Microbial life in the open ocean: a universe of tiny cells separated by empty space

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ABSTRACT

Marine microbes are fundamental components of food webs and the biogeochemical cycles that maintain the habitability of the planet. In the oligotrophic open ocean, these microscopic organisms live in a dilute environment separated from other cells by large distances at the microscale while surrounded by very few essential nutrient molecules. For ubiquitous sub-micron sized and non-motile microbes, cellular growth requirements for hundreds of millions (or more) of nutrient molecules are sustained predominantly by rapid molecular diffusion. Characterizing the interactions of cells and molecules in the "empty space" of the ocean remains central to understanding the drivers and consequences of oceanic biogeochemical cycles.

TEXT

Microscopic examination of a drop of seawater reveals the presence of millions of small cells, including Bacteria, Archaea, Protists, as well as viruses (Figure 1A). That microscopic view involves a filtration and concentration step that masks the remarkable distances between individual cells *in situ* in the oligotrophic ocean. At the microbial scale, hundreds of micrometers separate cells that themselves are less than a micrometer in size (Figure 1B). Despite their microscopic size and relative isolation, marine microbes catalyze chemical transformations at rates that are critical for maintaining the habitability of the planet. In the open ocean, but at a microbial scale, not only are cells (and viruses) distantly distributed, so are the organic compounds and inorganic nutrients that constitute the molecular building blocks of life. Recent studies have shown that there are multiple interactions between microorganisms, microorganisms and viruses (*1*), and microorganisms and molecules that occur at high daily rates despite extremely dilute concentration of nutrient molecules and large, relative distances between bacteria, algae, grazers and viruses of the sea. It is remarkable that every day, nitrogen molecules for example, must be supplied from a volume of seawater orders of magnitude greater than an individual nonmotile bacterial or cyanobacterial cell, in order to support microbial growth. By reviving a historical perspective combined with simple analyses and modeling of physical processes, we suggest that viewing the open ocean microbial world through the interwoven threads of space, time, and diffusion is critical for understanding how microbial interactions shape the biogeochemical cycles of one of the largest habitats on Earth.

Marine microbes were largely ignored in early considerations of marine food chains until epifluorescence microscopy showed that microbes were very abundant, typically almost 1 million bacteria cells per milliliter of surface seawater. The concept of the "microbial loop (*2*) made explicit the roles of microbes in nutrient recycling and funneling matter and energy into the protists and larger organisms of the oceanic food web. It was not possible to measure these processes at the scales relevant to cells, but only in large volume water samples that measure integrated rates of metabolism and growth of billions of cells. Early studies suggested that ocean algae (phytoplankton) grew at or near maximal growth rates despite extremely low concentrations of inorganic nutrients (*3*). One explanation was that microscale heterogeneities, or "patches" such as those made by grazing protists or zooplankton, provided localized high concentrations of nutrients that could be rapidly taken up by phytoplankton to support high growth rates in the ocean (*4*). This view of the microbial world as patchy and heterogeneous was extended to organic molecules and heterotrophic microbes (*5*) and, more recently, to tractable microscale experimental systems with bacteria (*6*). This conceptualization of what the microscale world looks like – a complex milieu of microbes and organic molecules in "hotspots" (*5*) – has become a common way of presenting the oceanic microbial world. These hotspots can result from exudation of organic molecules from active photosynthetic phytoplankton cells, which has been elegantly discussed in the context of the "phycosphere" (*7, 8*). Such microscale heterogeneities can be exploited by motile or particle-bound microorganisms that can rapidly take up localized elevated concentrations of organic matter (*9*). Yet, it is important to recognize that for many, if not most, of the microbial cells in the open ocean habitat, such hotspots or patches are "football fields" away, if considered on a human scale.

