Resourceful heterotrophs make the most of light in the coastal ocean

Mary Ann Moran and William L. Miller

Abstract | The carbon cycle in the coastal ocean is affected by how heterotrophic marine bacterioplankton obtain their energy. Although it was previously thought that these organisms relied on the organic carbon in seawater for all of their energy needs, several recent discoveries now suggest that pelagic bacteria can depart from a strictly heterotrophic lifestyle by obtaining energy through unconventional mechanisms that are linked to the penetration of sunlight into surface waters. These newly discovered mechanisms involve the harvesting of energy, either directly from light or indirectly from inorganic compounds that are formed when dissolved organic carbon absorbs light. In coastal systems, these mixed metabolic strategies have implications for how efficiently organic carbon is retained in the marine food web and how climatically important gases are exchanged between the ocean and the atmosphere.

Pelagic
Relating to or occurring in the water column.

Heterotrophic
The acquisition of metabolic energy by the consumption of living or dead organic matter.

Primary production
The original source of organic material in an ecosystem — plants, algae or chemosynthetic microorganisms.

Bacterioplankton
The bacteria that inhabit the water column of lakes and oceans, either freely suspended or attached to particles.

Department of Marine Sciences, University of Georgia, Athens, Georgia, 30602-3636, USA.
Correspondence to M.A.M.
e-mail: mmoran@uga.edu
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The two million prokaryotes that inhabit each millilitre of coastal seawater are much more familiar to us now than they were only a decade ago. Major advances in molecular taxonomy and ecological genomics have allowed numerous uncultured coastal bacteria to be named and, in some cases, functional roles to be assigned. It is hoped that understanding these tiny organisms better will allow us to make progress towards elucidating the main ecosystem-level processes — nutrient cycling, ocean–atmosphere exchanges and productivity — that occur in the pelagic marine environment. Our knowledge will be significantly advanced by understanding the biochemistry of the individual mediators of these processes. Recently, important advances have been made in our comprehension of the intricacies of the carbon cycle, particularly the efficiency with which organic carbon is processed by marine microorganisms in the surface waters of the ocean.

The conversion efficiency of organic carbon by heterotrophic microorganisms is determined by the balance between cellular respiratory losses (which provide energy for the organism by the oxidation of organic carbon to CO_2) and the synthesis of biomass (which builds new cellular material). This value has surprisingly important implications for how coastal oceans function in the Earth's carbon cycle. For example, we do not yet understand the conditions under which coastal oceans can be net contributors to the atmospheric CO_2 pool — when they release more CO_2 through respiratory processes than they fix through primary production — versus conditions under which they can be net sinks — when they fix more CO_2 than they respire. Respiration by heterotrophic bacteria and archaea is the main source of CO_2 in estuaries and coastal ocean waters[1] and therefore has a major effect on the net carbon balance and, ultimately, on the magnitude of the release of CO_2 into the atmosphere. The productivity of marine food webs and the factors that affect food yields is also of prime scientific and economic importance. The efficiency with which marine bacteria and archaea incorporate organic carbon into microbial biomass at the base of the marine food web is crucial for determining the food yield at the top[2].

Our concepts of carbon processing and the microbial conversion efficiency in the coastal ocean have changed recently owing to insights that have been gained from the application of molecular biology and genomic techniques to oceanography. Previously unknown strategies by which heterotrophic bacterioplankton can obtain energy have begun to alter paradigms for the pathways of carbon cycling in the coastal ocean[3]. It is now acknowledged that energy might be available to heterotrophic microorganisms from processes that do not consume organic carbon and, in some cases, do not generate CO_2.

Although examples of newly discovered metabolic strategies, or combinations of strategies, can be found for most types of marine environments[4,5], here, we specifically examine those that are directly or indirectly driven by the penetration of solar radiation into ocean surface waters. We consider how elucidating these bacterial

Biomass burning
The burning of living and dead vegetation, including the human-initiated burning of vegetation and natural, lightning-induced fires.

K_m
The substrate concentration at which the reaction is half of the maximal rate.

Cultured representative
A member of a bacterial taxon that is capable of growth in the laboratory, typically reaching high densities on microbiological media.

ametabolisms could affect our understanding of carbon processing in the upper waters of the coastal ocean and how climatically important gases are exchanged with the atmosphere (FIG. 1).

CO oxidation

CO has an important role in the Earth's atmosphere as an indirect greenhouse gas. Although not a strong absorber of terrestrial thermal energy itself, CO reacts with hydroxyl radicals that would otherwise oxidize methane and nitrous oxide, thereby contributing to a longer half-life for these potent greenhouse gases[6]. Terrestrial sources (from the biomass burning of grasslands and other fire-dependent ecosystems) and anthropogenic activities (such as fossil-fuel combustion) are the dominant sources of CO in the atmosphere[7], but ocean surface waters are ubiquitously supersaturated with internally generated CO (up to 1,200%[8,9]) and therefore also contribute to atmospheric pools.

The amount of CO that is emitted annually from the ocean to the atmosphere has been much debated, with estimates spanning two orders of magnitude (BOX 1). This surprisingly large range in global CO-flux estimates reflects several uncertainties, but one of the most important is whether ocean-surface bacteria consume CO before it can participate in ocean–atmosphere exchange. CO is formed by non-biological processes during the photochemical degradation of organic molecules in sunlit surface waters[10] (FIG. 2). As supersaturating concentrations of CO accumulate in seawater during daylight hours[7], any consumption of CO by bacteria will decrease ocean venting of CO to the atmosphere.

