Redox chemistry as a constraint for modeling Earth's microbially driven biogeochemical cycles

Emily J. Zakem, Department of Biological Sciences, University of Southern California, Los Angeles, CA, USA

Martin F. Polz, Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

Michael J. Follows, Department of Earth, Atmospheric, and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, MA USA

Abstract

Microbial activity mediates the global flow of carbon, oxygen, nitrogen, and other elements, including climatically significant gases. However, non-photosynthetic microbial activity is typically not resolved dynamically or mechanistically in global models of the marine and terrestrial biospheres, inhibiting predictive capability. Understanding the global-scale impact of complex microbial community activity requires a consistent framework with which to constrain the parameterizations of diverse metabolisms. Here, we describe how the key redox chemistry underlying specific metabolisms can be exploited to parameterize diverse metabolic strategies. By quantitatively relating metabolic yields to chemical gradients, the growth and respiration of microbial biomass is systematically related to stoichiometries of substrate consumption, oxidation, and reduction that constitute biogeochemical fluxes. Linked with parameterizations of resource acquisition rates, whole organism metabolism can be integrated into trait-based modeling frameworks as metabolic functional types. Benefits of this approach include prognostic metabolic biogeography and what we term `gene-fluent' predictions of community metabolism. The theoretically grounded, electron-balanced framework progresses the description of microbial ecosystems towards conservation of energy as well as mass.

Introductory quote

"Nothing is less real than realism. It is only by selection, by elimination, by emphasis, that we get at the real meaning of things." – Georgia O'Keefe

Introduction/Overview

Diverse, complex microbial activity mediates global biogeochemical cycling[1]. Photoautotrophic microorganisms are responsible for about half of CO_2 fixation and O_2 production on earth, and heterotrophic microorganisms are responsible for much of the "return" reaction, the oxidation of organic matter back into CO_2. The temporal and spatial separation of anabolism and catabolism in the global environment mediates the biological sequestration of carbon, the reduction of atmospheric CO_2, and the maintenance of elevated atmospheric and oceanic O_2[2–5]. Chemoautotrophic microorganisms also fix CO_2 and, together with anaerobic heterotrophic metabolisms, carry out diverse chemical transformations including the fluxes of nitrogen to and from biologically available states and the formation of the potent greenhouse gas nitrous oxide (N_2O). Biogeochemical cycles respond to, and feedback on, changes in climate, and so describing microbial activity accurately and mechanistically at global scales is important for global climate and carbon cycle science. However, understanding and predictions of these processes are limited in part due to oversimplified representation of microbes in earth system models.

How can we connect microscale and global-scale processes to understand biogeochemical-climate feedbacks? Ecosystem models combined with global circulation and climate models become useful tools because they integrate concurrent fluxes from microbial growth and respiration, chemical reactivity, and physical transport to give solution states that are

not always intuitive. In earth system models, much attention has been given to phytoplankton – the photoautotrophic microorganisms responsible for primary production in the sunlit surface ocean -- and their small zooplankton predators[6–8]. Trait-based functional type models of these organisms provide tools for probing how these organisms interact with each other and the environment to set biogeography, biodiversity, and biogeochemical cycles[9–11].

However, the bacterial and archaeal activities responsible for much of the remineralization of organic matter back into inorganic form and other significant transformations are typically parameterized crudely and empirically in multi-dimensional biogeochemical models[12], typically not resolving the active populations of non-photosynthetic bacteria and archaea (with exceptions, e.g. ref 9). Such models have limited prognostic capability. For example, many current models prescribe the environmental niche of active metabolisms with imposed thresholds, such as fixed critical oxygen concentrations for anaerobic metabolisms and maximum light levels for nitrification. Additionally, models often assume static, average elemental ratios of non-photosynthetic microbial transformations, although flexible stoichiometry has been demonstrated to impact biological carbon sequestration and atmospheric CO_2[13]. In the past, these approaches were largely necessary due to the difficulties of characterizing the taxonomy and metabolic capabilities of natural microbial communities. However, the rapid expansion of "-omics" sequencing capability has enabled a clearer view of microbial biogeography and activity in the environment. In consequence, computational biogeochemistry is opening up the `black box' of remineralization and other microbially mediated processes in marine and terrestrial environments[14–22].

In the midst of this progress, it has become necessary to think about how to represent microbial activity in ways that can be useful for predicting unobserved and future environments.

Though sequencing methods have provided an enormous amount of information, genes themselves can be thought of as intermediates between the forces that drive microbial metabolism and their resulting function, and so we seek fundamental constraints. For example, describing phytoplankton nutrient uptake kinetics as a function of cell size has resulted in theoretical estimates of uptake parameters that concur with empirical data, and broaden the applicability of the parameterizations and reduce the number of free parameters in models[23,24]. By linking cell size to nutrient uptake and affinity as well as to other traits such as maximum growth rate, cell size has become an organizing principle – a "master trait" – for understanding and predicting activity across diverse environments[8,25–29].

How can we similarly organize descriptions of metabolic diversity as we expand biogeochemical models to include the full metabolic potential of microorganisms? Unlike phytoplankton that harness light energy, non-photosynthetic organisms oxidize chemical species to meet energetic demands of biomass synthesis, and thus their respiration – and associated chemical fluxes – becomes biogeochemically significant. Redox chemistry provides an additional organizing principle required to resolve assimilatory and respiratory fluxes: microbes are the redox-based "engines that drive biogeochemical cycles"[1]. While not yet exploited in global biogeochemical models, this view has been advocated for such applications[30,31], and has been embraced and employed in the field of environmental biotechnology, such as in the interpretation and modeling of wastewater bioreactors[32]. Just as models of ocean and atmospheric circulation are constrained by conservation of energy and potential vorticity, complementing mass balance with powerful redox and energetic constraints enables self-consistent descriptions of the efficiencies and stoichiometry of diverse microbial metabolisms. In

this way, the activity of microbes within an ecosystem ought to be sufficiently predictable to flexibly and prognostically anticipate large-scale biogeochemical dynamics.

