Microbial Metabolites: Hidden Currencies of the Ocean Carbon Cycle

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One-quarter of Earth's photosynthesis-derived carbon rapidly cycles through a pool of seawater metabolites that is generated from the activities of microbes. While accounting for only a minor fraction of the total reservoir of marine dissolved organic carbon (DOC), the production and fate of these rapidly-cycling molecules dictate the role the ocean plays in sustaining life on our planet. Here we consider the sources of microbial metabolites in the surface ocean, their roles in ecology and biogeochemistry, and how a better understanding of these molecules can be developed using strategies that integrate chemistry, biology, modeling, and data science. Though this perspective is ocean-focused, chemical currencies underlying microbial interactions are central to the understanding of all microbiomes that contribute to the workings of our planet.

Organic molecules dissolved in seawater are cycled through the sunlit ocean by the billion marine microbes living in each liter of surface water[1] (Fig. 1). This process involves almost half of the ocean's annual net primary production (NPP), and therefore even minor changes in its functioning are significant on a global scale. Yet much remains unknown about the chemicals passing through the labile DOC pool, largely because they are almost simultaneously produced and consumed by diverse marine microbes. Indeed, the surface ocean labile DOC pool has a turnover time of ~3 d (ref[2]) with steady-state concentrations in the range of nanomolar (nM) to picomolar (pM)[3,4] for those abundant enough to be measured with existing methods, and below this range for many others. This leads to the dilemma that compounds supporting one of the most significant fluxes in Earth's carbon cycle are among the most challenging to study.

What are the marine sources of labile DOC?

Most of the labile DOC of marine origin comes from one of three sources: actively photosynthesizing phytoplankton, senescing and dead phytoplankton, or heterotrophic organisms. The first source occurs when healthy marine phytoplankton cells release metabolites, forming a pool of carbon referred to as 'extracellular release'[5] or 'dissolved primary production'[6] (Fig. 2). These released compounds represent potential resources now lost from phytoplankton cells, inspiring hypotheses as to the mechanisms behind their release[7]. For example, the 10^6-fold concentration differential between the inside and outside of phytoplankton cell membranes[8] could drive a molecular diffusion process by which healthy cells continually leak a fraction of their internal metabolites into seawater[9]. In this passive process, low molecular weight (<600 Da) and hydrophobic compounds (due to cell membrane composition) are more likely to leak[7,9,10]. In active mechanisms of extracellular release, labile DOC is excreted rather than lost from living phytoplankton, allowing for variability in rates and composition of

released metabolites. Physiological stresses imposed by photosynthesis are likely drivers of carbon export, and several mechanisms have been suggested. For example, when CO_2 is limiting, the principal carbon fixation enzyme binds oxygen instead, resulting in the production and release of toxic photorespiration products[11], such as glycolate[12]. On the other hand, when nutrients are limiting but inorganic carbon and irradiance are not, carbon fixation can outpace macromolecule synthesis[13] and cause extracellular release of the excess fixed carbon in the form of polysaccharides and organic acids, referred to as 'photosynthetic overflow'[13-16]. Nutrient stress has also been proposed to drive organic carbon excretion as a byproduct of cells increasing their ATP/ADP ratio (i.e., reaching a higher intracellular energy state) in order to facilitate the free energy costs of transport of low concentration nutrients[17]. These nutrient limitation-driven mechanisms should favor excretion of carbon-rich compounds[17]. At points in the diel cycle and regions in the photic zone where light shock can occur, extracellular release from phytoplankton may occur in response to a redox imbalance[18,19]. Lastly, phytoplankton actively release molecules that trigger behavioral or physiological changes in neighboring microbes. These metabolites often have distinctive chemical structures and can function as defense compounds[20], pheromones[21], or toxins[22,23]. Given the different mechanisms and drivers of release, the chemical composition of phytoplankton exometabolites (those that are released to the labile DOC pool) should differ from the composition of endometabolites (those maintained within the cell). This has indeed been observed in the case of amino acids, carbohydrates, and carboxylic acids[24-26]. Regardless of the release mechanism, the tight coordination in daily activity patterns between phytoplankton and heterotrophic bacteria that track the diel irradiance cycle[18,27,28] establishes a major role for extracellular products of photosynthetic activity in carbon transfer in the surface ocean (Fig. 3).

The second major source of labile DOC is the liberation of endometabolites during phytoplankton senescence and death[29,30] (Fig. 2). Protist and zooplankton predation results in the loss of dissolved organic matter from phytoplankton prey, liberating 10-30% of prey carbon as DOC via 'sloppy feeding' (escape of organic matter during grazing) or egestion (release of organic matter remaining undigested in guts and food vacuoles of predators)[31-33]. Viral lysis releases metabolites from phytoplankton cells[34,35] and their composition can be altered due to host reprogramming during infection[36,37]. Recent evidence suggests that microparasitic fungi induce lysis of phytoplankton cells, similarly modifying the composition of released metabolites[38]. Senescence or 'autocatalytic cell death' triggered by nutrient limitation or other stresses is also a source of metabolites[10,29] and may be particularly important in declining blooms[39,40].

Along with the sources linked to phytoplankton life and death processes, a third major source is the excreted metabolic byproducts and lysates of heterotrophs, including bacteria, archaea, protists, and zooplankton[32,41-43] (Fig. 2). Viral infection has also been shown to modify bacterial endometabolomes[44] via reprogramming of host metabolism[44,45], a potentially important impact on labile DOC given estimates that 1 in 3 ocean bacteria are infected at any time[34]. Organic molecules originating from heterotrophic protists and zooplankton have been found to be rich in organic nitrogen[46] and readily scavenged by bacteria[33]. Heterotrophic marine bacteria and archaea release potentially thousands of different molecules[47-50] with potential to serve as bacterial substrates[51]. Liberation of labile compounds also occurs during bacterial solubilization of polymeric components of particulate detritus[52], such as proteins and polysaccharides, whose degradation products diffuse from the particle surface[53-56].

What is the contribution from each source?

Each year, heterotrophic marine bacteria process ~20 Pg C from the labile DOC pool (Fig. 1), a value supported by both geochemical methods based on DOC reactivity[2] and ecological approaches based on bacterial carbon demand[57]. This routing of recently fixed carbon to bacteria through the labile DOC pool is one of the largest and most rapid fluxes of organic carbon in the biosphere. Two early estimates of marine carbon processed through the microbe-metabolite network were 10-50%[58] and 40%[57] of ocean NPP, and a recent compilation of bacterial carbon demand values is consistent with these (52%[59] of nNPP). Partitioning of inputs among the three major sources was estimated from published values for labile DOC release by microbial and zooplankton activities and suggested that ~40% of labile DOC originates directly from phytoplankton as extracellular release from photosynthesizing cells, ~40% is released from phytoplankton death processes (senescence, sloppy feeding, viral and fungal lysis), and ~20% is released from heterotroph excretion and death processes[59]; the value of this last source is constrained by the fact that the DOC is derived from secondary production and therefore from organic carbon pools diminished by respiration[60].

What is the molecular composition of labile DOC?

The molecules making up the ocean's labile DOC pool have been challenging to identify. Early studies proposed amino acids, carbohydrates, osmolytes, and small carboxylic acids (particularly the photorespiration product glycolate) as the major substrates for surface ocean bacteria[1,14,61,62]. These compound classes were suggested by extrapolating from plankton biochemistry, with the idea that internal metabolite pools could be considered proxies for released pools. Metabolites in intracellular

pools are easier to measure than external, typically having concentrations several orders of magnitude higher than surrounding seawater[63] (Table 1; μM to mM[14,63-66] internal versus pM to nM[3,4,66] external). Further, endometabolite samples can be concentrated by capturing cells on filters. However, paired analysis of endo- and exometabolites in phytoplankton cultures show that internal molecule pools do not correspond closely to external molecule pools[66], although viral lysis and sloppy feeding may be exceptions. This selective release of phytoplankton metabolites is not surprising but limits the benefit of using endometabolomes to predict labile DOC composition. Nonetheless, endometabolite analysis of marine plankton has yielded new candidates for the labile DOC inventory (Table 1). Examples include quaternary amines (choline, dimethylglycine, trimethylamine-*N*-oxide), organic sulfur compounds (gonyol, cysteate, dimethylsulfonioacetate, sulfolactate) and amino acid derivatives (homarine, trigonolline, ornithine)[18,64,66-75] (Table 1).