The widely held view that marine bacteria have little or no role in the consumption of oceanic CO originated from both microbiological and chemical studies. Carboxydotrophic bacteria that oxidize CO to CO_2 (by the enzyme CO dehydrogenase) and that can fix a portion of the CO_2 into biomass (by the enzyme ribulose-1,5-bisphosphate carboxylase) have been successfully cultured from aquatic environments and soils[11]. However, although this phenotype might seem perfect for an oceanic CO oxidizer, physiological studies have revealed that many cultured carboxydotrophic bacteria — for example, *Oligotropha carboxidovorans* and *Hydrogenophaga pseudoflava* — require concentrations of CO that are several orders of magnitude higher than those present in ocean waters to sustain growth. For example, the K_m for CO oxidation is typically 400–1,000 nM for carboxydotrophs in the laboratory[12]. However, the peak CO concentration in coastal environments is only ~15 nM and even less CO is present in the open ocean (only ~2–3 nM)[13]. Carboxydotrophs that grow autotrophically using CO require high concentrations of this substrate to sustain reasonable growth rates, owing to the low energy yield from the oxidation of CO. Although bacteria of this type might thrive in niches that contain high local concentrations of CO, for example waterlogged soils[14], their physiology suggests that they are unlikely to be capable of growth using the CO concentrations that are present in the ocean. Some chemical oceanography studies supported this hypothesis, with field measurements of the CO oxidation rates so low that it would take more than 100 days for bacteria to consume the ambient CO pool[15]. Thus, calculations of the ocean–atmosphere CO flux have usually indicated little or no biological participation.

By contrast, other efforts to estimate global CO budgets have indicated that microbial oxidation must be a major source of the CO loss from the ocean surface waters. Extensive field studies that generated system-specific values for photochemical efficiency (BOX 1) have led to the calculation that as much as 90% of CO that is produced in ocean surface waters was internally recycled by CO-oxidizing marine bacteria before it could escape to the atmosphere[16], which would represent a major shift in the CO paradigm.

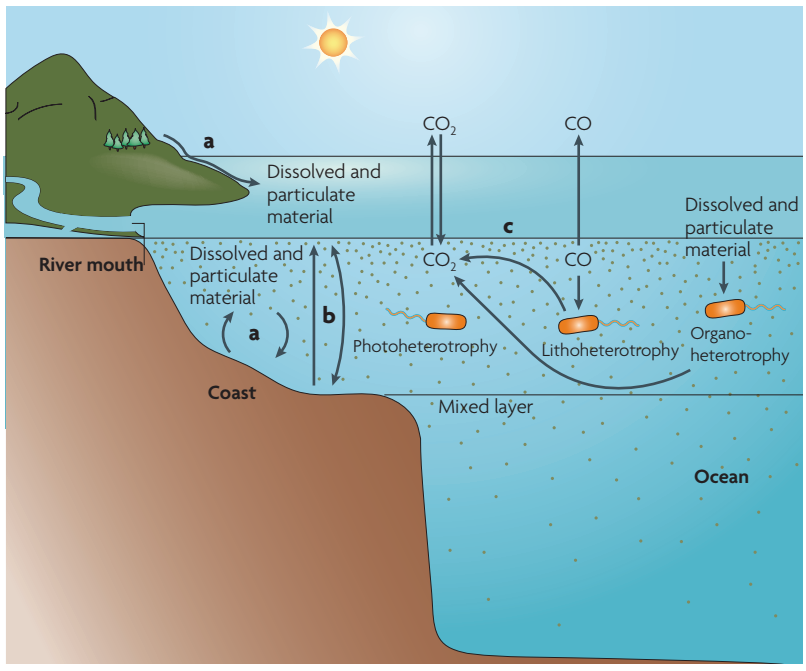


Figure 1 | **Light-linked bacterial and archaeal metabolisms in the coastal ocean environment.** Inputs from terrestrial systems and bottom sediments result in higher concentrations of organic matter in coastal environments than in the open ocean, which controls light penetration (particularly in the ultraviolet range, which is 280–315 nm) and dissolved organic carbon (DOC) availability (**a**). The water column in the coastal ocean is well mixed, allowing solar radiation and ocean–atmosphere gas exchange to directly affect a large proportion of the water column (**b**). Carbon inputs and optical properties of the surface water determine the importance of energy generation from the DOC pool (from organotrophy), inorganic photodegradation products similar to CO (by lithotrophy) and direct light harvesting (by phototrophy) (**c**). Energy that is generated from organotrophy and CO lithotrophy results in CO_2 formation, but energy from phototrophy does not. The relative importance of these processes has implications for the efficiency with which organic carbon is incorporated into the marine food web and the formation and release of the climatically important gases CO_2 and CO.