The goals of a redox-based approach are in line with those of trait-based modeling: to advance ecological modeling beyond species-specific descriptions to those that matter for biogeochemical function, and ultimately linking ecosystem modeling to the evolution of functional communities. Kiørboe *et al.* 2018 highlight three "life-form transcending" traits: body size, resource acquisition, and defense[8]. To this list, we suggest adding "metabolic potential," in the sense of the ability to carry out a particular set of metabolic pathways to acquire energy.

When incorporating this approach into ecosystem models, microbial function emerges from underlying physics and chemistry as a consequence of interactions between the metabolic functional types and the environment. Resulting dynamic, theoretically grounded ecosystem models independently predict microbial growth, respiration, and abundances in ways that we can compare with observations, including sequencing datasets. Reducing complex behavior to chemical and physical constraints results in more universally applicable descriptions of microbial activity.

Here, we outline the conceptual basis of this organizing principle and its translation into quantitative descriptions of microbial activity that are simple enough for global earth system models. We first review the arguments for predictability of microbial functional diversity at large scales. We then discuss the "macro" vs. "micro" perspective, and illustrate the practical approach. Finally, we use ecosystem model examples to list and discuss the benefits, implications, limitations, and possible future developments of the metabolic functional type approach.

"Macro-scale" predictability: Microorganisms as dissipators of chemical energy

From one perspective, the microbial community is best characterized by interactions at the micro-scale: gene expression, enzymatic capabilities, metabolites, species-specific interdependencies, etc., as well as the physical and chemical environment surrounding small cells[33–39]. The information from sequencing in particular has allowed for a huge expansion of insight into the *in situ* activity of uncultivated species[40–48]. When focusing on global-scale impact, how much of this small-scale detail might be neglected? Or must we incrementally build a description of microbial ecosystems from "the micro-scale up"?

An alternative "macro-scale" perspective relates ecosystem function as a whole -- both abiotic and biotic components -- to the chemical gradients that organisms appropriate for energy[30,49,50]. All organisms must acquire chemical energy from reduction-oxidation (redox) reactions to fuel reproductive and maintenance metabolism. Respiration by living organisms increases the entropy of the environment by dissipating concentrated sources of chemical energy in accordance with the Second Law of Thermodynamics[50–52]. For example, it is well known that microbial activity organizes in accordance with the `redox tower'— the ranking of half-reactions by chemical potential -- in sediments and anoxic zones[21,53,54].

Given the notorious diversity and complexity of microbial systems[55], how can we be assured that they aggregate predictably? Recent studies have concluded that the microbial community as a whole converges to carry out predictable biogeochemical activity[18,56]. Moreover, Frentz *et al.* 2015 provide direct evidence of deterministic behavior by demonstrating that external conditions cause the seemingly random fluctuations observed in microbial growth, rather than stochastic variation in gene expression[57]. Thus, the authors conclude, microbial systems can in principle be determined by macroscopic laws.

How is this determinism manifested? If microbial communities can respond relatively quickly and predictably to changes to their local environment, they may predictably optimize the exploitation of locally available resources. Dispersal in microbes is thought to be a highly efficient process such that microbial communities can in effect draw from an extensive seed bank[58,59], as captured in the phrase "everything is everywhere, the environment selects"[60]. However, recent evidence also shows that gene acquisitions and deletions happen quickly enough to allow for horizontal gene transfer to dominate bacterial adaptation[61–65], implying that evolution can occur within few generations and thus on timescales similar to ecological interactions. Perhaps consequentially, similar geochemical environments have been demonstrated to have high microbial functional redundancy despite different taxonomic compositions[66]. This may be interpreted with the hypothesis that physics and chemistry selects for metabolic traits, and that these traits can be housed in different organisms with taxonomic composition shaped by micro-scale interactions[18,66,67].

This "macro-scale" approach has a long history in microbiology[30,31,68–78], and is conceptually similar to redox-reaction-based approaches to understanding microbial activity in sediments, soils, subsurfaces, and aquatic systems[16,17,21,53,76,79–82]. Quantitatively understanding microbially mediated rates of conversion of substrates has practical implications for wastewater treatment, and thus the field of biotechnology has established methodologies for this approach in textbook form[32]. Flux balance analysis (FBA) models can be considered as much more highly detailed analogues that resolve the electron flow among a multitude of chemical reactions within a cell[83–85], but perhaps with more complexity than necessary for understanding earth system dynamics. Illustrating the power of a "macro-scale" perspective, anticipating microbial

metabolism from chemical potential resulted in a prediction that anaerobic ammonium oxidation (anammox) should exist decades before it was observed[86,87].

Specific approach: Metabolic functional types

We can resolve microbial activity dynamically in ecosystem models using metabolic functional types modeled as populations. This allows for systematic quantification of substrate consumption, biomass synthesis, and excretion of respiration products.

In the examples here, a particular set of redox reactions distinguishes each functional type, such as aerobic heterotrophy and aerobic ammonia oxidation. This considers all aerobic heterotrophs as one type, and so aggregates the diverse, mixed community of many species that are subsisting on the same metabolism. Such aggregation has been deemed a useful strategy for representing the biogeochemical impacts of the microbial community[88,89]. However, integration with trait-based frameworks can allow for mechanistic resolution of multiple functional types among any one of these redox-based metabolisms (such as primary vs. secondary heterotrophic consumers and ecotypes of ammonia-oxidizing archaea).