In the open ocean, bacterial and cyanobacterial communities are dominated by nonmotile cells of two ubiquitous species – *Prochlorococcus marina* and *Pelagibacter ubique*. *P. marina* is the smallest free-living autotroph on the planet, with abundances typically on the order of a hundred million per milliliter and are responsible for the daily production of ~25% of the world's oxygen. *P. unique* is the most abundant microbe in the ocean, a heterotrophic bacterium essential to the microbial loop that can be 25-50% of total microbial abundances. To emphasize the remarkable balance between cellular needs and supply, consider the rates of nutrient fluxes and uptake needs for growth by single cells. Ammonium ions, the preferred inorganic source of nitrogen (N) for most phototrophic algae, are typically at 10 nanomolar concentrations or less. Thus, ammonium molecules are distributed at a distance of about 0.6 micrometers, similar to the cross-sectional dimension of an open ocean bacterial or cyanobacterial cell (Figure 1B), which means that only a handful of molecules are near each cell. A seawater volume equivalent to a large microbial cell of radius 0.5 micrometers (~0.5 μm^3) would contain only a few (<5) molecules of ammonium. However, the N requirement for microbial cell division is much greater than in the water displaced by the cell; e.g. one *Prochlorococcus* cell requires 4 x 10^8 N atoms per day to divide (*10*). In other words, in order to reproduce, a microbial cell needs to harvest the ammonium from *hundreds of millions of times* its cell volume.

Two mechanisms that could expose individual cells to this number of ammonium molecules from a large volume of surrounding seawater are active swimming or passive movement (via Brownian motion) (*9*). A microbe of radius 0.25 micrometers swimming for one day at a velocity of 30 $\mu m\ s^{-1}$ would access ~0.5 nanoliters containing ~3 x 10^6 ammonium molecules, which is <1% of the daily requirements. In contrast, Brownian motion is much, much faster– the

diffusivity of molecules ($D_{mol} = 10^{-5}cm^2\ s^{-1}$), is 1000 times greater than that of the Brownian motion of the microbial cells. Thus, molecular diffusion of nutrients leads to far more frequent encounters than would active motion of the cells themselves.

The stirring number – defined by $(lv)/D_{mol}$, where D_{mol} is the molecular diffusivity of the ammonium ions (*11*) – provides a benchmark for comparing the encounter efficiency of swimming to that of molecular diffusion. At low stirring numbers, there is little to no enhancement of nutrient uptake by swimming. For ammonium at 10 nanomolar concentration with intermolecular spacing of $l = 0.6$ μm, molecular diffusion is >50 times more efficient than is cellular swimming at $v = 30$ μm/s. Molecular diffusion alone generates a potential flux of ~$2x10^9$ N atoms per day per cell – four-times the daily N requirement for *Prochlorococcus*. Thus, diffusive processes alone can fuel the growth and productivity of abundant, free-living unicellular microorganisms in the open ocean. This demonstrates why lack of motility, and free-living cells, in a dilute environment is ecologically successful in oligotrophic oceans.

Although such "simple" calculations can resolve the problem of how individual cells can grow in the nutrient-limited open ocean, they explain how these cellular-scale processes cascade to transform ecosystem functions, including global biogeochemical cycles. Sources of nutrients are primarily recycling by bacteria (either free-living cells or in particles) or grazers (protistan micrograzers or metazoan zooplankton) that decompose organic matter and liberate ammonium. Early studies (*4*) on inorganic nutrients, and more recent studies on organic matter (*8*) have analyzed how microscale heterogeneities of elevated concentrations of inorganic or organic nutrients (patches or hotspots) can affect microbial growth and activity. Small scale patches of elevated concentrations of nutrients, e.g., on the order of a few to hundreds of micrometers in spatial extent, presumably left as a result of lysis by viruses or grazing and excretion by grazers, could facilitate significantly higher uptake rates by microorganisms (*4*). However, early modeling studies (*12*) contended that such patches of inorganic nitrogen diffuse too quickly and are too short-lived to be effective in inorganic nutrient uptake by open ocean nonmotile microbes. Our re-analysis based on recent estimates of micrograzer sizes, which are smaller than those used by Jackson (*12*) , suggests that a 3 micrometer diameter micrograzer swimming at 100 μm per second and feeding and recycling ingested particulate nitrogen at a rate commensurate with 1 doubling per day, might leave a plume of remineralized nitrogen of only 5-80 nanomolar (assuming that the plume was not dispersed). The elevated concentrations of nutrients in microscale patches such as micrograzer plumes are insufficient to supply dispersed non-motile cells such as *Prochlorococcus* and do not nearly overcome the >50-fold advantage of molecular diffusion relative to swimming to patches in the oligotrophic ocean. Similarly, the nonmotile heterotrophic *Pelagibacter ubique* depends on diffusion for the organic molecules needed for food. The nature of molecular diffusion also provides a mechanistic explanation for why the most abundant organisms in the open ocean, *Prochlorococcus* and *Pelagibacter,* are non-motile (*13*). In essence, swimming towards evanescent hotspots or phycospheres is not the dominant mechanism for supporting productivity of small cells living in nutrient-depleted environments – the situation most common in the open ocean.