Labile DOC components can be coarsely categorized based on their physiological and ecological roles in the ocean's microbe-metabolite network. Here, we define three categories that capture several of these roles: substrates, facilitators, and ecological signals (Box 1). Substrate metabolites are defined as the compounds actively assimilated by marine bacteria for carbon and energy, and transferred between microbes in quantities that sustain growth, reproduction, and the cycles of carbon and other elements. Amino acids[76,77], polyamines[78,79], carbohydrates[80-82], sulfonate and sulfonium compounds[18,68], carboxylic acids[71,74,75,83] ,and nucleosides[69,71,74] all function as bacterial substrates in surface seawater (Table 1). Molecules in this category contribute most directly to flux by serving as the conduits, or 'currencies' that move carbon between marine microbes. Facilitator metabolites are defined as molecules that enable biochemical reactions and can be re-used and exchanged between microbes. Those that have been identified in seawater include the soluble B vitamins (B1, B7, B12)[84-86], iron siderophores[87,88], and other biosynthesized metal-binding molecules[89,90], all of which have steady state concentrations at the limit of bacterial uptake kinetics (<1 to 10s of pM[91-94]), though concentrations can fluctuate in time and space. Both substrate and facilitator molecules are also considered 'public goods' if they are energetically expensive to synthesize and released by only a subset of the microbial community[95]; public goods can set the stage for metabolic dependencies within the ocean's microbial network[96]. Finally, ecological signal metabolites or 'infochemicals' are defined as compounds orchestrating specialized microbial interactions under specific conditions[97,98]. Sourced and shared by microbes, they serve as the medium for interactions by altering community physiological or behaviorial features through chemicals exchanged between members (Box 1). Microbially-produced hormones (e.g., indole acetic acid[99]) and quorum sensing molecules (e.g., acyl homoserine lactones[100]) are among the

ecological signal compounds thus far discovered in ocean communities or marine microbial cultures. Molecules that inhibit growth or cause mortality [42,74,101] are also categorized as ecological signal compounds here because of their role in modifying physiology (albeit negatively) of other microbes. Both facilitator and ecological signal metabolites can contribute indirectly to labile DOC cycling by affecting rates and routes of carbon flux without necessarily making substantive contributions themselves.

What are the challenges to progress?

Though expertise in marine metabolites is growing, we cannot yet unravel the roles of labile DOC molecules in carbon flux and fate in the surface ocean[102]. Roadblocks to progress can be boiled down to one (long) sentence: *Hidden among the hundreds of thousands[103] of mostly unknown organic molecules embedded in a million-fold higher salt concentration are the currencies of a very large carbon flux through a very small carbon reservoir*. Here, we pull apart this sentence and explain in more detail the specific challenges it captures.

Hidden in the highly complex marine DOC pool…

Each of the tremendous number of distinct microbial organisms that occupy surface seawaters[104,105] can release 10s to 1000s of different molecules [47,48,50,66,106-108]. Genome-scale metabolic models based on flux balance analysis (FBA) agree that microbial cells simultaneously maintain many hundreds of different endometabolites[109] that can potentially be lost or exported as labile DOC. Some of these are predicted to be 'costless' metabolites, such as byproducts of anabolic and catabolic pathways released without any fitness costs to the microbe[110]. Untargeted mass spectrometry (MS) and nuclear magnetic resonance (NMR) analyses have indeed uncovered hundreds of thousands of distinct organic features in marine DOC[111,112], of which only ~1-5% can be identified[113-115]. Manually-intensive, low-throughput identification pipelines are currently the primary approach for converting these unidentified compounds into chemical insight. Of course, the biological reactivities of all the unknown compounds are also unknown and therefore biogeochemically important molecules cannot be singled out of this multitude. In short, we can't identify which of these compounds are important substrates, facilitator metabolites, or ecological signal metabolites, or are otherwise uninvolved in the labile DOC cycle.

…embedded in a salty matrix…

Salt is a frustrating problem for the analysis of marine DOC. In mass spectrometry, non-volatile salts interfere with ion formation and degrade spectral quality[116]. In nuclear magnetic resonance (NMR) analysis, salts reduce probe sensitivity[117]. Accordingly, development of methods to remove organic compounds from their salty matrix has been an essential endeavor in chemical oceanography. Strategies that have been explored include water and salt removal via tangential flow filtration (TFF)[118], reverse osmosis/electrodialysis (RO/ED)[119], and metabolite capture on solid-phase extraction (SPE) resins[120,121]. Yet only 10 to 40% of marine DOC is captured with these methods, with a bias towards moderate (>300 Da) and high (>1000 Da) molecular weight compounds. In particular, small and polar metabolites, which are characteristics of many labile biomolecules, are lost during tangential flow filtration or RO/ED and are not well-retained on solid-phase extraction resins such as the styrene−divinylbenzene polymer PPL[121]. Other saline fluids that are more easily analyzed for metabolite content have higher concentrations of molecules in a lower concentration of salt, for example millimolar metabolite concentrations (up to 10^6-fold higher than seawater) in 1 - 10 ppt salt solutions (up to 30-fold lower than seawater) characteristic of blood and urine. For now, the state-of-the-art techniques used to isolate organic molecules from seawater remain strongly biased against the low molecular weight and polar exometabolites[121], the majority components of labile DOC.

…is a small metabolite reservoir with a very large flux

Labile DOC release is largely balanced over time by its consumption by heterotrophic bacteria, maintaining individual compounds in the pM to low nM concentration range[18,92,122-124], or lower. Specific uptake affinities for DOC components in oligotrophic marine bacteria are among the highest reported, for example 150-fold higher than for well-studied model bacteria[92]. Oligotrophic bacteria (those that can survive on low organic matter concentrations) are so efficient at depleting the labile DOC pool that calculations suggest they must simultaneously scavenge for at least 34 different substrates to grow at one generation per day[91]. Copiotrophic marine bacteria (those that require higher organic matter concentrations) have lower substrate affinities but higher maximum uptake rates that allow them to rapidly draw down local substrate spikes on the order of 100 nM to 1 μM[125]. It may be possible for a single bacterial taxon to switch between these life history strategies[126,127], but it is more likely that genetic makeup (genome size, content, regulation) locks a heterotrophic bacterium into either a high or low affinity strategy[128-130]. Regardless of how affinity is apportioned among cells, the communities of marine bacteria in aggregate are capable of both high affinity and fast uptake to maintain low concentrations of labile DOC components in seawater, and are considerably better at detecting DOC

than our state-of-the art chemical methods. While targeted chemical methods have the sensitivity to directly measure some classes of metabolites in seawater, for example amino acids[131], sugars[132], and organic sulfur molecules[68], untargeted methods that aim to maximize the number and novelty of detected molecules are challenged by the low natural concentrations[4].

The difficulty of characterizing the 'small reservoir' of labile DOC within the total DOC pool is intertwined with its 'large flux'. Cycling rates of metabolites are not predictable by their concentration in seawater and may even be inversely related[133]. For example, dimethylsulfoniopropionate (DMSP) has been estimated to support up to 10% of total bacterial carbon demand (i.e., bacterial secondary production plus respiration) in the marine photic zone[134] yet has a steady-state concentration in the surface ocean of just ~3 nM[135] (Box 2). The most common method for estimating flux of recently fixed carbon into bacterial cells is to measure exudates released from ^{14}C-labeled phytoplankton cells in the absence of bacterial uptake[32], the so-called 'dissolved primary production'. These estimates of carbon available for bacterial scavenging span a very wide range (from 4 – 47% of NPP[15]) and provide no information on the specific compounds involved. Another approach captures the combined flux of labile DOC from all principal sources by determining the bacterial carbon demand as a percentage of ecosystem NPP. Results from this methodology suggest ~40%-60% of primary production is accessible to heterotrophs across a variety of marine environments, but with considerable uncertainties[57,59]. Moreover, these methods cannot directly observe carbon flux on the time scales (seconds to minutes) and space scales (microns) of many microbial processes[136,137,138].

What are some ways forward?