Each functional type can be represented as a population in a multi-dimensional environmental model (e.g. an ocean simulation) with biomass concentration B (mV^{-1}), with a differential equation describing its rate of change as a function of its growth rate μ (t^{-1}), its loss rate L ($mV^{-1}t^{-1}$), and physical transport, such as according to velocity $\mathbf{u}$ (Lt^{-1}) and diffusion coefficient $\boldsymbol{\kappa}$ (L^2t^{-1}), as:

$$\frac{dB}{dt} = \mu B - L(B) - \underbrace{\nabla \cdot (\mathbf{u}B)}_{\text{advection}} + \underbrace{\nabla \cdot (\boldsymbol{\kappa}\nabla B)}_{\text{diffusion}} \quad (1)$$

The loss rate function represents a combination of processes including predation, viral lysis, maintenance, and senescence, which remain largely unconstrained, although efforts are made to relate losses to cell size and community dynamics[24,90–93]. Growth rate is limited or co-limited by a subset of multiple required substrates, where V_i (t^{-1}) is the specific uptake rate of substrate i, and yield y_i is the biomass yield with respect to that substrate (i.e. amount of biomass per unit substrate consumed). Yields for the different substrates and elements are interlinked by redox chemistry as discussed below. Typically, Liebig's Law of the Minimum is employed, and so the limiting growth rate is described as:

$$\mu = \min(V_i y_i) \tag{2}$$

The yield partitions the amount of substrate taken up by the population into that used for growth, $V_i y_i$, versus that exiting the cell in modified form as a waste product (Fig. 1). For heterotrophs, the yield for organic carbon directly relates to respiration and emission as CO_2, while the uptake of organic nitrogen and excretion of NH_4^+ can also be represented with the balanced reaction (Table 1). Accounting for maintenance energy decreases the ratio of growth to respiration, most significantly at low growth rates[94]. Non-limiting substrates are consumed in proportion to the limiting resource according to the stoichiometry of synthesis and may accumulate in the form of storage molecules. When neglecting maintenance energy and accumulation for clarity, the uptake of each substrate i relates to the limiting growth rate as $V_i = \mu y_i^{-1}$, with excretion in modified form as $V_i(1 - y_i)$.

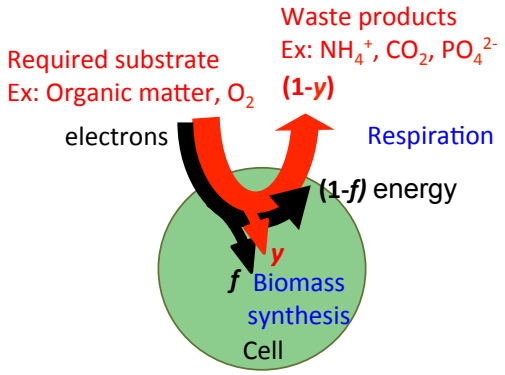

Figure 1: Schematic of a single cell represented as a metabolic functional type. The electron fraction *f* represents the partitioning of electrons into growth vs. respiration (neglecting maintenance costs for simplicity). The yield *y* represents the partitioning of substrate, which is a function of *f* and the stoichiometry of redox reactions.

Consider two contrasting model metabolic types: an aerobic heterotroph and a chemoautotrophic ammonia-oxidizer, as listed in Table 1. For each metabolism, an electron-balanced description consists of three half-reactions: biomass synthesis, oxidation of an electron donor, and reduction of an electron acceptor[32,71,74]. The aerobic heterotroph uses organic substrate as the source of both elements and electrons the synthesis of biomass and energy production, and oxygen serves as the electron acceptor. (Note that this simple description may be expanded to incorporate the assimilation of other substrates including inorganic nutrients such as ammonium.) For the ammonia-oxidizer, ammonium provides both electrons for energy and the elemental N for synthesis, while cellular carbon is assimilated through CO_2 reduction.

Table 1: Simplified equations describing two metabolic functional types. For each, half-reactions combine to form the catabolic and anabolic full reactions[32]: the oxidation of an electron donor (R_D; here either organic matter or ammonium), the reduction of an electron acceptor (R_E), and biomass synthesis (R_S). The total reaction (R_T) sums each of these three multiplied by a factor of f, the fraction of electrons partitioned into the synthesis reaction vs. respiration. Denominator d represents the number of electron equivalents that correspond to the oxidation states of the inorganic constituents of that synthesis: with a microbial biomass composition of $C_5H_7O_2N$, $d_B = 4(5) + 1(7) - 2(2) - 3(1) = 20$. Organic matter oxidation and synthesis equations are written without H_2O on the left- and right-hand side, respectively, for conciseness. Example whole organism metabolic budgets are calculated using the listed example efficiencies for marine organisms, an average marine organic substrate composition of $C_{6.6}H_{10.9}O_{2.6}N$[140], and the above biomass composition.