Lithoheterotrophy

Although it had been deemed unlikely that the carboxydotrophs are the main producers of oceanic CO oxidation, the microbial taxa that perform this process remained elusive. A hint at their identity came from the genome sequence of *Silicibacter pomeroyi*, which is a cultured representative of an abundant alphaproteobacteria taxon in the coastal ocean — the marine Roseobacter group. *S. pomeroyi* has two CO dehydrogenase proteins[17] and

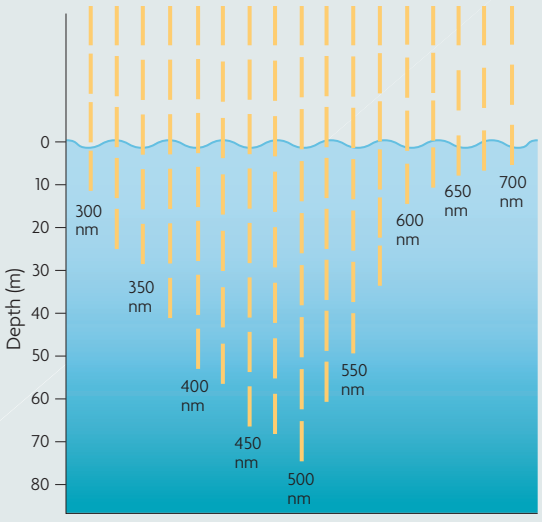
can oxidize CO at low nanomolar concentrations that are consistent with those found in the coastal ocean[18]. This bacterium differs from the previously characterized carboxydotrophs by one important characteristic: it has no genes for CO_2 fixation and, therefore, only uses CO as a supplemental energy source during heterotrophic growth on organic substrates. This metabolic strategy is termed lithoheterotrophy, which means that inorganic compounds (in this case CO) provide energy to organisms that nonetheless require organic matter as a source of carbon. Genome sequences of approximately 12 other Roseobacter group strains have indicated that genes for CO oxidation are common among cultured members of this group[19].

The discovery of CO lithoheterotrophy in an ecologically relevant marine bacterium suggested a resolution to the problem of how such a low concentration of CO can support bacterial growth — it does not necessarily have to. The hypothesis that lithoheterotrophs, rather than autotrophs, mediate a major fraction of the CO oxidation in ocean surface waters was supported by two findings. First, none of the CO that was oxidized by marine bacterioplankton communities seems to be incorporated into biomass[20] and, second, diverse heterotrophic CO oxidizers from coastal seawater were successfully cultured[13]. One possible exception to this hypothesis has been found — a *Stappia* strain (from the alphaproteobacteria) that contains genes for both CO oxidation and CO_2 fixation, although the two processes have not yet been shown to be physiologically coupled in this isolate[21]. Abundance estimates indicate that CO-oxidizing bacterioplankton are not a novelty in surface waters; they have been estimated to account for 1 in every 14 cells in the oligotrophic Sargasso Sea[18], with similarly high frequencies estimated in the north-eastern coastal waters of the United States[13].

Our improved understanding of the ecology of marine CO oxidizers not only provides a biological framework for understanding the ocean–atmosphere CO flux[16], but also indicates that there is a need to re-examine current hypotheses about the roles of heterotrophic marine bacterioplankton. Because organic carbon is a scarce commodity in the ocean, being present as a dilute mixture of dissolved compounds and small particles, CO might serve as a much needed supplemental energy source (FIG. 1). This would enable more of the organic carbon to be routed into bacterial biosynthetic pathways, with less metabolized for energy. How much of an advantage this would give a CO-oxidizing bacterium would depend on the availability of CO (FIG. 2), the amount of energy that is obtained from CO oxidation (probably 1 molecule of ATP per molecule of CO oxidized[11]) and the scarcity of organic carbon. We examine this issue quantitatively later.

Box 1 | **Microbial control of sea-to-air CO exchange: a new hypothesis**

Supersaturation and diurnal cycles of CO in marine waters were reported almost 40 years ago[54]. Studies done since that time have confirmed its source (photochemistry) and sinks (bacterial consumption and ventilation to the atmosphere), but quantifying CO cycling in the ocean has proven difficult, as none of these processes are well defined on a global scale. Photoproduction values, either inferred from sea-to-air flux estimates or calculated from photochemical data and optical models, have ranged from 4–820 teragrams of CO–C per year[16]. This ~200-fold difference can be traced back to problems with the extrapolation of freshwater photochemical data from marine systems, as well as the practice of summing estimates of two variable sinks (bacterial consumption and atmospheric ventilation) to derive approximate production estimates.



The first attempt at a large scale (~40°N to ~60°S in the Pacific) analysis of the photochemical production and bacterial consumption of CO in the same marine water samples was reported by Zafiriou and colleagues[16]. These data provided a global estimate for CO production of ~50 teragrams of CO–C per year, which was much lower than almost all other published estimates. The difference reflected the establishment of appropriate photochemical efficiency spectra from measurements of CO formation, as a function of wavelength, thereby allowing the complex light field of ocean waters to be considered in the calculations of production rates (see the figure). Remarkably similar efficiency spectra were subsequently measured for North-Atlantic waters[55].

The penetration of photons into seawater is dependent on wavelength. The depth at which 99% of photons at the ocean surface have been attenuated (that is, the 1% light level) owing to absorption and scattering by water, dissolved constituents and particles is shown in the figure, for a range of wavelengths, for example, coastal site Global Ocean Survey station 14 (REF. 41; FIG. 3). Oceanic CO-formation estimates are derived from applying wavelength-resolved photochemical efficiency measurements of CO production to the intricate light field of ocean waters. Together with concurrent measures of bacterial CO consumption rates, these lower estimates of the CO source in seawater made it clear that bacterial consumption is the dominant sink for global CO cycles, recycling approximately 80–90% of the CO produced before it can be exchanged with the atmosphere.

Oligotrophic
An aquatic environment that has low levels of nutrients and algal photosynthetic production (for example, high mountain lakes or the open ocean).

