved, orthologous proteins in piezophilic *Colwellia* strains were enriched in tryptophan, tyrosine, leucine, phenylalanine, histidine, and methionine compared with those of piezosensitive strains (Peoples et al. 2020). The amino acid species enriched in piezosensitive *Colwellia* strains were glutamic acid, aspartic acid, asparagine, and serine (see the sidebar titled Hydrostatic Pressure and Low Temperature).

Comparing the relative abundances of clusters of orthologous genes, Peoples et al. (2020) found a higher percentage of genes for replication, recombination, and repair; cell wall and membrane biogenesis; cell motility; extracellular structures and translation; and ribosomal structures in piezophilic *Colwellia* strains than in piezosensitive *Colwellia* strains. Piezophilic and psychrophilic strains have a higher content of polyunsaturated fatty acids to increase membrane fluidity under high hydrostatic pressure and low temperature (Peoples et al. 2020). Another apparent adaptation to high hydrostatic pressure is the high abundance of glycosyltransferases, enzymes promoting extracellular polysaccharide synthesis (Peoples et al. 2020).

HYDROSTATIC PRESSURE AND LOW TEMPERATURE

The mesopelagic and (particularly) bathypelagic global ocean is characterized by low temperatures, ranging from 0°C to 4°C. Only the deep waters of the Mediterranean Sea, the Red Sea, and the Sulu Sea have higher deep-water temperatures (up to 20°C). Hence, piezophilic prokaryotes should typically also be adapted to low temperatures. The commonly accepted definition of piezophily is that the maximum growth rate of piezophiles occurs at pressures above the atmospheric pressure of 0.1 MPa. Typically, however, the piezosphere is considered to be depths below 1,000 m, corresponding to 10 MPa (Yayanos 1986). Consequently, piezophiles exhibit maximum growth rates at >10 MPa (Jannasch & Taylor 1984). As stated recently, only 86 prokaryotic isolates with a growth optimum at >0.1 MPa are currently available (Scoma 2021).

In a general sense, hydrostatic pressure influences the intermolecular distances and thereby affects the conformation of polynucleotides (DNA and RNA), lipid bilayers, and the tertiary structure of proteins (Oger & Jebbar 2010, Scoma 2021). Thus, it seems logical that piezophilic prokaryotes should have a selective advantage over non-piezophilic prokaryotes in the deep ocean. In a meta-analysis, Scoma (2021) took into account the interactive effect of hydrostatic pressure and temperature, determining the growth optimum based on the initial work of Yayanos (1986) to define piezophiles. Scoma (2021) differentiated three functional groups based on temperature: piezopsychrophiles, piezomesophiles, and piezothermophiles. A competitive advantage of piezophiles over piezosensitives is predicted to begin at 10 MPa and to exist consistently irrespective of temperature at hydrostatic pressures above 20 MPa. Hyper-piezopsychrophiles are specific to hadal trenches, and their competitive advantage over piezopsychrophiles begins at a hydrostatic pressure above 50 MPa (Scoma 2021).

Based on the results described above, it appears that a piezophilic lifestyle of heterotrophic prokaryotes requires many changes throughout the cell. Many piezophile enriched genes are located near areas of genomic variability and could be shared among piezophiles via horizontal gene transfer.

FUTURE ISSUES

1. Dark-ocean prokaryotic heterotrophic production has been routinely measured; however, the leucine-to-carbon conversion factor required to estimate production rates is rarely determined in the deep ocean. The few data available on leucine-to-carbon conversion factors for the deep-ocean prokaryotes are highly variable.
2. While there is a fairly large data set available on heterotrophic production, there is a severe lack of measurements of respiration, which typically contributes much more to the heterotrophic prokaryotic carbon demand than biomass production. Thus, there is a major uncertainty in the estimates of the prokaryotic heterotrophic carbon demand in the dark ocean. Metabolic rate measurements on deep-sea microbes should be performed under in situ pressure conditions. The fraction of piezophilic and piezotolerant microbes in the deep sea should be determined, since we do not know how large the fraction of piezophilic and piezotolerant microbes actually is.
3. New sampling tools and techniques are required to selectively sample detrital particles (marine snow) in the meso- and bathypelagic waters, as these particles are largely very fragile but densely populated by prokaryotes. Metabolic rate measurements on these

particle-associated prokaryotes should be performed, as one might expect that most of the prokaryotic activity in the deep ocean is associated with these particles.

4. There is still uncertainty about the biomass of macrofauna in the meso- and bathypelagic ocean and its interaction with the microbial community. Also, symbiotic interactions between deep-sea metazoans and microbes might be more common than assumed thus far.
5. There is a major gap in our knowledge of the energy sources fueling the dark ocean's dissolved inorganic carbon fixation. Rate measurements of the uptake of potential electron donors are also largely missing.
6. We are currently unable to close the carbon budget for the dark ocean, as the apparent carbon demand is higher than the sinking flux of particulate organic matter. This apparent mismatch might be caused, at least partly, by a temporal or spatial mismatch in measurements of particulate organic matter flux versus microbial activity.

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The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Errata

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