Advances in chemical methods

While there are now more than 18,500 metabolites catalogued in the KEGG compound database[139], an untold number including many with central roles in biogeochemical cycling are still missing. A strategic approach is needed to focus on those currencies most relevant in driving the ocean's carbon cycle. Molecular-level characterization of marine DOC has indeed been a goal for decades in chemical oceanography and organic geochemistry, yet has been thwarted by the salt and concentration challenges detailed above as well as by instrument limitations. Metabolite identification has emerged as a significant bottleneck in marine metabolomics because the complex mixtures contain numerous isobaric and isomeric compounds that are poorly represented in current reference databases biased towards human- and human-associated metabolites. Major advances in both MS and NMR have now

begun to crack this DOC "black box" (Fig. 4). The advent of electrospray ionization (ESI; ref[140]) enabled the transfer of polar molecules directly into mass spectrometers. Mass resolution and accuracy have improved dramatically with Fourier-transform based analyzers such as Fourier-transform ion cyclotron resonance cells[141] and Orbitrap detectors[142], now routinely enabling sub-ppm mass accuracy for thousands of molecules within complex mixtures. Chromatographic columns such as hydrophilic interaction chromatography (HILIC) and new mixed-mode resins can deliver specific fractions of the labile DOC pool for characterization[143]; picomoles of individual compounds is sufficient to trigger fragmentation spectra for identification. Although typically less sensitive than MS for molecular detection, NMR provides structural information that better enables identification. Higher field NMR magnets[144] and advanced small-diameter probes provide better analyte detection with lower salt sensitivity[145]; nanomoles of individual molecules are currently required for structural identification. Two-dimensional NMR approaches[146] have been developed to obtain complete covalent geometry of molecules, sometimes with stereochemistry[147]. Further advances in MS and NMR technology offer promise for improved DOC characterization in the near future, including better data deconvolution strategies[148] and improved methods for integrating data for unknown compound identification[149]. The compounds that have been successfully identified in marine metabolomes (Table 1) represent only a small fraction of total metabolite diversity.

While MS and NMR offer the best potential for identifying biologically labile molecules in marine DOC, the salt problem has yet to be solved. Derivatization protocols show excellent promise for this challenge, particularly those that target functional groups common in biologically produced compounds (e.g., alcohol and amine groups[4] and carbonyl moieties[150]). Recent application of derivatization methods are enabling detection in seawater at nanomolar (NMR; GC/MS)[151] to picomolar (LC/MS)[4] concentrations. Identification of polar metabolites by direct injection of seawater into mass spectrometers has also been demonstrated recently for marine culture media with a limit of detection averaging 600 nM (range: 10 nM - 3.2 µM) for 73 compounds[152].

Chemical methods are now better able to measure bacterial uptake of labile metabolites. Isotopically-labeled compounds can be tracked into individual cells using nano-secondary ion mass spectrometry (NanoSIMS)[153,154] and chemical tagging tools[155-158]. As sampling volume requirements decrease and instrument sensitivities increase, single-cell measurements of internal and external metabolites will draw tighter associations between microbes and molecules[159,160]. High resolution magic-angle spinning NMR probes provide access to real-time metabolism *in vivo*, even in the presence of high

salt concentrations[161]. Flux measurements at the required temporal and spatial scales are on the horizon, with advances in tracking isotope incorporation through intracellular metabolic pathways[162,163], cellular uptake of individual metabolites[161], and fluid-flow devices coupled to high-resolution imaging[136]. These multiple approaches chip away at barriers to progress.

Biological screening for lability

Labile metabolites are defined as those susceptible to microbial transformation, making evidence of biological processing an effective operational definition for labile DOC. Biology-based screening approaches fall into two categories: those that use biological signals to generate hypotheses regarding important compounds, and those that couple biological signals with chemical analysis. In the former category, gene, transcript, and protein inventories suggest which molecules are produced and consumed. These biosensor strategies do not require disruption of steady state interactions, instead surveying intact natural communities (using metagenomics, metatranscriptomics, metaproteomics) or model organism systems (using genomics, transcriptomics, proteomics). This approach has offered insight into metabolite flux in phytoplankton blooms[164,165], oligotrophic seawater[166,167], model communities[65,73], and ocean-wide surveys[168,169]. It has been used to construct co-occurrence networks of microbes and metabolites[170,171], and provide insight into the choreographed daily cycles of metabolite-driven phytoplankton-bacteria interactions in the surface ocean[18,27,166,172] (Fig. 3). The approach can be readily expanded to regional, global, and full ocean depth scales to illuminate large scale patterns in labile DOC transformations. Biological screening methods that rely solely on microbial response, however, are ultimately constrained by inadequate and slowly advancing gene annotation, an area for which investments in new technology and strategies are critically needed.

The second category of biological screening is coupled to chemical analysis. This typically requires steady-state disruption, altering either the accumulation or utilization of DOC by modifications to the microbial community (for example, removing phytoplankton or bacteria[67,74,99] or adding viruses[44]) or by manipulation of environmental parameters (for example, irradiance[173]), followed by chemical analysis of the altered pools. Drawdown studies introduce bacteria into a DOC pool and rely on chemical analysis to identify features that are depleted[69], such as characterization of bacterial substrate use by different species[69]. Mutant screening studies introduce bacteria with disrupted genes into a DOC pool and rely on chemical analysis to identify features that are no longer depleted, for example identification of the phytoplankton exometabolite dihydroxypropanesulfonate (DHPS) from its accumulation in a transporter mutant assay[68]. Enzymatic activity assays use selective digestion by high specificity bacterial

enzymes to quantify labile compounds within complex mixtures, for example hydrolases that degrade laminarin into diagnostic sugar units that are readily measured[174]. Other biological screening strategies currently used primarily in the field of biochemistry have promise for adoption in ecological studies. Vesicular transport assays embed transporters in synthetic membrane vesicles in an inside-out orientation, trapping target metabolites in the vesicle for chemical analysis[175]. Metabolite-protein binding assays detect enzymes that bind to known metabolites, for instance identification of substrates of *Escherichia coli* enzymes that previously lacked functional annotation[176]. Finally, activity-based protein profiling (ABPP) uses chemical probes with reporter tags that mimic metabolites and form covalent bonds with microbial enzymes, for instance to identify novel catabolic enzymes that degrade cellulose[177]. The coupling of biological screening with chemical tools holds promise for pinpointing the hidden chemical currencies of the surface ocean and the genes and enzymes that transform them.

Modeling microbes

Modeling approaches are being used to extract carbon-cycle relevant insights from observations of the ocean's microbe-metabolite network. The challenge for models is to bridge a spatial scale spanning 13 orders of magnitude, from cell metabolism at the scale of 10^{-6} m to ocean flux at the scale of 10^{7} m. Component models that focus on specific portions of this spatial scale already exist or are being developed. At the microbial end, earlier models of DOC release by phytoplankton sought a mechanistic understanding based on parameters such as phytoplankton size, nutrient status, and photosynthetic output[178-180]. More recent models leverage genomic data to address the biochemical basis for metabolite production and consumption. FBA-based metabolic models[181] typically optimize for generation of new biomass, but could optimize for other physiological or ecological traits[182] such as metabolite release, abiotic stress tolerance, or carbon use efficiency[183]. Phylometabolic modeling integrates comparative genomics with insights from biochemistry and ecology to reconstruct metabolic innovations that affect metabolite production and consumption, such as complementary organic matter exchange between phytoplankton and bacteria[17]. Multi-cell metabolic models for microbes in colonies or multi-species communities[184] uncover rules governing metabolite exchange and provide parameters for models working at regional to global scales[185,186].

In the transition from cellular scales to regional and global scales, hard-fought details of metabolite chemistry and biology must be simplified yet not trivialized. This need to identify the optimal balance between excessive detail and oversimplification has emerged as a crucial barrier to incorporating microbial processes into global models[187], along with high computational costs, limited

conceptual foundation, and lack of data to formulate and evaluate the more complex models. Nonetheless, global and regional DOC dynamics can be captured through nutrients – phytoplankton – zooplankton – detritus (NPZD) modules, widely used to compare historical and future climate change[188,189]. These models approximate the details of microbe-metabolite networks with bulk functions (such as Michaelis-Menten equations for substrate uptake) and use simplified rules to track substrates and energy through core metabolic pathways[188]. Conceptual convergence between models at different scales can potentially be leveraged, for example by linking cell growth output from steady-state FBA models with resource allocation rules applied to NPZD models.