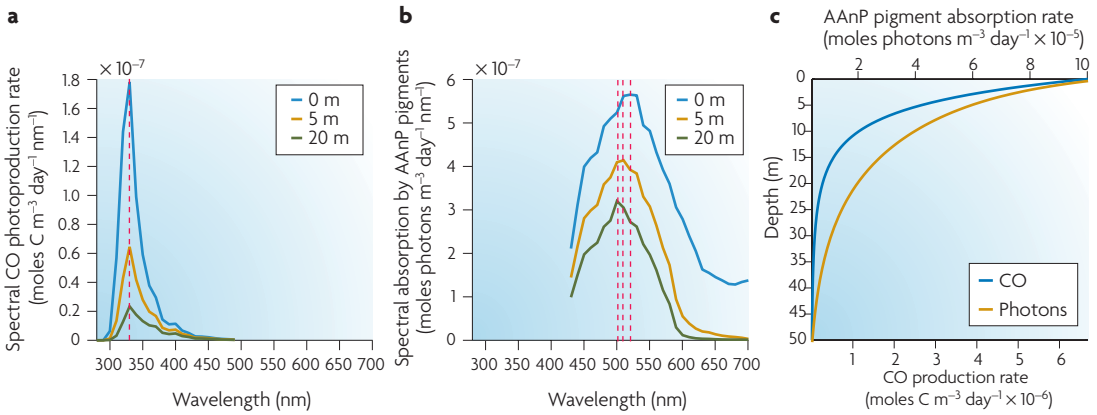


Figure 2 | **Light-dependent processes at different ocean depths.** Wavelength and depth dependence of CO formation (panels **a** and **c**) and aerobic anoxygenic phototroph (AAnP)-based phototrophy (panels **b** and **c**) in coastal water at Global Ocean Survey station 14 (REF. 41) modelled for July. Panels **a** and **b** show that the rates and dominant wavelengths for these light-driven processes vary with depth (shown for 0, 5 and 20 m), depending on the number of incident photons at each wavelength and the degree to which each wavelength can penetrate. The differences in the wavelength of peak production between CO and AAnP pigments (shown as red dotted lines) are due to the properties of the chromophores that are involved in each process. Panel **c** shows estimates of the total CO formation and AAnP-based phototrophy at this site as a function of depth.

Bacteriochlorophyll in seawater

Aerobic bacteria that photosynthesize without producing oxygen — the aerobic anoxygenic phototrophs (AAnPs) — were discovered several decades ago[22]. However, until recently AAnPs were considered to be ecological oddities from specialized habitats. In 2000, a discovery was made that surprised the whole oceanography community — the primary pigment that is associated with anoxygenic phototrophy, bacteriochlorophyll *a* (Bchl*a*), was found to be ubiquitously distributed in ocean surface waters[23]. The bacterioplankton taxa that are responsible for synthesizing the Bchl*a* and carrying out this previously unrecognized ocean process were at first completely unknown.

Early culturing attempts indicated that the marine AAnPs might be Bchl*a*-containing members of the genus *Erythrobacter*[23], but the low representation of this taxon in 16S ribosomal RNA (rRNA)-based marine bacterioplankton surveys led to doubts that they were truly important ecologically. The first glimpses of the identity of the AAnPs were revealed by metagenomic approaches, which uncovered the genes that are responsible for the synthesis of Bchl*a* and the associated photosynthetic reaction centres in the 40-kb segments of DNA that were retrieved directly from uncultured ocean bacterioplankton[24]. Genome fragments from a range of bacterial taxa were found to contain AAnP genes, including members of the marine Roseobacter group (this was unsurprisingly as members of the Roseobacter group were among the first AAnPs described[22]) as well as quite distant beta- and gammaproteobacteria groups for which cultured representatives were not available[24]. Subsequent genomic and metagenomic efforts have greatly increased our knowledge of the bacteria that are capable of carrying out anoxygenic phototrophy in the ocean. For example, the AAnPs in the gammaproteobacteria, which were first recognized by Béjà and colleagues[24] through metagenomic sequencing, now have a cultured representative[25]. Evidence for many additional (and mostly uncultured) taxa that encode AAnP genes has also been found based on a metagenomic survey of Atlantic and Pacific Ocean surface waters[26]. Thus, many major oceanic bacterioplankton taxa have representatives with AAnP genes, and the ~50 genes that are required for this process seem to have been horizontally transferred with ease among the major marine groups[25].

Apart from the excitement of unearthing a previously unknown process in the ocean, the discovery of aerobic anoxygenic photosynthesis has implications for understanding how carbon is processed in the ocean. Importantly, and unlike classical photosynthesis, the light energy that is harvested by AAnPs is not thought to fuel carbon fixation in autotrophic cells[27,28]. Hence, calling this process photosynthesis might be technically incorrect[29] and instead this metabolic strategy is termed photoheterotrophy, which means that sunlight can provide energy to organisms that nonetheless require organic matter as a source of carbon (FIG. 1).

The global significance of this process ultimately depends on how many marine bacterial cells obtain energy by this mechanism, and what percentage of their energy needs can be met. Indeed, direct microscopic counts of Bchl*a*-containing cells indicate that AAnPs account for only 1–10% of the chlorophyll *a* (Chl*a*)-based plankton counts in ocean surface waters[28,30,31]. However, in oligotrophic environments populations might be considerably larger and comprise a bigger fraction of the Chl*a*-based plankton counts[32]. Ratios of Bchl*a* to Chl*a* in the various marine surface waters suggest that the energy that is generated by AAnPs forms less than 1% of the total light-driven electron flux that is derived from standard photosynthesis[33] (FIG. 2).

A direct comparison of the energy capture by the two processes does not account for the fact that AAnP-derived energy is directly used by heterotrophic cells,

Phototroph
An organism that derives energy from sunlight.