The example of nitrogenous nutrients and *Prochlorococcus* demonstrates the importance of considering space, time and diffusion in understanding major microbial processes of the dilute ocean. But there are multiple implications of these types of small-scale processes in the open

ocean microbial world. Submicrometer-sized viruses are 10s of micrometers apart (*14*), eukaryotic algae and micrograzers 100s of micrometers apart and interactions occur over relatively large microscale distances (e.g. (*15*)). Although we used nitrogen as an example, the relationship of space, time, and diffusion applies to many other aspects of the microbial loop. The spatial distributions of phosphorus-containing molecules and iron, both essential nutrients, are much greater. Furthermore, recent studies suggest that metabolic exchanges between species are important in microbial interactions in the marine microbiome (*16*) but such exchanges are also controlled by the time-space considerations described here. The secretion or exchange of molecules in the dilute open ocean ("public goods") is problematic because of the great dilution in time and space in the oligotrophic ocean. Instead, mutualisms – cell-to-cell collaboration – that reshape the environment and provide energetic advantages to organisms may help explain long-term evolutionary adaptations linking the behavior of ubiquitous autotrophs, like *Prochlorococcus*, and heterotrophs like *Pelagibacter* (*17*).

The non-motile marine microbes at the base of the ocean food chain, like *Prochlorococcus* and *Pelagibacter*, transform and sustain ocean life. Such organisms may be small, even relative to other ocean microbes, and non-motile but they are hardly simple. Adaptive release of extracellular compounds, the distribution of ecologically distinct subspecies across different light, temperature, and mixing regimes ("ecotypes"), and day-night synchronization of activity (*18*) suggest some of the dynamic processes that contribute to the evolutionary fitness of these tiny microbes that drive biogeochemical cycles of the oligotrophic oceans. Many questions remain, including the interactions between viruses and grazers, the extent of the leakiness of the microbial loop and, in turn, the export of carbon to the deep ocean. Even without microscale complexity, chemotaxis and motility, it is essential to understand how the abundant microorganisms in the dilute habitat of the open ocean interact, and how they interact with hotspots and motile microorganisms. To do so requires that we recognize how dilute spatial distributions and molecular diffusion, at scales relevant to marine microbes, act in ways that may not seem intuitive to us [non-microscopic humans], yet are critical for understanding how the oceans and the global Earth system work. Recent discoveries, new techniques for measuring rates of chemical transformations at the microscale, genetic and genomic analyses of single cells and visualization and experimentation at the scale of milliliters rather than liters, make it possible to examine the microscale processes in the open ocean that affect oceanic biogeochemical processes at the ecosystem scale.