The recently developed 'emergent' models in which microbial community structure and function emerge from a wider set of possibilities combine microbial genomic or physiological data with a dynamic physical/chemical ocean model to observe biogeochemical outcomes. Outputs from emergent models have revealed, for example, predictable assembly of communities based on functional repertoire rather than taxonomic affiliation[190] and matched distributions and abundances of model microbes with their real-world counterparts across ocean light and temperature gradients[191]. An inherent struggle with these models is parameterizing the bioenergetic cost of a gene or gene function[190,192,193]. More work is needed to overcome the associated modeling challenges, but it is clear that successful modeling of labile DOC flux at regional and global scales will close one of the largest knowledge gaps in the global carbon cycle.

Computational data science approaches

Software solutions that respond to the needs for interdisciplinarity and integration in microbiome science are emerging[194-197], including those that build microbe-metabolite networks with machine learning tools[198]. Other strategies address metabolite-related gene annotation by merging information on 'genes without a metabolite' with information on 'metabolites without a gene'. For example, the Metabolite Annotation and Gene Integration (MAGI)[199] method identifies patterns of metabolite-gene associations by scoring the consensus in occurrence between the two data sets. Emerging open source community-driven analysis platforms, such as the crowd-sourced Global Natural Product Social Molecular Networking (GNPS)[200] database, improve metabolite identification and annotation by enabling comparisons of fragmentation spectra. As annotations improve, *in silico* reconstructions can serve as knowledge repositories that facilitate data integration of reactions and pathways and enable predictions of microbial biosynthetic capabilities as environmental conditions, genetic perturbations, and fitness functions[110,201,202] vary. These informatic-centric approaches offer key

starting points for improved inventories of the microbial metabolites and genes playing important roles in surface ocean carbon flux.

Despite both progress and interest, the ability to co-investigate chemical compounds and their genetic determinants across space and time remains a significant bottleneck to characterizing microbe-metabolite networks. Data sharing is imperative among marine chemists and microbiologists in order to enable discoverability, integration, and interoperability of data across software tools; data exchange through software is necessary to leverage a growing public database that merges marine bacterial, archaeal, and eukaryotic genomes[203,204], metagenomes[205-207], metatranscriptomes[208], metaproteomes[209], and annotation resources for chemical oceanography[200,210]. Integrative strategies will lead to characterization of the genes that link microbial activity to the production and consumption of key metabolites.

Microbial currencies in a changing ocean

The well-recognized downward export of particulate organic carbon from the surface ocean to deep ocean waters and sediments (the "biological pump") is globally significant because it isolates carbon from the atmospheric pool for hundreds to thousands of years[211,212]. The microbial remineralization of labile carbon to inorganic form is globally significant because it diverts carbon from the biological pump, reducing net community production and influencing air-sea CO_2 fluxes[213,214]. The quantitative importance of labile DOC remineralization becomes clear by considering that it constitutes only 0.03% of the total DOC pool yet accounts for 86% of total DOC turnover[2]. This implies that rapidly-cycled microbial metabolites are among the most important individual conduits of ocean carbon flux.

Recent studies have provided a framework for predicting how phytoplankton-derived components of labile DOC might be altered under future climate scenarios, despite often complex and species-specific responses. There is evidence that increased ribosome efficiency under higher temperatures will decrease phosphorus demand[215,216,217], lowering the N:P stoichiometry of labile DOC as rRNA synthesis needs are lessened. Mismatches between phytoplankton photosynthetic flux (weakly affected by temperature) and metabolism (strongly affected by temperature)[217] could increase release of carbon-rich carbohydrates when CO_2 fixation is in excess of phytoplankton requirements[15]. Elevated CO_2 concentrations can decrease photorespiration rates and alter the release of photorespiration products such as glycolate[218,219]. Finally, a warming climate is predicted to favor smaller phytoplankton cells better able to compete for nutrients in a stratified ocean[220,221], shifting labile DOC chemistry

towards metabolite profiles characteristic of cyanobacteria and green lineage phytoplankton taxa[18,222]. As these critical predictions are emerging, so is recognition of the microbial functions underpinned by the production and cycling of the substrates, facilitators, and ecological signals exchanged via the labile DOC pool.

The details of the metabolic currencies that transfer carbon between microbes to sustain the surface ocean carbon cycle have been largely invisible to scientists in the past. Yet climate-carbon feedbacks mediated through the labile DOC reservoir depend on this microbe-metabolite network. Indeed, the resilience of our changing ocean relies on responses of the network to temperature increases, ocean acidification, and the many linked environmental changes. With advances in the chemical, biomolecular, and data sciences, more previously invisible molecules and their roles in the ocean carbon economy are being recognized. The future promises rapid scientific advances in knowledge of the chemical currencies of the surface ocean carbon cycle at a time when they are needed to safeguard an increasingly human-perturbed ocean.

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All authors contributed ideas. MAM, EBK, and WFS wrote the manuscript with substantial input from all authors.

Conflict of Interest Statement

The authors declare no competing interests.

Figure Legends

Figure 1. Microbial metabolites drive the carbon cycle in the surface ocean. Metabolites in the labile dissolved organic carbon pool (labile DOC) are synthesized by phytoplankton and other microbes and released into seawater through exudation, leakage, sloppy feeding, and lysis. Within hours to days, they are consumed primarily by heterotrophic bacteria for growth and energy. The respiration of labile DOC adds CO_2 back to the surface ocean, influencing air-sea exchange. A small fraction of labile DOC is converted to non-labile forms and participates in long-term carbon storage in the deep ocean (MCP; microbial carbon pump). Fixed carbon that is not processed as labile DOC includes living microbes and metazoans; particulate detritus; and refractory DOC, with transport to the deep ocean via the biological carbon pump (BCP).

Figure 2. Major sources of the microbial metabolites that form labile DOC in the surface ocean. Green arrows indicate substrate metabolites derived from primary production, and brown arrows indicate those from secondary production. The contributions of the three main sources can only be hypothesized at this time (see text) given large reported ranges in the percent of primary production released in dissolved form[5,223]; biases from measurement artifacts (e.g., cell disruption during filtration[5], differential radiolabeling of microbial intracellular pools[19]); and influences of environmental factors such as photosynthetic rate[19,223], irradiance levels, nutrient limitations, bloom stage, and temperature[32]. Dotted gray arrows indicate facilitator and ecological signal metabolites that can contribute indirectly to carbon flux through influence on microbial activity.

Figure 3. Diel synchrony in microbial synthesis, release, and utilization of metabolites. a) Periodicity in expression of a diatom gene (*SDH*) in the 2,3-dihyhdroxypropane-1-sulfonate (DHPS) biosynthesis pathway (top) is correlated with DHPS concentrations in phytoplankton metabolomes (bottom) in

surface waters of the North Pacific Subtropical Gyre. Expression data are transcipts L^{-1}, normalized to the mean (redrawn from ref.[18]). b) Periodicity in relative gene expression by *Trichodesmium* (closed symbols) is correlated with expression by its associated microbiome (open symbols) for nitrogen fixation and metabolism genes (top) and carbon fixation and respiration genes (bottom) in the North Atlantic (redrawn from ref[172]). c) Gene expression patterns for key metabolic processes by dominant members of a California coast microbial community, averaged over 6 diel light cycles. Top, photosynthetically active radiation. Bottom, time of peak relative expression for individual genes. Colored symbols represent genes with statistically significant diel patterns. Gene expression by the dominant primary producer (*Ostreococcus*, green symbols) is shown separately for (top to bottom) photosynthesis, carbon fixation, and translation. Gene expression by five dominant heterotrophic bacterial groups is shown for translation. Gray shading represents night (redrawn from ref[27]).

Figure 4. The marine DOC spectrum. Chemical analysis strategies for marine DOC are targeted to molecules of different size and hydrophobicity, and typically begin with extraction and concentration from seawater. SPE includes common resin types such as C18, C8, HLB and PPL. SPE, solid phase extraction; HLB, hydrophilic-lipophilic balance; PPL, priority pollutant; TFF, tangential flow filtration; ESI/LC-MS, electrospray ionization-liquid chromatography/mass spectrometry, NMR, nuclear magnetic resonance spectroscopy, FT-MS, Fourier transform mass spectrometry (includes Orbitrap mass spectrometers and Fourier transform ion cyclotron resonance mass spectrometers); GC/MS, gas chromatography/mass spectrometry.