Autotrophic
An organism that synthesizes organic carbon from the fixation of inorganic carbon, for example, by photo- or chemosynthesis.

thereby bypassing a trophic level and the associated energy losses in the microbial food web. For example, a classical bacterial heterotroph might route only 30% of the available organic carbon substrate into macromolecule biosynthesis and cell growth, because 70% is respired to meet cellular energy needs. By contrast, an AAnP that fulfils one-fifth of its cellular energy requirement from light-driven ATP synthesis[23] could increase its cell biomass by 15% using the same amount of organic carbon, by routing 35% of the organic carbon to biosynthesis. Therefore, phototrophic electron flux by aerobic anoxygenic phototrophy might be more appropriately viewed as a supplement to heterotrophy rather than as a competing photosynthetic process. Many questions must be answered before a full understanding of the biogeochemical relevance of aerobic anoxygenic phototrophy in the marine carbon cycle can be assessed. For example, we still have little information about the factors that regulate the production of Bchl*a* and the related light-harvesting proteins by AAnP cells.

Unexpectedly, light has a negative effect on pigment formation in some AAnPs, for example, some *Erythrobacter* strains[34], whereas light has a positive effect on other AAnPs, including gammaproteobacteria OM60 strains[25]. Oxygen generally stimulates the formation of phototrophic pigments during growth in the laboratory[35], which is not the case for the anaerobic relatives of AAnPs. A decrease in the availability of organic carbon also stimulates photopigment formation[36], which might indicate that AAnPs can better survive when substrates are scarce. The identity of the reduced compounds that are used by oceanic AAnPs as electron donors is not clear, but their anaerobic relatives typically use reduced sulphur compounds[37], which are scarce in ocean surface waters. Exuded reductants from active oxygenic phototrophs might serve as electron donors, which could partially explain the observed correlations between Bchl*a* and Chl*a* in ocean waters[23].

Proteorhodopsin

The detection of a genome fragment from an uncultured bacterioplankton cell from Monterey Bay, California, led to the unexpected discovery of a new mechanism for light-driven energy generation in the ocean[38]. This DNA fragment, which was derived from a member of the uncultured SAR86 lineage, contained a gene that encoded a rhodopsin-type protein. On absorption of a photon of light, this protein pumps protons to the outside of the bacterial membrane, and the resulting membrane potential is used by the cell to generate ATP[38]. Subsequent studies established that proteorhodopsin proteins are spectrally tuned to absorb the most dominant wavelength of light in coastal versus open ocean or deep waters (green versus blue), simply by a substitution at a single amino-acid position that functions as a spectral tuning switch[39,40].

The ATP-generating function of rhodopsins was already well known when the oceanic proteins were discovered, but these photoproteins were thought to be confined to highly salt-tolerant Archaea from unusual environments. It was inconceivable at the time that rhodopsin-based energy generation could be a mainstream process for energy acquisition in marine surface waters. Now we know that proteorhodopsin genes are extremely abundant in the genomes of ocean bacterioplankton, with almost 4,000 sequences containing these genes recovered from the Global Ocean Survey metagenome[41]. Additionally, proteorhodopsins are more broadly distributed taxonomically than the

Box 2 | Modelling ecosystem-level effects of lithoheterotrophy and phototrophy in the coastal ocean

Our model used SeaWiFS (sea-viewing wide field-of-view sensor) monthly binned ocean-colour data that was recorded continuously over a 9-year period (1997–2006; OceanColor Web, see Further information) to estimate light attenuation and chromophoric dissolved organic matter (CDOM) absorbance and the STAR (system for transfer of atmospheric radiation) model[56] to estimate the downwelling spectral scalar irradiance (in moles of photons m^{-2} s^{-1} nm^{-1}) just below the sea surface for the Middle and South Atlantic Bights (17.5°N–41.5°N latitude, 68°W–82°W longitude)[57]. Diffuse attenuation coefficients and CDOM absorption coefficients over the 280–490 nm spectral range were used to model the depth-resolved rates of photon absorption by CDOM in the mixed layer (typically 10–60 m in depth, but varying with season and latitude). The average of two quantum yields for CO photoproduction[16,55] was then used to estimate the efficiency of CO production per absorbed photon. We assumed that 86% of the CO that is produced daily in the mixed layer by photochemistry was consumed by bacteria[16] and we also assumed a stoichiometry for moles of CO oxidized:moles of ATP produced of 1:1 (REF. 11).

The bacteriochlorophyll *a* (Bchl*a*) concentration was estimated from the chlorophyll *a* (Chl*a*) concentration according to Goericke[33]. Downwelling scalar irradiance just below the surface, diffuse attenuation coefficients and the absorption coefficients of the phototrophic bacterium *Thiocapsa roseopersicina*[58] (including absorption by both the Bchl*a* and accessory pigments) in the 400–700 nm range (photosynthetically active radiation) were used to quantify the rate of photons absorbed. We assumed a stoichiometry for moles of photons absorbed:moles of ATP produced of 0.67:1. Photoinhibition by ultraviolet radiation was ignored, as was the absorption of infrared photons by the phototrophic bacteria (Bchl*a* has absorption peaks at over 800 nm); these should be minor for an areal measurement.