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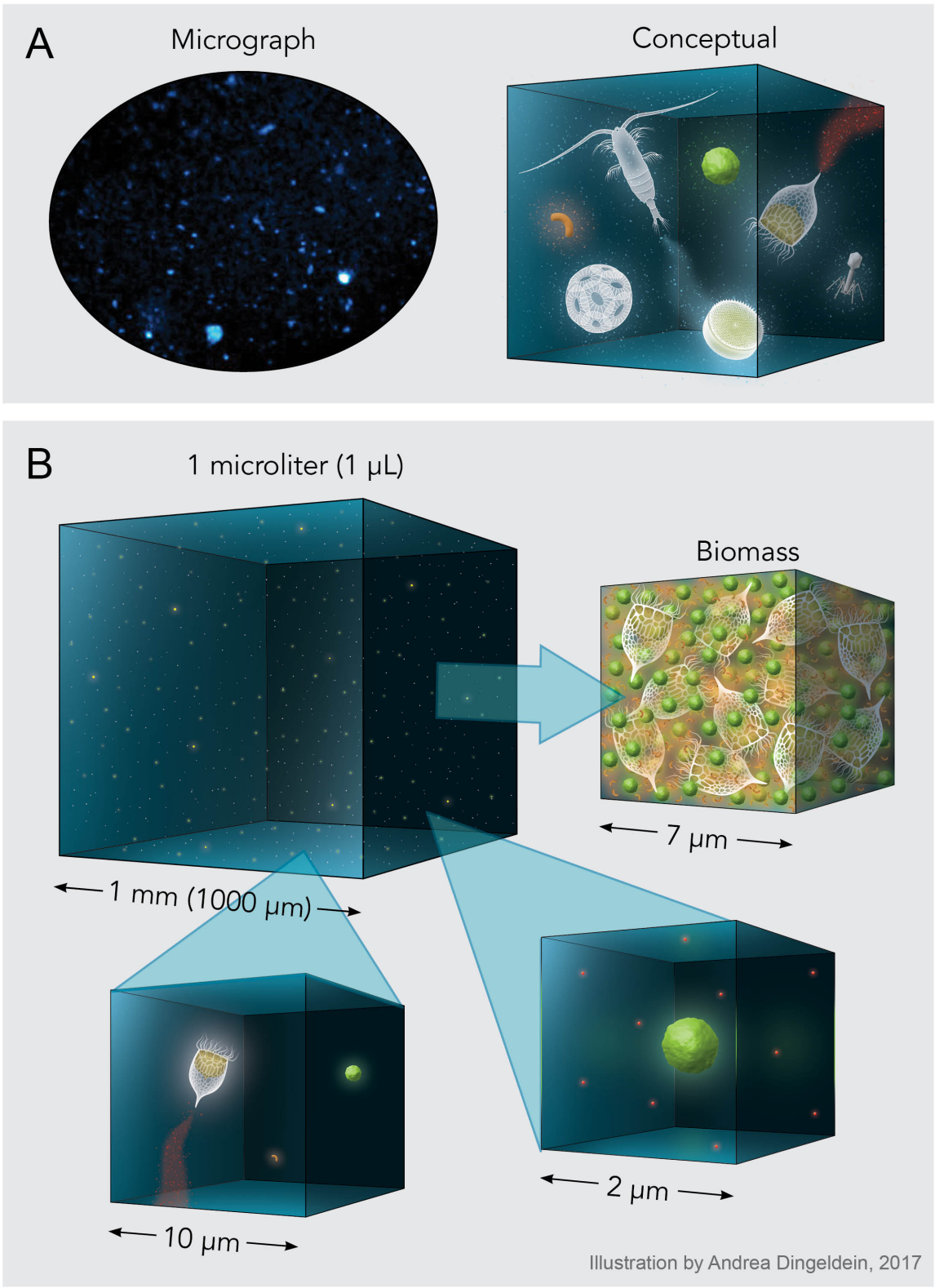
Figure 1. The components, sizes and distances in the microbial engine that drives Earth's biogeochemical cycles in the open ocean. A. Conceptual representation of microbial components that are not to scale but emphasize biological complexity, and a typical epifluorescence microscopic image of marine microbes from 0.2 nanoliter of seawater concentrated onto a polycarbonate filter. B. Scaled representation of microbe size and distances between them in the open ocean microbial world. Upper left: Scaled microliter with typical *Prochlorococcus* concentrations. *Prochlorococcus* cells are magnified approximately 3X, in order to be visualized at the same scale. Upper right: All of the microbial biomass from 1 μL (1 cubic millimeter, or microliter) fits in a 7 micrometer cube, just a fraction of space of a microliter. Lower left: Protist micrograzer in relation to size of prey (*Prochlorococcus* and other microbes) and plume of nutrients that are recycled for microbes. Lower right: *Prochlorococcus* cell with recycled ammonium molecules (not to scale), showing the minute fraction of ammonium molecules available relative to the daily needs of a single cell for cell division.