	Aerobic heterotroph	Ammonia-oxidizing chemoautotroph
R_D (1)	$\frac{1}{d_D}C_{c_D}H_{h_D}O_{o_D}N_{n_D} \rightarrow \frac{n_D}{d_D}NH_4 + \frac{c_D}{d_D}CO_2 + H^+ + e^-$	$\frac{1}{6}NH_4^+ + \frac{1}{3}H_2O \rightarrow \frac{1}{6}NO_2^- + \frac{4}{3}H^+ + e^-$
R_E $(1-f)$	$\frac{1}{4}O_2 + H^+ + e^- \rightarrow \frac{1}{2}H_2O$	$\frac{1}{4}O_2 + H^+ + e^- \rightarrow \frac{1}{2}H_2O$
R_S (f)	$\frac{n_B}{d_B}NH_4 + \frac{c_B}{d_B}CO_2 + H^+ + e^- \rightarrow \frac{1}{d_B}C_{c_B}H_{h_B}O_{o_B}N_{n_B}$	$\frac{n_B}{d_B}NH_4 + \frac{c_B}{d_B}CO_2 + H^+ + e^- \rightarrow \frac{1}{d_B}C_{c_B}H_{h_B}O_{o_B}N_{n_B}$
R_T	$\frac{1}{d_D}C_{c_D}H_{h_D}O_{o_D}N_{n_D} + \frac{(1-f)}{4}O_2 \rightarrow \frac{f}{d_B}B + \left(\frac{n_D}{d_D} - \frac{n_B f}{d_B}\right)NH_4 + \left(\frac{c_D}{d_D} - \frac{c_B f}{d_B}\right)CO_2$	$\left(\frac{1}{6} - \frac{f}{d_B}\right)NH_4 + \frac{c_B f}{d_B}CO_2 + \frac{(1-f)}{4}O_2 \rightarrow \frac{f}{d_B}B + \frac{1}{6}NO_2$
e^- donor yield	$y_D = f\frac{d_D}{d_B} \approx f$	$y_{NH_4^+} = \left(1 + \frac{d_B}{6f}\right)^{-1} \approx \frac{6f}{d_B}$
Example efficiency	$f = 0.1 - 0.2$; Marine bacteria (Robinson 2008)	$f = 0.02 - 0.04$; Marine archaea (Zakem et al. 2018)
Example budget	$7.1C_{6.6}H_{10.9}O_{2.6}N + 47O_2 \rightarrow B + 6.1NH_4 + 42CO_2$	$112NH_4 + 5CO_2 + 162O_2 \rightarrow B + 111NO_2$

Following Rittman and McCarty 2001[32], the ratio of anabolism and catabolism is represented by the fraction f of electrons fueling cell synthesis vs. respiration for energy (Table 1, Fig. 1). Fraction f reflects the cost of cell synthesis, the free energy supplied by the respiration pathway, and an estimate of the energetic inefficiency of each process. The stoichiometry of the whole organism metabolism and the projected yield y_i of each required substrate are then functions of f and the coefficients of the half-reactions.

This methodology suggests quantitative differences between the heterotroph and the chemoautotroph. First, for the same electron fraction f, the electron donor yield for the heterotroph is a few factors larger than that of the ammonia oxidizer (Table 1). This reflects that the oxidation of one mole of ammonia for energy yields less electrons than the oxidation of one mole of the specified organic substrate. Second, f itself will be lower for the chemoautotroph when accounting for the energetic demand of reducing CO_2 for assimilation.

Eqn. 2 suggests a correlation between growth rate μ and yield y (though maintenance energy will contribute to some decoupling), but there also exists a trade-off between uptake rate and yield particularly when considering the length of a metabolic pathway. One example is the trade-off between growth rate and yield of ATP production when considering the transition between aerobic respiration and fermentation[95–97]. Full aerobic respiration maintains a higher ATP yield, but must allocate enzyme towards the cellular machinery required to use oxygen, while fermentation sacrifices the higher yield and allocates enzyme towards the machinery for faster substrate uptake[96]. Thus using Eqn. 2, the state of full aerobic respiration can be described with a lower maximum uptake rate and a higher yield as compared to fermentation. Below, we discuss tradeoffs among these traits more quantitatively.

Benefits and implications

Resolving microbial activity with underlying chemical potential obviates the need to impose critical or threshold concentrations that determine the presence or absence of metabolism in global biogeochemical models. Rather than being empirically imposed, metabolic biogeography emerges dynamically from ecological interactions. This flexibility expands model applicability to diverse and unobserved environments and aids in understanding metabolic thresholds more fundamentally.

We illustrate this and other benefits of the electron-balanced approach using two ecosystem models as examples (Figs. 2 and 3). In both models, all chemical transformations are explicitly represented by the growth and respiration of metabolic functional types. Fig. 2 illustrates a vertical water column model of a stratified, oxygenated marine environment in which the three successional steps of the remineralization of organic nitrogen to nitrate (Organic $N \rightarrow NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^-$) are carried out by distinct metabolic functional types: aerobic heterotrophs, ammonia-oxidizing chemoautotrophs, and nitrite-oxidizing chemoautotrophs, respectively[19]. Fig. 3 illustrates a model simulating a zonal transect through the S. Pacific Ocean, which contains an anoxic oxygen minimum zone. The ecosystem model resolves emergent metabolic activity along a gradient in oxygen with (for simplicity of illustration) just two resolved microbial metabolisms: aerobic and anaerobic (denitrifying: $NO_3^- \rightarrow N_2$) heterotrophy.