Areal bacterial production (BP) rates for the mixed layer were estimated from the modelled net primary productivity (NPP)[59,60], based on SeaWiFS monthly binned ocean-colour data, using the areal relationship between BP and NPP in marine systems provided by Cole and colleagues[51] and assuming that the BP is equally distributed through the water column. Bacterial respiration (BR) rates were assumed to equal 70% of the total bacterial carbon demand (BP + BR) for strict heterotrophic metabolism, and respiration was assumed to yield 38 moles of ATP per 6 moles of carbon oxidized (a degree of reduction that is equivalent to hexose).

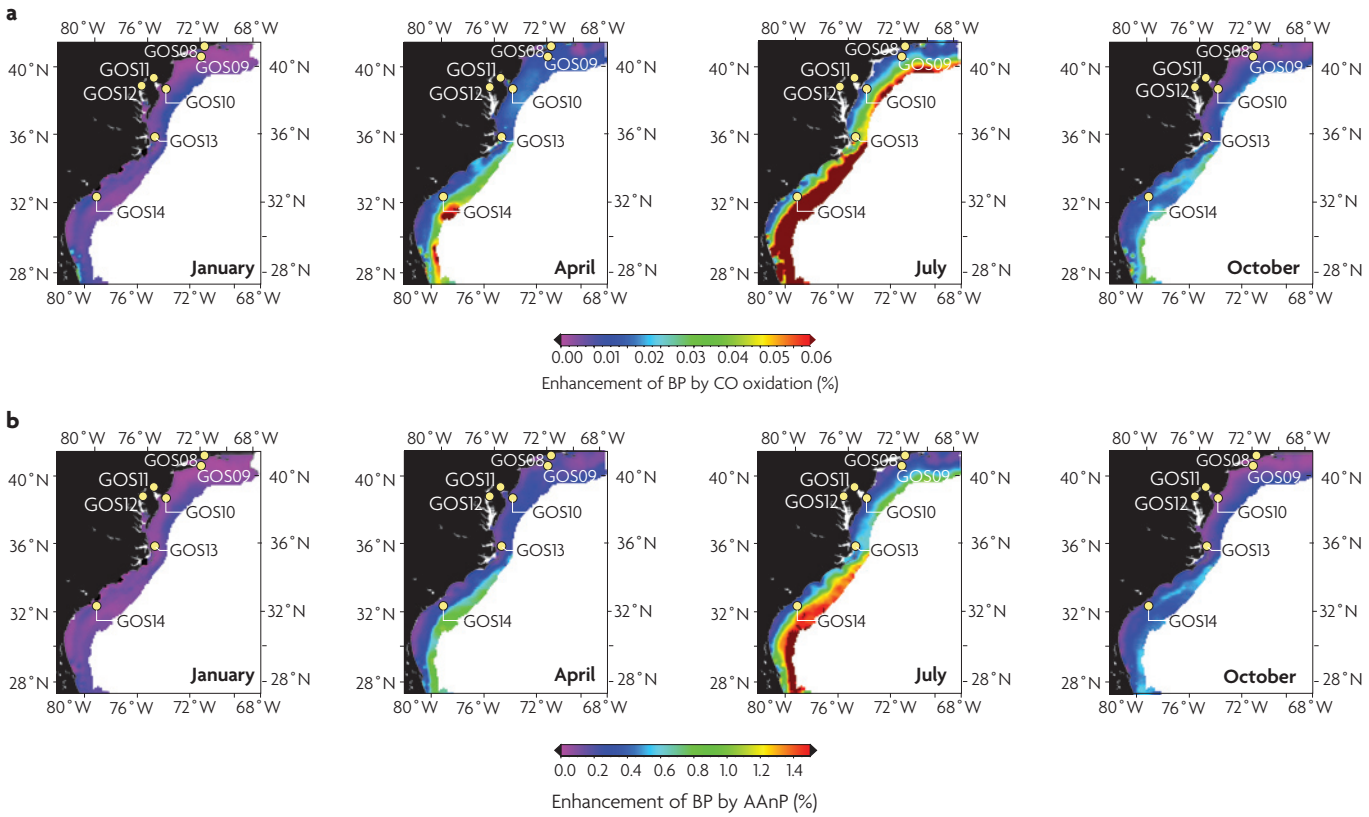


Figure 3 | **Light-supplemented bacterioplankton productivity in coastal communities.** The estimated percentage enhancement of mixed layer-integrated coastal bacterioplankton production for 4 selected months based on the energy that is derived from CO oxidation (**a**) and aerobic anoxygenic phototrophy (**b**) relative to strict organoheterotrophy. The modelled region is the Mid- and South-Atlantic Bights to the 500 m isobath. The seven locations where metagenomic data were collected as part of the Global Ocean Survey[41] are indicated with yellow dots. BP, bacterial production rate; AAnP, aerobic anoxygenic phototrophy.

'proteo' in their name would imply, as taxa from both within and outside the Proteobacteria have now been found to contain proteorhodopsin orthologues. These include the gammaproteobacteria (SAR86 and at least one other unidentified gammaproteobacteria group[42]), the alphaproteobacteria (SAR11[43]), the Bacteroidetes[44,45] and, most probably by the acquisition of the gene through horizontal gene transfer, the marine Euryarchaeota[46]. The recently sequenced genome of one member of a difficult-to-culture Roseobacter lineage (alphaproteobacterium HTCC2255) now adds the Roseobacter group to the list of marine alphaproteobacteria taxa that contain proteorhodopsin genes.