Box Legends

Box 1. Ecological classes of microbial exometabolites. Metabolites are small molecules that are direct products of metabolism. Chemically, they hail from a wide variety of structural classes and span a range of solubilities, molecular weight, and functional groups[224]. Metabolites can also be classified based on their ecological role in microbial communities; in the marine microbiome they typically have one of three main roles:

Substrate metabolites sustain biomass production and element cycling in microbial communities. In the surface ocean, molecules in this category include carboxylic acids[225,226], glycerols and fatty acids[225,227], nitrogen-containing compounds (such as polyamines)[78,168,225], C_1-compounds [168,225,227], carbohydrates (such as glucose)[7,228], and sulfonates and sulfonium compounds (such as DHPS)[18,68,229]. Substrate metabolites are likely to be synthesized in core biochemical pathways during microbial growth and be conserved across diverse taxonomic groups.

Facilitator metabolites enable or enhance chemical reactions and include molecules such as vitamins (such as B_7) and siderophores (such as catecholate siderophores)[86,230]. (Enzymes are considered macromolecules rather than metabolites and are not included here). Facilitator metabolites are also likely to be synthesized in core biochemical pathways.

Ecological Signal metabolites alter the phenotype of neighboring microbes and are typically secondary metabolites produced to support non-growth activities. In the marine microbiome, ecological signal metabolites include chemical cues or “infochemicals” (such as homoserine lactones involved in quorum sensing), microbial pheromones, and antimicrobials and algicides (such as tropdithietic acid)[231-235]. Marine bacteria can devote considerable genomic resources to the synthesis of ecological signal metabolites[236,237].

Box 2. Hunting a Marine Metabolite. Determining the role of an ocean metabolite is a complex and multidisciplinary process, as revealed by the decades of research dedicated to learning the biogeochemistry of just one metabolite: dimethylsulfoniopropionate (DMSP). Early research established DMSP as a major phytoplankton osmolyte[238], an important substrate for marine bacteria[239], and the precursor of dimethylsulfide (DMS), which is the dominant volatile in ocean-atmosphere sulfur flux[240,241]. Yet the biochemical mechanisms of DMSP synthesis and degradation remained unknown until genomic data enabled gene discovery beginning in 2006[242-245]. Since then, the configuration of the microbial-DMSP network, initially considered simple flux from phytoplankton to bacteria (gray arrows), has been revealed as a highly complex web of synthesis and utilization (black arrows). New network edges have been discovered, such as discovery that bacteria also synthesize DMSP[242] and phytoplankton also assimilate it from the environment[246]. New nodes have been discovered, such as chemical relatives of DMSP that affect its fate[70,247] and roles for viruses in release and transformation[248,249]. New functions for DMSP have been discovered, such as ecological signals for bacterial chemotaxis[250] and pathogenesis[251]. DMSP may be the highest-flux single metabolite of the surface ocean carbon cycle[72,134], yet biological and chemical studies over decades were required to unravel intricacies of its dynamics. Factors that regulate the fate of DMSP, including what controls its transformation to climate-active DMS, are yet to be resolved[162,252,253]. DMSOP, dimethysulfoxoniumpropionate.

Table 1. Example marine microbial exometabolites identified in seawater and culture medium, and endometabolites identified in plankton communities and cultured cells. Recognizing the metabolites and metabolite classes that play central roles in ocean carbon flux is not yet possible, but is a key goal of future research. References are as follows: [18]Durham et al., 2019; [68]Durham et al., 2015; [69]Ferrer-González et al., 2020; [70]Gebser et al., 2020; [64]Dawson et al., 2020; [63]Boysen et al., 2021; [74]Shibl et al., 2020; [75]Uchimiya et al. 2021; [66]Fiore et al. 2015; [238]Matrai and Keller 1994; [72] Kiene et al., 2000; [115]Johnson et al., 2020; [254]Johnson et al., 2016; [3]Weber et al., 2020; [4]Widner et al., 2021; [255]Longnecker et al., 2018.

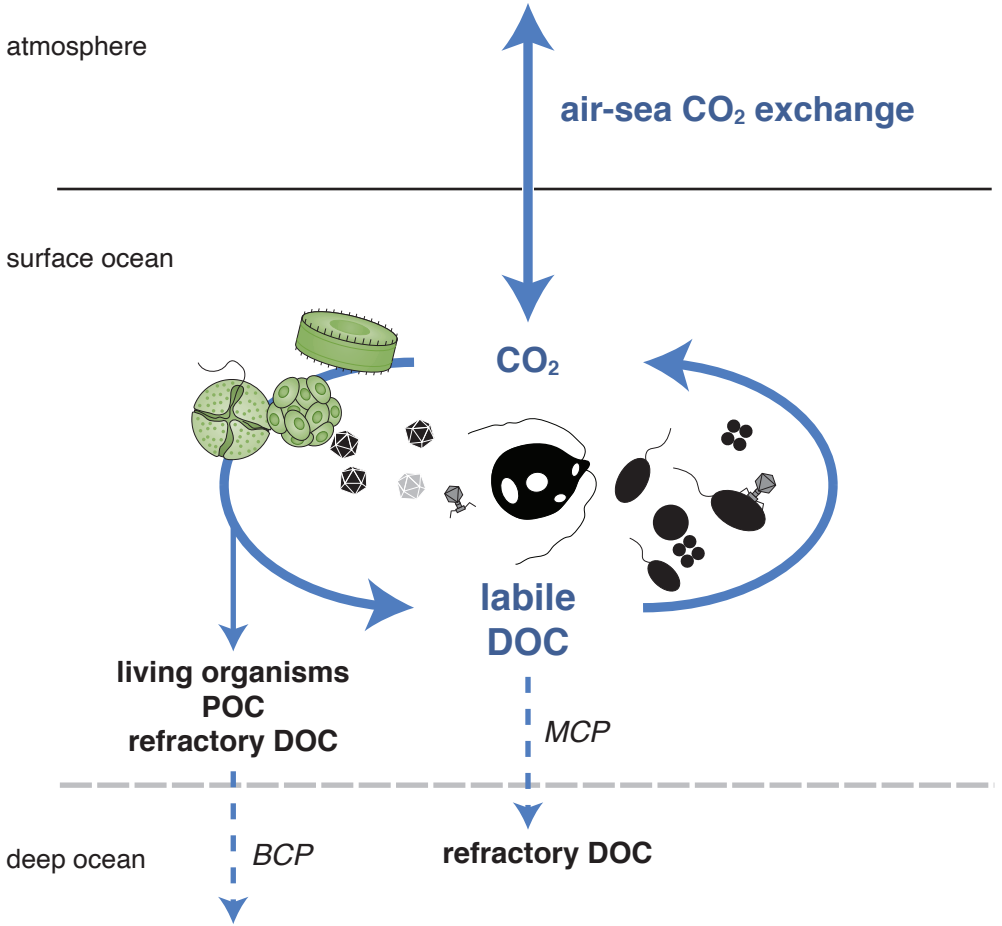
Metabolite	Exometabolite in seawater	Exometabolite in culture	Endometabolite in marine plankton	Endometabolite in culture	Reference
1-Methylhistidine		x			74
2'-Deoxyguanosine		x			4
2,3-Dihydroxybenzoate	x				255
3-Dehydroshikimate		x			254
3-Hydroxybutyrate				x	75
3-Mercaptopropionate	x	x			66, 255
3-Phosphoglycerate		x			74
4-Acetamidobutanoate		x			74
4-Amino-5-aminomethyl-2-methylpyrimidine (AmMP)					3
4-Amino-5-hydroxymethyl-2-methylpyrimidine (HMP)	x	x			4
4-Aminobenzoate	x	x			3, 255
4-Aminobutanoate		x			74
4-Hydroxybenzaldehyde		x			74
4-Hydroxybenzoate	x	x	x		66, 115, 3, 255
4-Hydroxyphenylacetate		x		x	74, 75
4-Hydroxyphenylglycine		x			74
5'–Methylthioadenosine (MTA)		x	x	x	115, 254, 3
5'- Uridine monophosphate (UMP)	x	x			4
6,-Phosphogluconate	x				255
(6R)-5,6,7,8-Tetrahydrobiopterin		x			4
7-Dehydrocholesterol			x		63
Acetate				x	75
Acetyltaurine	x				3
Aconitate			x		63
Adenine	x	x			63, 3, 4
Adenosine	x	x	x	x	69, 63, 74, 66, 115, 3, 4, 255
Adenosine monophosphate (AMP)			x		69, 63, 66, 115, 4
Aminobutyrate			x		63
Alanine	x	x	x	x	64, 63, 75, 4
Arachidonate			x		63
Arginine	x	x	x	x	69, 64, 63, 75, 66, 115, 3, 4
Asparagine	x	x	x	x	63, 75, 4
Aspartate	x	x	x	x	64, 63, 75
Azelaiate		x			74
Biotin	x		x		115, 255