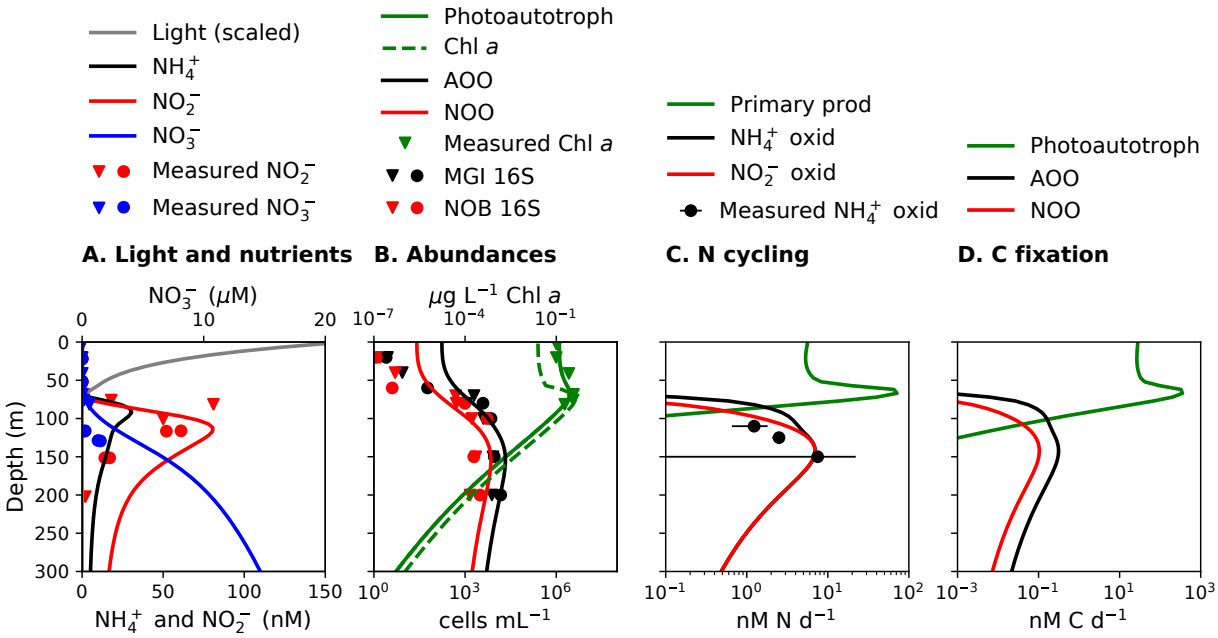

Figure 2: Vertical water column model simulation and observations of the nitrification system in the North Pacific. Biogeochemistry is driven by microbial metabolic functional types: phytoplankton, heterotrophic bacteria, ammonia-oxidizing organisms (AOO), and nitrite-oxidizing organisms (NOO), and a zooplankton grazer. Solid lines are model solutions, and marked points are observations. Observations are from Santoro et al. 2010 and Santoro et al. 2013, from cruise stations 67.115 (triangles) and 67.155 (circles) in the Pacific Ocean[107,138]. **A.** Solar irradiance ("Light", normalized to the width of the box) and dissolved inorganic nitrogen species. **B.** Cell abundances, converting from modeled biomass concentration with 0.1 fmol N $cell^{-1}$ and one gene copy per cell. Observed abundances are of the 16S rRNA abundances of archaeal Marine Group I (MGI) and of *Nitrospina*-like organisms (NOB). **C.** Rates of N cycling, where primary production is the rate of NH_4^+ assimilation into phytoplankton biomass. **D.** Modeled rates of C fixation by the three autotrophic functional types.