None of the cultured proteorhodopsin-containing bacteria has recognized pathways for carbon fixation[43,45]. Therefore, the beneficiaries of proteorhodopsin-driven ATP acquisition seem to be bacterioplankton that require organic carbon for biosynthetic pathways, making this another photoheterotrophic metabolic strategy (FIG. 2). The increased growth yields of proteorhodopsin-containing cells compared with the growth yields of strict organoheterotrophs has been experimentally challenging to demonstrate[47], but was recently achieved for the Bacteroidetes *Dokdonia* strain MED134 (REF. 45).

Resourceful heterotrophs

All of these newly discovered microbial processes in the marine carbon cycle have one thing in common: they are mechanisms by which heterotrophs can obtain energy from something other than organic carbon (either from inorganic compounds or sunlight) and thereby depart slightly from a strictly heterotrophic lifestyle. The logical assumption would be that these alternatives to strict organoheterotrophy, in which organic matter supplies both energy and carbon, are beneficial[3], considering the ecological costs of maintaining the required genes and synthesizing the necessary proteins. For example, CO oxidation requires at least three genes to encode a large dimeric CO dehydrogenase, together with several accessory proteins[4,14]. Aerobic anoxygenic phototrophy requires approximately 50 genes to encode the synthesis of the light-harvesting complex, the reaction centre, Bchl*a* and carotenoids[48].

Given that an individual cell probably benefits from these processes, it is less clear what effects these metabolic processes might have on the carbon budget of coastal ecosystems. We used a modelling approach to estimate the impact of litho- and photo-heterotrophy on bacterial production and CO_2 formation in coastal waters of the eastern United States. Monthly average estimates (BOX 2)

Table 1 | **Frequency of homologues to bacterial genes important in carbon cycling**

Site	Location	Depth (m)	Aerobic anoxygenic phototrophy	CO oxidation	Proteorhodopsin	*recA*
GOS8	Newport Harbour, Rhode Island	1	4 (7)	10 (19)	39 (72)	54 (100)
GOS9	Block Island, New York	1	1 (3)	4 (12)	20 (61)	33 (100)
GOS10	Cape May, New Jersey	1	1 (3)	4 (13)	23 (77)	30 (100)
GOS11	Delaware Bay, New Jersey	1	2 (8)	10 (40)	20 (80)	25 (100)
GOS12	Chesapeake Bay, Maryland	13.2	1 (2)	7 (13)	19 (35)	55 (100)
GOS13	Off Nags Head, North Carolina	2.1	1 (3)	10 (27)	29 (78)	37 (100)
GOS14	South of Charleston, South Carolina	1	1 (1)	10 (12)	47 (58)	81 (100)

The table shows the frequency of homologues (actual number of homologues in the dataset) to genes that encode CO oxidation-based lithoheterotrophy (*coxL*), aerobic anoxygenic phototrophy (average of *pufL*, *pufM* and *bchX* as suggested by Yutin and colleagues[26]) and proteorhodopsin-based proton pumping (a proteorhodopin gene) at seven coastal stations in the United States sampled in the Global Ocean Survey (REF. 41; FIG. 3). The frequency of homologues of *recA* (a single-copy essential gene) is provided as a comparison; all bacterioplankton genomes are expected to contain one copy of *recA*. Sampling biases that might arise owing to the differences in gene size have been corrected by dividing the number of homologues by the ratio of the length of the selected gene to the length of *recA* (1,059 nucleotides). Values in parentheses represent estimates of the percentage of bacterial cells that have a homologue assuming that all of the genes are present in single copies, calculated as: (homologue frequency × 100)/*recA* frequency). GenBank accession numbers for query translated sequences: CoxL, AAV95654 and AAV94,806; proteorhodopsin, Q9F7P4 and EAQ40925; PufL, AAU00045; PufM, ABN14037; BchX, AAF24297; and RecA, P0A7G6. Homologues were identified as those with E (expect) values lower than 10^{-40} and the correct functions of closest hits in BLAST (Basic Local Alignment Search Tool) analyses of sequences against all the sequences in GenBank.

were made of the daily depth-integrated bacterial production for water depths of up to 500 m in coastal waters of the eastern United States (FIG. 3). The estimated bacterial production if energy is derived only from the oxidation of organic carbon (organoheterotrophy) was compared with the estimated bacterial production if CO oxidation (lithoheterotrophy) or aerobic anoxygenic phototrophy (photoheterotrophy) also provided energy. Currently, there is insufficient quantitative information available to include proteorhodopsin-based energy generation in the model.

The model indicates that only a small fraction of the total bacterial production in the study area could be supported by CO oxidation (a maximum of only 0.2% in July and considerably less in colder months; FIG. 3). Even if our predicted rates of CO formation are too low by tenfold, which is unlikely[16], the model still predicts that the energy that is available from CO oxidation is minimal. From an ecosystem perspective, this result suggests that bacterioplankton CO oxidation probably has a more important role as an oceanic sink for this greenhouse-relevant gas than as a mechanism for reducing CO_2 release from bacterial respiration. From a cellular perspective, the relative benefits to an individual cell would be increased because CO oxidation is not carried out by all cells in the community. For example, approximately 20% of bacterioplankton were estimated to be CO oxidizers for seven of the coastal sites in the United States that were included in the Global Ocean Survey[41] (TABLE 1). Nonetheless, the estimated gain for individual cells is still a minor fraction of their energy requirement. This paradox between the low supply rate of CO yet relatively high frequencies of bacterial CO oxidizers in the coastal ocean is an excellent example of how genomic and biogeochemical data can together enhance and challenge traditional views of carbon-cycle processes. An understanding of the depth-dependent shifts in bacterial activities and genes[49], or a better annotation of the large and complex collection of CO oxidation-like genes in bacterial genome sequencing projects[4], might help to resolve this discrepancy.