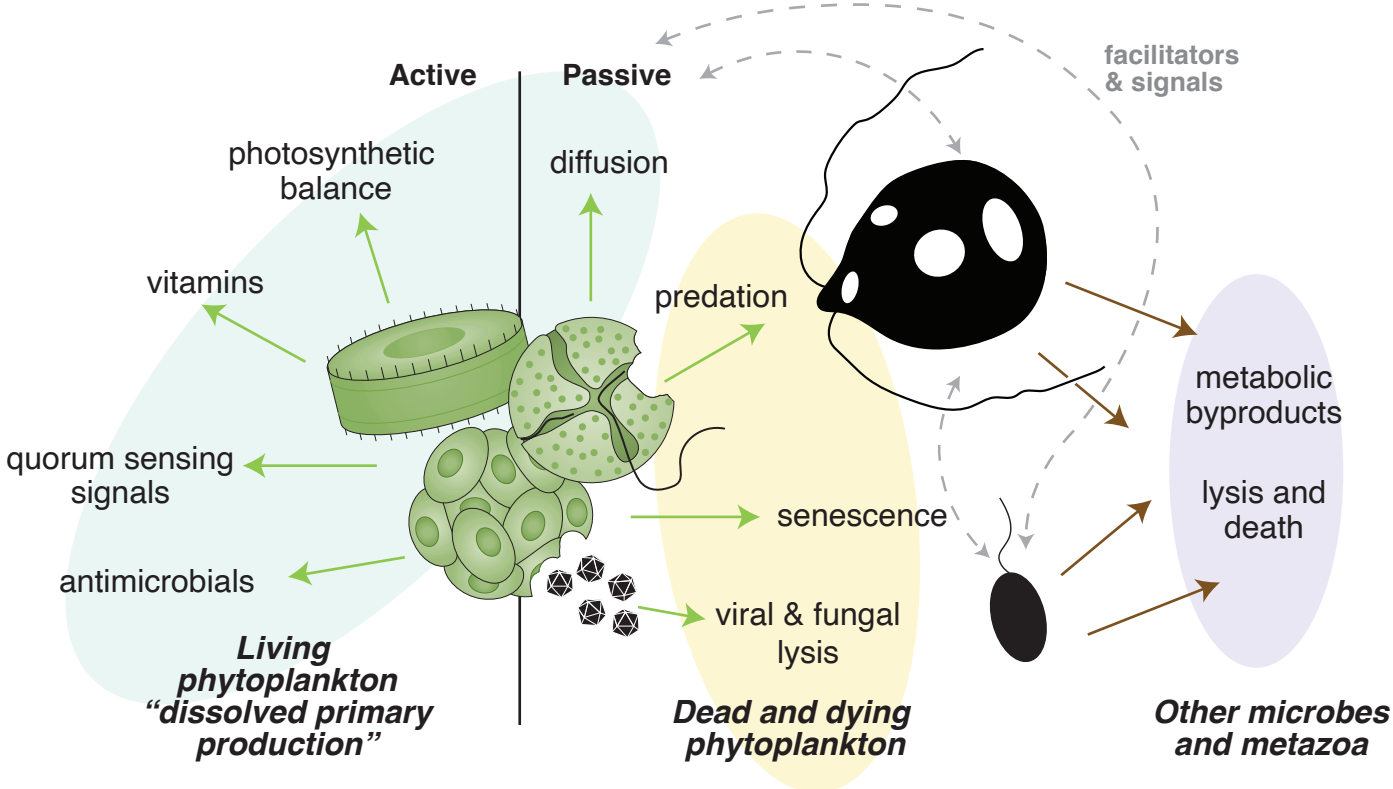
C_{16}-hydroxy-glycerophosphocholine		x			[74]
Caffeine	x		x		[63, 115, 3]
Carnosine		x			[74]
Cyclic guanosine monophosphate (cGMP)			x		[63]
Chitobiose	x	x	x	x	[69, 63, 66, 3, 4]
Chitotriose	x	x			[69, 3, 4, 255]
Choline	x		x	x	[64, 63, 75, 3, 255]
Ciliatine	x	x			[3, 4]
Citrate	x		x		[63, 3]
Citruline	x	x		x	[74, 66, 3]
Creatine			x		[63]
Cyanocobalamin	x	x			[66, 255]
Cystathionine			x		[63]
Cysteinolate			x		[18]
Cysteate	x	x	x	x	[18, 63, 4]
Cysteine	x	x			[4]
Cytidine	x		x		[63, 4]
Cytosine			x		[63]
Desthiobiotin	x				[3, 255]
Dihydroxyacetone phosphate	x				[3]
Dihydroxypropansulfonate (DHPS)	x	x	x	x	[18, 68, 64, 63, 75, 3, 4]
Dimethylglycine			x	x	[63, 75]
Dimethylsulfonioacetate (DMSA)				x	[70]
Dimethylsulfoniopropionate (DMSP)	x	x	x	x	[68, 64, 63, 75, 66, 238, 72, 11, 5, 254, 255]
Ectoine	x	x			[3, 4]
Ethanolamine				x	[75]
Folate	x		x		[115, 255]
Fosfomycin	x				[3]
Gamma-aminobutyrate (GABA)	x	x			[4]
Gluconate			x		[63]
Glucose				x	[75]
Glucose-6-phosphate	x			x	[66, 3, 255]
Glucosamine 6-phosphate	x	x			[4, 255]
Glucosylglycerol			x	x	[64, 63]
Glutamate	x	x	x	x	[64, 63, 75, 66, 3, 4]
Glutamine	x	x	x	x	[64, 63, 75, 254, 4]
Glutathione	x		x	x	[63, 66, 3]
Glycerol 3-phosphate	x		x	x	[63, 75, 3]
Glycerophosphocholine				x	[75]
Glycine	x	x		x	[75, 4]
Glycine betaine			x	x	[64, 63, 75, 66, 115]
Glyphosate	x				[255]
Gonyol			x	x	[70, 63]
Guanine	x		x		[63, 115, 3]