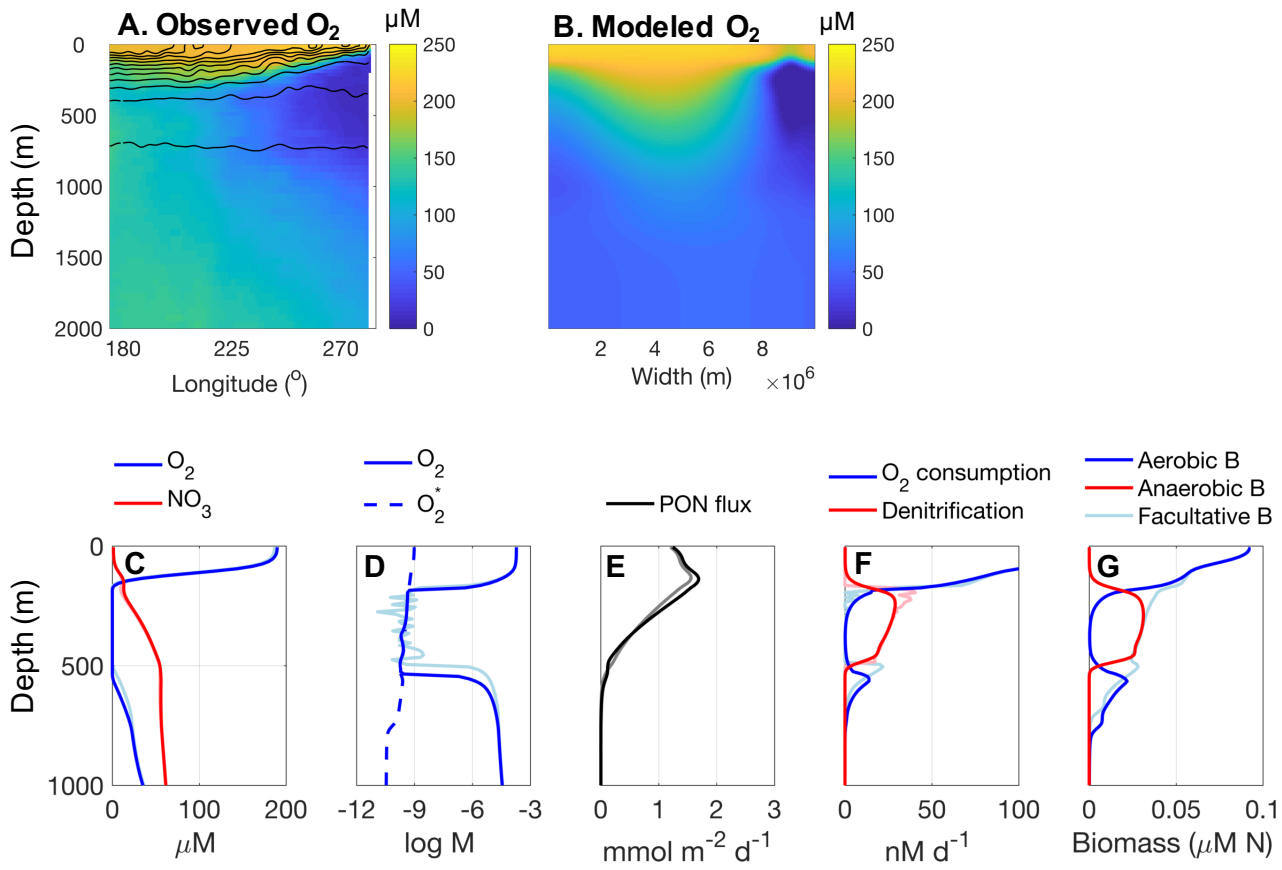

Figure 3: Ecosystem model of the 10°S transect across the South Pacific Ocean. Heterotrophic microbial functional types consume and respire organic matter with O_2 or DIN (dissolved inorganic nitrogen) as an electron acceptor, with no prescribed critical or inhibiting oxygen concentrations. **A.** [O_2] from the World Ocean Atlas (2013) with lines depicting isopycnal (density) surfaces[139]. **B.** Modeled steady state [O_2]. **C-G**. A profile of the solutions through the anoxic zone. Darker lines show solutions for a model version with two distinct metabolic functional type populations: obligate aerobic and anaerobic (denitrifying) heterotrophs. Lighter lines show solutions for a second model version with one facultatively aerobic population that consumes either [O_2] or DIN, depending on which allows for a higher growth rate at each time step in the model. For the respiration rates of the facultative type (**F**), averages over 100 days at steady state are illustrated.

1. Flexible and broadly applicable metabolic thresholds.

Many biogeochemical models prescribe the oxygen concentrations that inhibit metabolisms in accordance with observations of specific organisms or communities in experimental conditions. In contrast, in the oxygen minimum zone model of Fig. 3, the transition from aerobic to anaerobic metabolism emerges from the interactions. When oxygen supply is abundant, the denitrifying type is competitively excluded because growth using NO_3^- as an electron acceptor is lower than with oxygen, as predicted from the free energies of the underlying redox reactions. In the `eastern' half of the domain, the aerobic population depletes oxygen until it reaches its subsistence concentration[98,99]. In this oxygen-limited state, denitrification becomes a competitive metabolism, but the aerobic type can coexist, and continues to utilize any small amounts of oxygen supplied. This stable coexistence of aerobic and anaerobic metabolism can improve predictions of how much organic matter reaching anoxic zones is oxidized aerobically vs. anaerobically. This is relevant, for example, for understanding the emissions of N_2 and N_2O from the ocean that result from anaerobic respiration[100].

As a second example, in Fig. 2, nitrification is restricted from the sunlit surface ocean without an imposed light inhibition due to competitive exclusion by phytoplankton. Though light inhibition has been directly observed in many nitrifying microorganisms, nitrification has also been measured in sunlit waters[101,102]. This dynamic resolution improves our understanding and predictive power of where and why nitrification is sometimes feasible in the surface: a global simulation predicts active nitrification in surface waters where phytoplankton are not N-limited, such as at high latitudes where phytoplankton are limited by light[19]. The emergent exclusion of the nitrifiers from much of the ocean anticipates that many clades of nitrifying microorganisms

have adapted to long-term exclusion from the surface and consequentially lost (or did not develop) photoprotective cellular machinery.

2. Dynamic biogeochemical cycling.

The metabolic functional type approach can mechanistically represent microbial activity in dynamic steady-state or time-varying environments. This predictive power is of particular benefit for resolution of microbial processes in fine-grained ocean circulation models where flow can vary on the order of days, similar to the timescales of microbial growth.

However, solutions become dependent on the partitioning of metabolism among the functional types as the timescales of physical change approach the timescales of microbial growth. For example, in Fig. 3, the equilibrium state solutions of two versions of the ecosystem model – one with two obligate (aerobic and anaerobic) populations and the other with one facultatively anaerobic population – are nearly identical at steady state, but differ in the transient. Fig. 4 illustrates the differences in the time evolution of the oxygen concentration and the denitrification rate following a perturbation in which oxygen concentrations are increased. In the model with the two obligate types, anaerobic denitrification does not cease throughout the recovery period because a population of anaerobes remains after O_2 is supplied and competitive exclusion has not come to completion. In contrast, the facultative population switches to respiring O_2 when it is supplied, and so denitrification rates cease immediately. Real community dynamics likely reflect both solutions: current understanding is that heterotrophic microorganisms are generally facultatively anaerobic, while chemoautotrophic anaerobic ammonium oxidizing (anammox) bacteria, which accounts for roughly a third of the fixed N loss in pelagic zones, are understood to be obligate anaerobes[86,103–105].

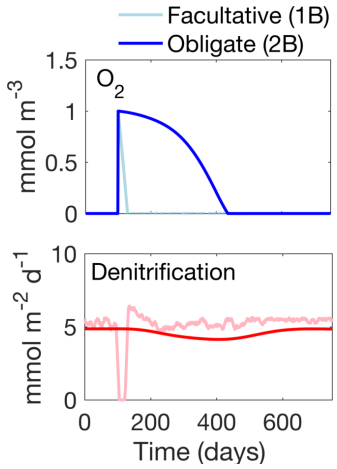

Figure 4. The time progression of $[O_2]$ in the core of the simulated anoxic zone (Fig. 3) and the integrated denitrification rate, with a perturbation at 100 days in which the model $[O_2]$ minimum was raised to 1 μM.

Thus, knowledge of how metabolisms are distributed among populations is required for interpreting transient states. Other species-specific time-varying phenomena such as the lag response of organisms to substrate availability also becomes relevant[106]. These are limitations on one hand, and so require further constraint. On the other hand, these differences may serve as a tool for parsing out how metabolisms are distributed by comparing models with observational time series. The different solution states in Fig. 4, for example, can serve as testable hypotheses against which the response of natural assemblages can be compared.

3. Relationships between microbial abundances, ambient rates, and nutrient concentrations.

The redox balance approach articulates that low biomass concentrations may be associated with high volumetric rates of chemical transformations. Thus, abundance has no simple correlation with biogeochemical impact. For example, the nitrification model predicts a three-fold difference in the abundances of the organisms responsible for each of the two steps of nitrification, although they carry out the same rate of subsurface N-cycling (Fig. 2B,C).