The system-level bacterial production that could be supported by deriving energy from aerobic anoxygenic phototrophy is approximately tenfold higher than from CO, with an estimated increase in bacterial production of up to 2% in the summer months (FIG. 3). This estimate is suggestive of the importance of Bchl*a*-based phototrophy, is consistent with previous assessments[33] and better reflects the estimated frequency of cells that contain AAnP genes in coastal oceans (2–8%; REF. 26; TABLE 1) between which the benefit would be spread.

Modelling can help us to understand coastal carbon-cycle processes. However, these studies are in their infancy. For example, although our model assumes that CO_2 fixation is not fuelled by litho- and phototrophic processes, owing to the absence of typical autotrophic pathways in cultured organisms that have these metabolisms, it is possible that alternate mechanisms of CO_2 fixation are operating. One possibility is enhanced anaplerotic CO_2 fixation, a mechanism that typically functions at low levels in prokaryotic and eukaryotic cells for the purpose of replenishing citric acid-cycle intermediates that are needed for biosynthesis (FIG. 4). It has been proposed

Anaplerotic mechanism
A cellular reaction that replaces intermediates of the citric acid cycle that have been siphoned off into biosynthetic pathways.

that members of the marine Roseobacter lineage that oxidize CO fix CO_2 by anaplerotic mechanisms[18]. This was based on measurements that revealed the high activity of the enzyme pyruvate carboxylase[50]. Enhanced anaplerotic CO_2 fixation has also been suggested for marine AAnPs[23], owing to the presence of a highly active phosphoenolpyruvate carboxylase[25,48] (FIG. 4). If CO_2 fixation does indeed occur, then these processes represent true autotrophy, rather than simply growth yield increases by heterotrophs living on limited concentrations of organic carbon.

Efforts to quantify microbial respiration and ocean–atmosphere CO_2 exchange often assume a close coupling between Chl*a*-based photosynthesis and heterotrophic bacterial production[51] or respiration[52]. Inherent in this assumption is the notion that primary production sets a limit on bacterioplankton activity. Indeed, this is assumed in our model to estimate bacterial production rates in coastal waters (BOX 2). Resourceful heterotrophs can affect this relationship and therefore affect our ability to predict and correctly balance organic carbon production rates versus oxidation rates on regional and global scales, and to estimate the export of organic matter to shelf sediments and the deep ocean. For lithoheterotrophic CO oxidation, the fact that the CO that is oxidized by bacterioplankton is formed from photosynthesis-derived organic compounds indicates that this relationship is not altered. However, for the photoheterotrophic metabolisms, the use of light as an independent source of energy partially decouples Chl*a*-based primary production rates from bacterial production and respiration. True autotrophy, if it exists, would also result in a more complete decoupling that, depending on its quantitative importance (FIG. 3), might be significant for ecosystem-level carbon budgets.

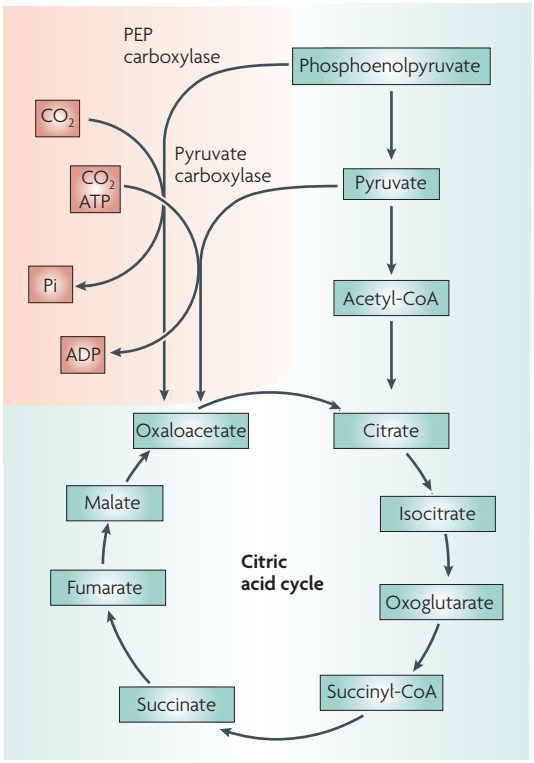


Figure 4 | **Anaplerotic pathways for CO_2 fixation.** The pathways of CO_2 fixation that are anaplerotic in bacteria and archaea are shown by red shading. These pathways typically operate at low levels to replace citric acid-cycle intermediates, but have been proposed to be more active in marine planktonic heterotrophs that supplement their ATP pool through litho- or photoheterotrophy. PEP carboxylase, phosphoenolpyruvate carboxylase.

Outlook

These newly recognized metabolic mechanisms for sunlight-linked bacterial energy acquisition are likely to affect the amount of organic matter that is retained in the ocean food web, stored in marine organic carbon reservoirs and released as CO or CO_2 from ocean surface waters[53]. However, all of these metabolisms are constrained by the penetration of solar radiation into coastal ocean surface waters (FIG. 1), which will be variable on spatial and temporal scales in this dynamic environment. In order to gain a comprehensive understanding of the carbon cycle, as well as the role of coastal systems in shaping the Earth's climate, a more in-depth understanding of all of these processes is required.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Genome: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome
alphaproteobacterium HTCC2255 | *Silicibacter pomeroyi*
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
MED134
Entrez Protein: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=protein
BchX | CoxL | proteorhodopsin | PufL | PufM | RecA

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