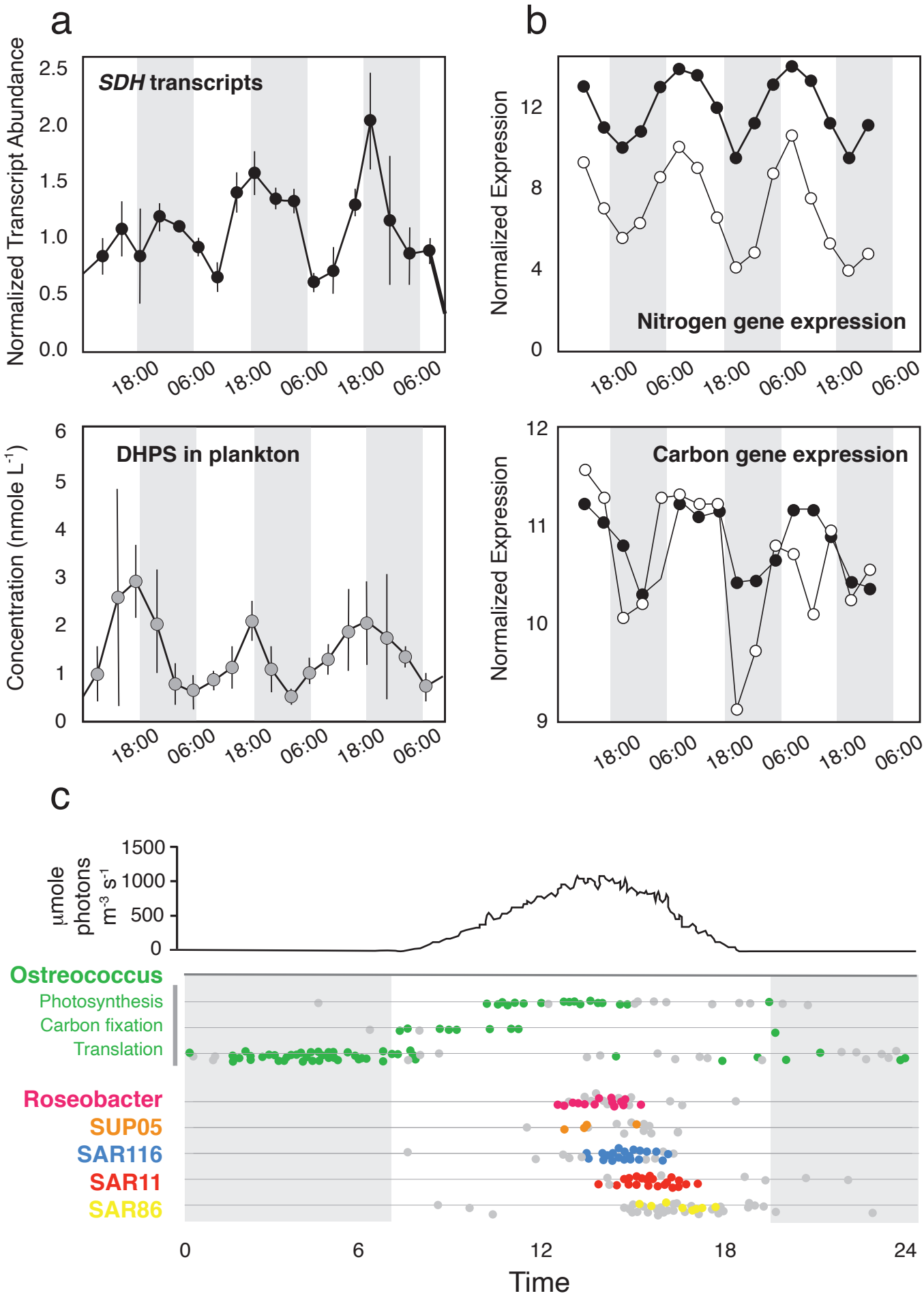
Guanosine	x	x	x	x	69 63 75 3 255
Guanosine monophosphate (GMP)			x		63
Histidine	x	x	x		63 4
Homarine			x	x	63 75
Homoserine	x	x			C
Homoserine betaine		x			4
Hydroxocobalamin			x		63
Indole-3-acetate	x	x	x		63 66 115 255
Indole-3-acetamide			x		63
Inosine 5'-monophosphate	x				255
Inosine	x	x	x		69 66 115 3 255
Isethionate	x	x	x	x	18 64 63
Isoleucine	x	x	x	x	69 64 63 75 115 255
Kynurenine	x	x	x		63 3 4 255
Leucine	x	x	x	x	69 63 74 75 115 3 4 255
Lysine	x	x	x	x	64 63 75 4
Malate	x	x			3 4
Methionine			x	x	63 115 254
Methylglutarate		x			74
Methyl indole-3-carboxylate			x		63
Muramate	x	x			4
N-(3-oxotetradecanoyl)-L-homoserine lactone		x		x	115
N-acetyl-galactosamine	x	x		x	74 66
N-acetyl-glutamate	x	x			66 255
N–acetylmuramate	x	x			3 4 255
N-acetyltaurine		x			66
N-tetradecanoylaspartate		x			74
Niacin			x		63
Nicotinamide adenine dinucleotide-hydrogen (NADH)	x				3 255
Nicotinamide adenine dinucleotide-phosphate (NADP)	x				255
Norvaline		x			74
Oleate		x			74
Ornithine	x	x		x	64 4
Pantothenate	x	x	x		63 115 3 4 255
Phenylacetate		x			74
Phenylalanine	x	x	x		63 66 115 3 4 255
Phosphoglycerate			x		63
Phosphorylcholine				x	75
Proline	x	x	x	x	69 64 63 75 66 115 3 4
Propionate		x		x	254
Putrescine	x	x			4
Pyridoxine	x		x		63 115 3
Pyridoxal			x		63
Pyridoxal phosphate			x		63

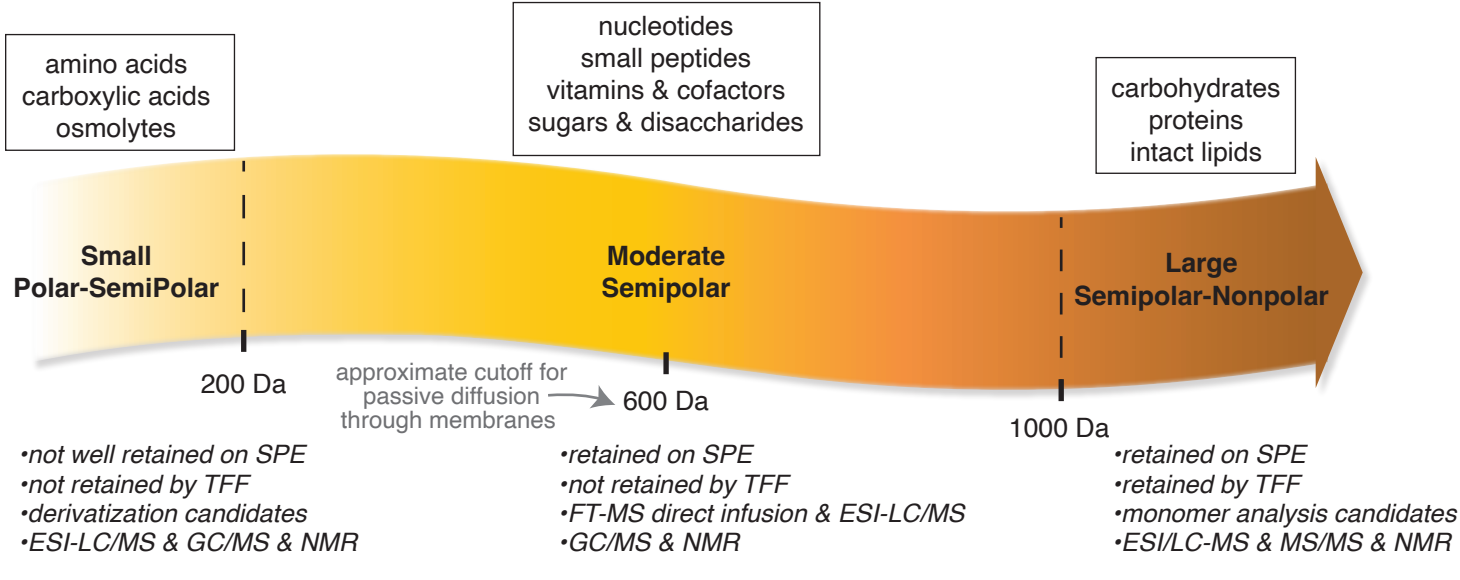
Quinolinecarboxylate		x			74
Riboflavin	x	x	x	x	63 115 254 3 255
Ribose 5 phosphate			x		63
Rosmarinate		x			74
S–5′-adenosyl–L–homocysteine	x	x	x		63 3 4
S-adenosyl methionine			x		63
Sarcosine	x	x	x		63 4
Serine		x	x		63 4
Shikimate		x			254
Sperimidine	x	x		x	66 4
Sphingamine		x			74
Stearate		x			74
Suberate		x			74
Succinate	x	x			66 3
Sucrose			x		63
Sulfolactate			x	x	18 63
Syringate	x				3
Taurine	x		x	x	18 64 63 4
Taurocholate	x	x			66 3 4 255
Thiamin				x	66
Thiamin monophosphate				x	254
Threonine	x	x	x		63 74 3
Thymidine	x	x	x		69 63 66 3
Thymine			x		63
Trehalose			x		63
Trigonolline			x		63
Trimethylamine-N-oxide				x	75
Tryptamine	x				3 255
Tryptophan	x	x	x		63 66 115 3 255
Tyrosine	x		x		63 3
Uracil				x	66
Uridine	x	x	x	x	63 75 3
Uridine 5-monophosphate	x				255
Uridine diphosphate-glucosamine			x		63
Uridine diphosphate-glucose			x		63
Valine	x	x	x	x	69 63 75 3 4
Vanillate			x		63
Xanthine	x		x		115 3 255
Xanthosine	x		x		63 3 4 255
α-aminoadipate		x			74
α-ketoglutarate	x				3
α-Ribazole		x			254
β-1,3-glucan				x	75