This is a clear example of the signature of chemical potential in microbial ecology. The three-fold difference in relative abundance between the nitrifying types is consistent with observed differences (Fig. 2D)[107], and reflects that the oxidation of one mole of NH_4^+ generates 3x the number electrons as the oxidation of one mole of NO_2^-, with a potential contribution also from differences in cell size[19]. The equal N-cycling rates instead reflect the rates substrate supply, which also is consistent with observations that show indistinguishable rates of ammonia and nitrite oxidation below the euphotic zone[108]. Also, these equal nitrification rates persist in the model despite different steady-state DIN concentrations (Fig. 2A)[19], matching many observations.

4. Relative elemental flows.

The approach imposes the coupling of elemental cycles by resolving the stoichiometry of whole organism metabolism (Table 1). For example, resolving nitrifying biomass quantifies the C fixation rates associated with nitrification rates (Fig. 2D). Though the approach itself does not explain differences in biomass stoichiometry between populations, it enables the investigation of the mass and energetically balanced consequences of such differences, such as of C-rich

phytoplankton relative to heterotrophic bacteria[109,110]. This explicit description of the ratios of elemental flow through the ecosystem and their variation from average values is critical for understanding climate-biogeochemical feedbacks[13,111–113].

5. Integration with trait-based modeling.

The approach presented is fully compatible with trait-based models. The yield y and uptake V in Eqn. 2 determine the population's fitness in an environment, and thus we may consider them as traits. For example, the chemoautotrophic nitrifiers have a very low biomass synthesis yield ($y < 0.01$ mol biomass N per mol DIN oxidized) and thus lose the competition for DIN against phytoplankton in the surface, for which we have assumed a nitrogen-based yield $y \approx 1$ (i.e. all nitrogen consumed is synthesized into biomass; Fig. 3). Furthermore, we can use these traits to identify trade-offs among microbial functional types carrying out the same metabolism, as has been previously demonstrated for diverse types of phytoplankton[114].

As another example, a tradeoff between a higher maximum growth rate and a lower subsistence concentration (R^*)[98,115–117] correspond to a dichotomy of "opportunists" vs. "gleaners," respectively[116], or, "r-selected" vs. "K-selected" species from the logistic equation for population dynamics[118]. When using a Michaelis-Menten form to describe uptake of resource R, the maximum growth rate is $\mu_{max} = yV_{max}$ and the resource subsistence concentration is:

$$R^* = \frac{K_m L}{yV_{max} - L} \quad (3)$$

where V_{max} is the maximum specific uptake rate (t^{-1}) and K_m is the half-saturation concentration. Fig. 5 illustrates the trade-off with two functional types – an "opportunist" and a "gleaner" – competing for substrate in a virtual chemostat. When substrate is supplied intermittently, one or the other may be excluded over time, or both may be sustained if the variable conditions prevent

competitive exclusion entirely (as in Fig. 5). In reality, we can understand that these tradeoffs may represent species characteristics among any one metabolism, and that conditions may select temporarily the most optimized from the species pool so that dynamic change can be expected on short time scales.

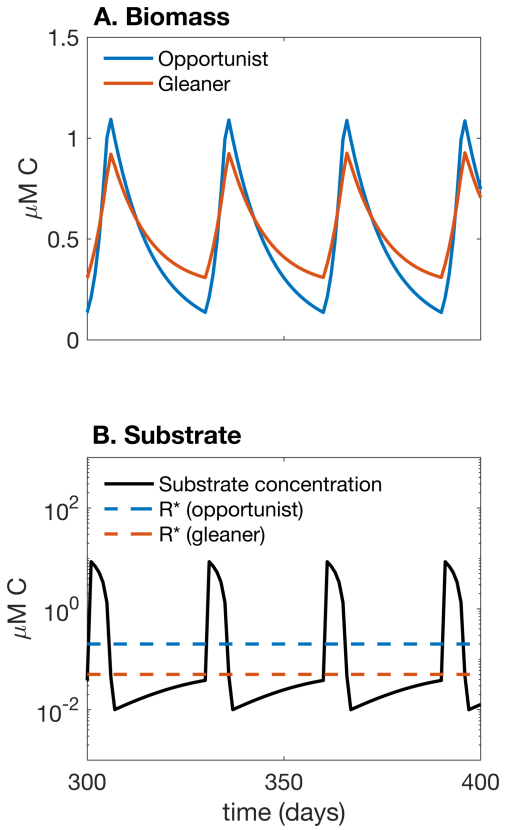

Figure 5: A model simulation of the competition between two functional types characterized by a tradeoff among traits, with resulting biomasses (A) and substrate concentration (B). The "opportunist" has a higher maximum growth rate and a lower substrate affinity than the "gleaner," which has a lower subsistence concentration (R^*). The two populations grow in a virtual chemostat with dilution rate 0.1 per day, with a continual incoming substrate concentration of 1 μM C and an additional 10 μM C pulse of substrate added every 30 days. Here, the "opportunist" is parameterized with a maximum uptake rate of V_{max} = 2 mol substrate C per mol biomass C per day and a half-saturation concentration k_S = 1 μM C. For the "gleaner," has V_{max} = 1 mol substrate C per mol biomass C per day and k_S = 0.1 μM C. For both, substrate yield y = 0.3.

6. "Gene-fluent," independent predictions

How do we relate metabolic functional type models to sequencing datasets measuring genetic, transcriptomic, and proteomic diversity? Connecting biogeochemical models with sequencing data is critical because this data provides an enormous amount of information. However, genes (or transcripts) themselves are not necessarily the most concise or useful currency given functional redundancies, unattributed function, and variation in gene dosage from horizontal gene transfer as well as growth rate[62]. Recent "gene-centric" models aim to resolve the abundances of key genes as proxies for a predetermined set of metabolic pathways[16,18,21], but the choice to resolve genes as representatives of metabolic reactions does not itself add a constraint to the models. Rather, the parameters used to describe metabolic pathways in these models are estimated similarly to the redox-balanced yields and efficiencies described here.