A
Micrograph
Conceptual
B
1 microliter (1 µL)
Biomass
7 µm
1 mm (1000 µm)
10 µm
2 µm
Illustration by Andrea Dingeldein, 2017

1. S. Sunagawa *et al.*, Ocean plankton. Structure and function of the global ocean microbiome. *Science* **348**, 1261359 (2015).
2. F. Azam *et al.*, The ecological role of water-column microbes in the sea. *Marine Ecology-Progress Series* **10**, 257-263 (1983).
3. J. C. Goldman, D. G. Peavey, Steady-state growth and chemical composition of the marine chlorophyte *Dunaliella tertiolecta* in nitrogen-limited continuous cultures. *Appl Environ Microbiol* **38**, 894-901 (1979).
4. J. J. McCarthy, J. C. Goldman, Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. *Science* **203**, 670-672 (1979).
5. F. Azam, F. Malfatti, Microbial structuring of marine ecosystems. *Nat Rev Microbiol* **5**, 782-791 (2007).
6. R. Stocker, J. R. Seymour, Ecology and physics of bacterial chemotaxis in the ocean. *Microbiol Mol Biol Rev* **76**, 792-812 (2012).
7. W. Bell, R. Mitchell, Chemotactic and growth responses of marine bacteria to algal extracellular products. . *Biological Bulletin* **143**, 265-277 (1972).
8. J. R. Seymour, S. A. Amin, J. B. Raina, R. Stocker, Zooming in on the phycosphere: the ecological interface for phytoplankton-bacteria relationships. *Nat Microbiol* **2**, 17065 (2017).
9. R. Stocker, Marine microbes see a sea of gradients. *Science* **338**, 628-633 (2012).
10. S. Bertilsson, O. Berglund, D. M. Karl, S. W. Chisholm, Elemental composition of marine Prochlorococcus and Synechococcus: Implications for the ecological stoichiometry of the sea. *Limnology & Oceanography* **48**, 1721-1731 (2003).
11. E. M. Purcell, Life at low Reynolds number. *American Journal of Physics* **45**, 3-11 (1977).
12. G. A. Jackson, Phytoplankton growth and zooplankton grazing in oligotrophic oceans. *Nature* **284**, 439-441 (1980).
13. D. Dusenberry, Minimum size limit for useful locomotion by free-swimming microbes. . *Proceedings of the National Academy of Sciences USA* **94**, 10949-10954. (1997).
14. C. H. Wigington *et al.*, Re-examination of the relationship between marine virus and microbial cell abundances. *Nat Microbiol* **1**, 15024 (2016).
15. D. A. Siegel, Resource competition in a discrete environment: Why are plankton distributions paradoxical? *Limnology & Oceanography* **43**, 1133-1146 (1998).
16. S. A. Amin *et al.*, Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* **522**, 98-101 (2015).
17. R. Braakman, M. J. Follows, S. W. Chisholm, Metabolic evolution and the self-organization of ecosystems. *Proc Natl Acad Sci U S A* **114**, E3091-E3100 (2017).
18. F. O. Aylward *et al.*, Microbial community transcriptional networks are conserved in three domains at ocean basin scales. *Proc Natl Acad Sci U S A* **112**, 5443-5448 (2015).

Supplemental Calculations for "How microbes survive in the open ocean" by J. Zehr, J.S. Weitz, and I. Joint

Ammonium molecules per micron cubed Given ammonium concentration of ρ in units of nM then the number of ammonium molecules per micron cubed, x, is defined as

$$x = \overbrace{\rho}^{[\text{nanomoles/L}]} \times \overbrace{10^{-9}}^{[\text{moles/nanomoles}]} \times \overbrace{10^{-3}}^{[\text{L/cm}^3]} \times \overbrace{10^{-12}}^{[\text{cm}^3/\mu\text{m}^3]} \times \overbrace{6.02 \cdot 10^{23}}^{[\text{molecules/moles}]} = 0.6\rho. \quad (1)$$

Hence for $\rho = 10$ nM then $x \approx 6$ molecules/μm^3.

Distance between ammonium molecules Given molecular density, x, then the typical distance l between ammonium molecules is $l \approx x^{-1/3}$. For $x \approx 6$ molecules/μm^3, then $l \approx 0.5$ μm.