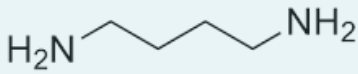
1124


atmosphere
air-sea CO_2 exchange
surface ocean
CO_2
labile
DOC
living organisms
POC
refractory DOC
MCP
BCP
deep ocean
refractory DOC


Active
Passive
facilitators
& signals
photosynthetic
balance
diffusion
vitamins
predation
quorum sensing
signals
senescence
antimicrobials
viral & fungal
lysis
metabolic
byproducts
lysis and
death
Living
phytoplankton
"dissolved primary
production"
Dead and dying
phytoplankton
Other microbes
and metazoa


a
b
c
SDH transcripts
Normalized Transcript Abundance
2.5
2.0
1.5
1.0
0.5
0.0
18:00
06:00
18:00
06:00
18:00
06:00
Normalized Expression
12
8
4
0
Nitrogen gene expression
18:00
06:00
18:00
06:00
18:00
06:00
DHPS in plankton
Concentration (nmole L-1)
6
5
4
3
2
1
0
18:00
06:00
18:00
06:00
18:00
06:00
Carbon gene expression
Normalized Expression
12
11
10
9
18:00
06:00
18:00
06:00
18:00
06:00
µmole photons m-3 s-1
1500
1000
500
0
Ostreococcus
Photosynthesis
Carbon fixation
Translation
Roseobacter
SUP05
SAR116
SAR11
SAR86
0
6
12
18
24
Time

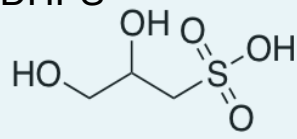

amino acids
carboxylic acids
osmolytes
nucleotides
small peptides
vitamins & cofactors
sugars & disaccharides
carbohydrates
proteins
intact lipids
Small
Polar-SemiPolar
Moderate
Semipolar
Large
Semipolar-Nonpolar
200 Da
approximate cutoff for
passive diffusion
through membranes
600 Da
1000 Da
•not well retained on SPE
•not retained by TFF
•derivatization candidates
•ESI-LC/MS & GC/MS & NMR
•retained on SPE
•not retained by TFF
•FT-MS direct infusion & ESI-LC/MS
•GC/MS & NMR
•retained on SPE
•retained by TFF
•monomer analysis candidates
•ESI/LC-MS & MS/MS & NMR



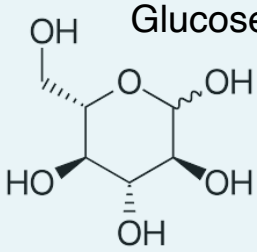
Polyamine

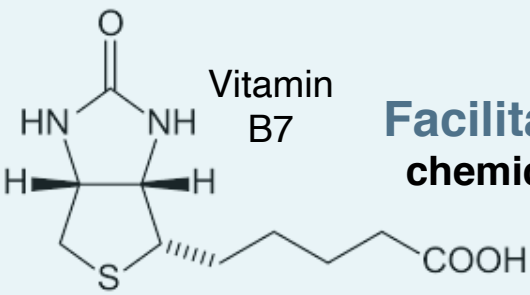
Substrates sustain biomass production and element cycling

DHPS



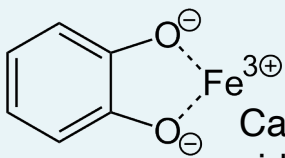
Glucose





Vitamin B7

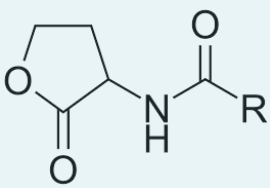
Facilitators enable chemical reactions

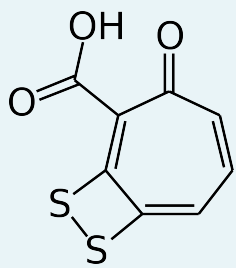


Catecholate siderophore

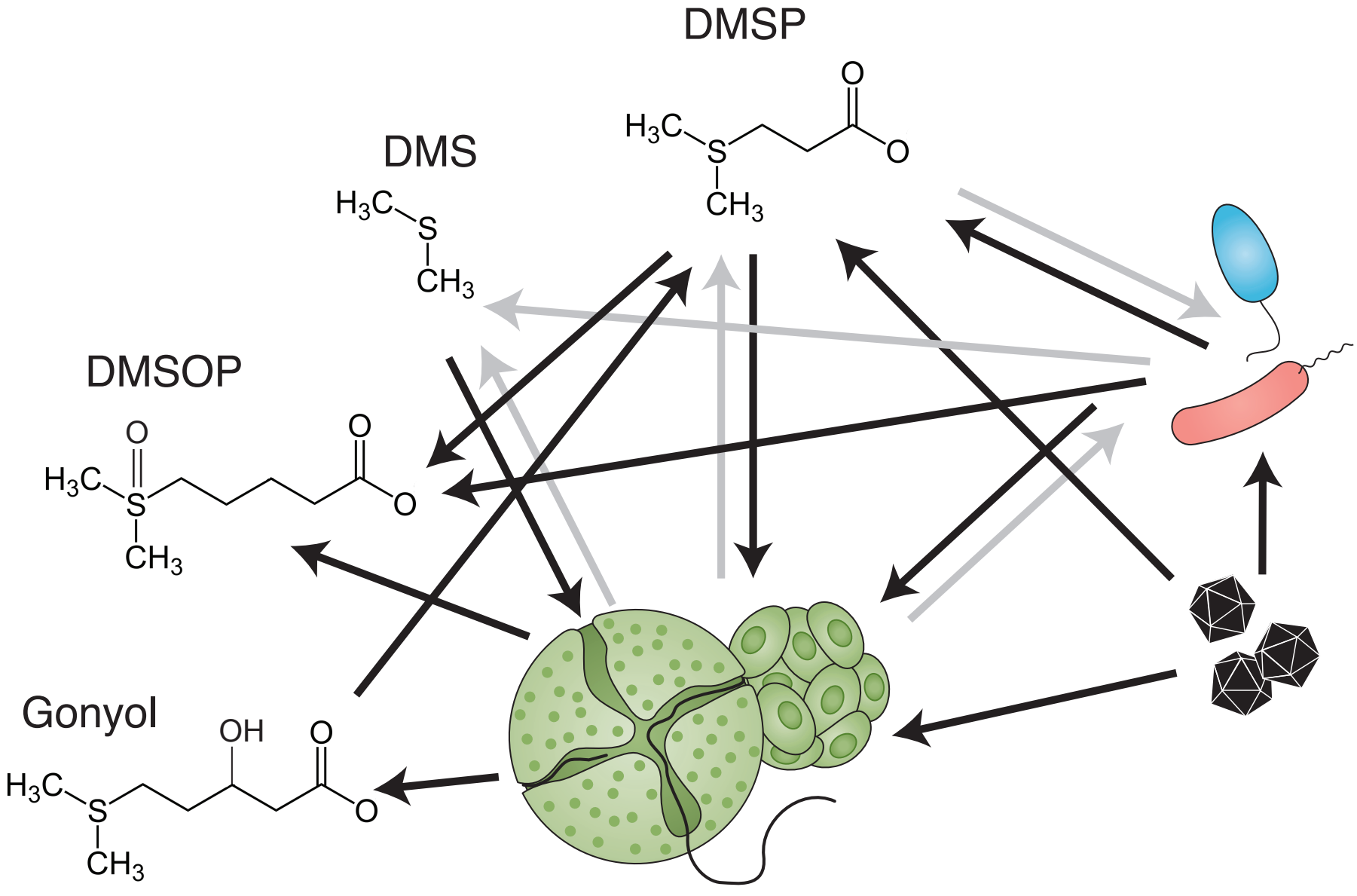
Signals alter microbial phenotypes

N-acyl homoserine lactone





Tropodithietic Acid


DMSP
DMS
DMSOP
Gonyol
OH