The redox chemistry-based functional type approach provides a consistent framework with which to estimate metabolic parameters while externalizing the conversion between predicted activity and sequencing information as a transparent process. In Fig. 2B, the predicted functional biomass of ammonia oxidizing population is related to archaeal Marine Group I (MGI) and *Nitrospina*-like 16S rRNA genes with two conversion factors: cell quota (1 fmol N $cell^{-1}$) and the number of cellular gene copies (1 $cell^{-1}$). Conversion error arises since cell mass and size vary with growth rate[119,120].

The innovation of gene-centric models is the sophisticated conversion of estimates of biogeochemical activity and biomass to genes. For example, the model of Coles et al. 2017 resolves biomass and nutrient concentrations prognostically, and then uses a three-part formula – representing constitutive, regulated, and steady state transcription -- to diagnostically calculate transcription rates from modeled biomass and growth rates[18]. Thus, the two types of modeling

are complimentary, with redox chemistry providing independent estimates of metabolic activity, and the careful calibrations between activity and sequencing providing a comparative metric.

Maintaining transparency of the conversion from predicted microbial activity to genes and transcripts allows interdisciplinary audiences to understand and critique the models. Furthermore, deprioritizing genes is in line with a perspective that considers genes as an intermediate step between forcing and function, with the detailed complexity of biological reality following the underlying chemical and physical constraints (analogous to the "form follows function" principle of architect Louis Sullivan). By perceiving genes as intermediaries, we suggest `gene-fluent' as a new term to describe this type of modeling.

Limitations and potential extensions

Using chemical potential as a theoretically grounding organizing principle for the resolution of diverse metabolisms can greatly improve the current state of microbial description in global biogeochemical models. However, the approach does have its limitations, which generally increase in significance with the temporal or spatial resolution of ecosystem dynamics.

As mentioned above, modeling metabolic diversity with functional type populations requires choosing how metabolisms are distributed among the populations. Redox chemistry does not alone inform these choices, and so additional knowledge or an additional constraint is required. One such constraint is the limited capacity of the cell and thus its allocation of proteome towards different function[121]. Combined with redox chemistry, considering enzymatic allocation allowed for the prediction of both the division of nitrification into a two-step process in mixed-environments and the combined, complete pathway in one organism ("comammox") in biofilms, which preceded observations of the latter[122–124].

Uncertainty in distributions of metabolism lies not only in the length of a metabolic pathway, but also in the degree of metabolic versatility (metabolic "mixotrophy"). Such versatility characterizes key players in large-scale biogeochemistry, such as nitrite-oxidizing bacteria and photoheterotrophs[125–127]. Mixotrophic lifestyles can increase the fitness of populations in their environments, impacting overall ecosystem function[128]. In one sense, the approach here provides a prediction of where we might expect such mixotrophy by resolving stable co-existences of diverse metabolisms. In Fig. 3, for example, syntrophic co-existence occurs at depth among heterotrophs, ammonia-oxidizers, and nitrite-oxidizers, and future work could investigate what determines which combinations of these co-existences remain as `passive' interactions, which develop into mutualistic dependencies as `active' interactions[129], and which evolve into mixotrophic phenotypes or endosymbionts. Additionally, by considering the potential to carry out a metabolism as a trait, we can use the current framework along with an additional constraint to investigate implications of metabolic mixotrophy. For example, Coles et al. 2017 impose a tradeoff between the degree of metabolic diversity of a single functional type and growth rate, enabling the exploration the consequences of distribution of metabolism on the biogeochemical state[18].

Also, the approach here resolves only active functional biomass, while evidence suggests that less than 10% to more than 75% of the microbial community may be inactive[130]. Some seemingly inactive populations may slowly metabolize over long timescales, requiring longer model integration times and careful attention to their loss rates for resolution, while some populations are periodically active as revealed by high-resolution observations in time[59].

The approach presented here does not predict photoautotrophic metabolisms from underlying energetics. However, further work could connect the supply of photons to available

energy for biosynthesis within the cell. Most biogeochemical models implicitly assume that photoautotrophs have very high efficiency (yields at or near one) for all inorganic substrates by assuming that most is assimilated into biomass, but it would be interesting to investigate the implications of when this assumption breaks down such as at the base of the euphotic zone, where some phytoplankton excrete nitrite due to incomplete reduction of nitrate[131,132]. Fortunately, parameterizations of phytoplankton are currently much more sophisticated than of bacteria and archaea in models, reflecting a longer history of comprehensive sets of observations.

As a more radical extension, can we progress past population modeling and model microbial consortia as one aggregate community biomass[52,133–137]? This may improve resolution of time-varying metabolic versatility. However, if both steps of nitrification were a part of such a consortia, would the accumulation of nitrite in Fig. 3 be predicted? We leave these questions for future research, and conclude that the best choice for the "resolution" of metabolism will depend on the specific research question and the available observations.

We have described a useful approach for understanding and anticipating microbial control of biogeochemical cycling that is suitable for global applications. The approach aims to represent microbial growth and respiration explicitly and consistently from knowledge of chemical gradients and other physical properties of the environment, towards a goal of building an independently constructed theoretical ecosystem model that can then be compared to observations. Describing microbial community with underlying energetic constraints connects metabolisms dynamically with global geochemical distributions, such as carbon dioxide, oxygen, and biologically available nitrogen. This deepens our understanding of microbial ecosystems and enables the incorporation of the feedbacks of microbial activity to changes in global biogeochemistry and the climate system.

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