Nitrogen atoms in *Prochlorococcus* We use a consolidated estimate of $m_{Pro} \approx 10$ fg per *P. marina* cell following Bertillson et al. (2003), hence the number of nitrogen atoms per cell, x_{Pro}, is approximately

$$x_{Pro} = \overbrace{m_{Pro}}^{[\text{fg}]} \times \overbrace{10^{-15}}^{[\text{g/fg}]} \times \overbrace{(1/14)}^{[\text{moles/g}]} \times \overbrace{6.02 \cdot 10^{23}}^{[\text{molecules/mole}]} = 4.3 \times 10^7 m_{Pro}, \quad (2)$$

such that $x_{Pro} \approx 4.3 \times 10^8$ nitrogen atoms per cell.

Daily volume swept by swimming microbe The daily total volume of water, V (nL), explored by cellular swimming and neglecting the effects of diffusion, is approximated as the product of the cellular cross-section, πr^2 where $r \approx 0.3$ μm for *P. marina*, and the distance traveled $d = vt$, i.e., given a hypothetical value of $v = 30$ μm/sec,

$$V = \pi \overbrace{(0.3)^2}^{[\mu\text{m}^2]} \times \overbrace{30}^{[\mu\text{m/sec}]} \times \overbrace{86400}^{[\text{sec}]} \times \overbrace{10^{-12}}^{[\text{cm}^3/\mu\text{m}^3]} \times \overbrace{10^6}^{[\text{nL/cm}^3]} \approx 0.7\text{nL}. \quad (3)$$

Ammonium molecules per nL Given ammonium concentration of ρ in units of nM then the number of ammonium molecules per nL is

$$x_{nL} = \overbrace{\rho}^{[\text{nanomoles/L}]} \times \overbrace{10^{-9}}^{[\text{moles/nanomoles}]} \times \overbrace{6.02 \cdot 10^{23}}^{[\text{molecules/mole}]} \times \overbrace{10^{-9}}^{[\text{L/nL}]} = 6 \cdot 10^5 \rho, \quad (4)$$

such that for $\rho = 10$, then $x_{nL} \approx 6 \times 10^6$ (molecules/nL).

Diffusive flux of ammonium into microbial cells The daily maximum diffusive flux for a microbial cell of radius r given concentration ρ is $J \approx (4\pi D r \rho \cdot 86400)$ where D is the diffusion rate of the molecule. We note that if $x \approx 6$ molecules/μm^3 then the number of molecules per cm^3 is $\approx 6 \times 10^{12}$, and

$$J = 4\pi \times \overbrace{10^{-5}}^{[\text{cm}^2/\text{sec}]} \times \overbrace{0.3 \cdot 10^{-4}}^{[\text{cm}]} \times \overbrace{6 \cdot 10^{12}}^{[\text{molecules/cm}^3]} \times \overbrace{86400}^{[\text{sec}]} \approx 2 \times 10^9. \quad (5)$$

Plume-generated remineralization of ammonium Consider a micrograzer of diameter 3 μm swimming at 100 μm/sec releasing a plume of diameter 20 μm, representing a volume $(10/0.3)^2 \times (100/30) \approx 4000$-fold higher than in Eq. (3), equivalent to 2800 nL in one day. Following Caron et al. (2017), we assume a micrograzer (or nanoflagellate) has $n_{grazer} \approx 50 - 200$ fg nitrogen per cell. Assuming an equivalent amount of nitrogen is remineralized this would correspond to a plume concentration:

$$\rho_{plume} = \frac{\overbrace{n_{grazer}}^{[\text{fg/nL}]}}{2800} \times \overbrace{10^{-15}}^{[\text{g/fg}]} \times \overbrace{1/14}^{[\text{moles/g}]} \times \overbrace{10^9}^{[\text{nL/L}]} \times \overbrace{10^9}^{[\text{nanomoles/mole}]} \approx 0.026 \cdot n_{grazer}, \quad (6)$$

such that ammonium concentrations in the plume would range from $\rho_{plume} \approx 1 - 5$ nM for micrograzers with nitrogen content ranging from 50-200 fg, respectively, if the plume did not disperse. These concentrations are less than background, however if the plume diameter were 6 μm and given content of 200 fg then the resulting plume concentration would be $\rho_{plume} \approx 60$ nM. Note that larger grazers releasing small, concentrated excretions could provide local opportunities for significant enhancement of uptake via active motility.

References:

S. Bertillson et al. *Limn. Ocean.* 48:1721 (2003). D.A. Caron et al. *Deep-Sea Research I* 121:14 (2017).