



REVIEW

Contributions of anoxygenic and oxygenic phototrophy and chemolithotrophy to carbon and oxygen fluxes in aquatic environments

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ABSTRACT: Estimates of aquatic primary productivity at local, regional or global scales commonly concentrate on oxygenic photolithotrophy. The analysis presented here briefly considers the occurrence and metabolism of other autotrophs *sensu lato*, i.e. not just the organisms with an autotrophic inorganic carbon assimilation machinery. These other autotrophs include chemolithotrophs and anoxygenic photolithotrophs, of which some use the photosynthetic carbon reduction cycle as do oxygenic photolithotrophs, while others use one of 4 other pathways. The category of other autotrophs also includes organisms that possess photochemical energy transduction machinery but lack autotrophic carbon assimilation; light stimulates the growth rates of these autotrophs and/or increases the fraction of the organic carbon substrate used that is converted into cell material when using light energy. Organisms lacking autotrophic inorganic carbon assimilation influence food webs by increasing the rate, or efficiency, of conversion of dissolved organic carbon ultimately derived from autotrophic inorganic carbon assimilation into particulate organic carbon. Chemolithotrophs and anoxygenic autotrophs today depend on the activities of oxygenic chemolithotrophs for one or more of their growth substrates, and thus contribute to global net primary productivity but not to global gross primary productivity. Global net aquatic primary productivity by oxygenic photolithotrophs is at least 50 Pg C yr⁻¹, while chemolithotrophs and anoxygenic photolithotrophs together contribute about 0.40 Pg C yr⁻¹. Before the occurrence of oxygenic photolithotrophs in the Archaean, chemolithotrophs and anoxygenic photolithotrophs had a net primary productivity of about 3.4 Pg C yr⁻¹, which is higher than the present values largely because anoxygenic photolithotrophs were able to inhabit the euphotic zone worldwide.

KEY WORDS: Archaean · 3-hydroxypropionate pathway · Inorganic carbon concentrating mechanisms · Photosynthetic carbon reduction cycle · Reductive tricarboxylic acid cycle · Resource use · Ribulose biphosphate carboxylase-oxygenase

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INTRODUCTION

Marine net primary productivity, estimated from measurements of the conversion of inorganic to organic carbon that are as direct as possible, has been estimated at about 50 Pg C yr⁻¹, with almost all of this conversion carried out by oxygenic phytoplankton organisms (Field et al. 1998). The corresponding value for terrestrial systems is about 55 Pg C yr⁻¹ (Field et al. 1998), with a contribution from inland waters that is

globally very small but locally highly significant (Raven & Maberly 2005). Estimates of gross aquatic (almost all marine) primary productivity based on respiration rates, assuming that organic carbon inputs into rivers each year are equal to marine sedimentation of organic carbon, are about 170 to 200 Pg C yr⁻¹ (del Giorgio & Williams 2005). For the open ocean, where the most direct comparisons are possible, and allowing for possible underestimates of net primary production and the need to convert this to gross primary produc-

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tion for comparison with respiration, the range (100 to 130 Pg C yr⁻¹) overlaps with that of open ocean respiration (110 to 160 Pg C yr⁻¹). Regardless of which estimates are closer to the actual value, aquatic primary productivity is clearly a major biogeochemical process. The diversity of mechanisms that underlie the inorganic carbon acquisition and assimilation process, and their responses to environmental factors, are of great interest with regard to assessing the limitations for their relative contributions to global primary productivity, both today and on the early anoxic Earth.

One important finding on aquatic primary productivity is that a much greater diversity of organisms, at higher taxonomic levels, are involved in this process than is the case on land (Chapter 1 of Falkowski & Raven 2007). This diversity of organisms is paralleled by a greater diversity of mechanisms (summarized in Tables 1 & 2) through which inorganic carbon assimilation is energized, and by which inorganic carbon is converted into organic carbon, in aquatic compared with terrestrial autotrophs. The aim of this review is to summarise the present state of knowledge of the phylogenetics, spatial distribution and global significance of the various mechanisms of primary productivity,

with particular emphasis on how genomics and metagenomics have contributed to our understanding of these mechanisms and how such studies might contribute in the near future (see also Gasol et al. 2008, Höfle et al. 2008).

In synthesising the available information, a wide definition of autotrophs is used here. In addition to well-established oxygenic and anoxygenic photolithotrophs and chemolithotrophs, organisms capable of photochemical energy transformation but with no clear evidence of the capacity for autotrophic inorganic carbon assimilation are also considered (Falkowski & Raven 2007, Francis et al. 2007, Moran & Miller 2007). As will be seen when considering the significance of chemolithotrophs and anoxygenic photolithotrophs in global primary productivity, some of the organisms in these trophic categories make poorly constrained and probably very small contributions to global energy flows and elemental cycles in aquatic environments, although they are of interest in relation to local food webs and to biodiversity. An underlying theme of this review is the dominance of oxygenic photolithotrophs, and the involvement of their activities in restricting or facilitating the activities of the other groups of

Table 1. Energy (NADPH or reduced ferredoxin, ATP) costs and affinity for CO₂ [reciprocal of the K_{1/2}(CO₂)] of the 5 known autotrophic pathways of reductive CO₂ assimilation. RTCAC: reductive tricarboxylic acid cycle; PCRC: photosynthetic carbon reduction cycle; PCOC: photorespiratory carbon oxidation cycle

Pathway	NADPH/CO ₂	ATP/CO ₂	K _{1/2} (CO ₂) mol m ⁻³	Source
Total synthesis of acetate	2	1 ^a	-40 ^b	Rusching et al. (1976), Müller (2003)
RTCAC	2	1.67	~1.3 ^c , 2 ^d	Furdui & Ragsdale (2000), Kanao et al. (2002), Lebedeva et al. (2002), Raven et al. (2008a)
3-hydroxy-propionate	2	2	0.01 ^e	Hügler et al. (2003), Raven et al. (2008a)
3-hydroxy-propionate/ 4-hydroxy-butyrates	2	3	0.01 ^e	Furdui & Ragsdale (2000), Hügler et al. (2003), Berg et al. (2007), Raven et al. (2008a)
Dicarboxylate/ 4-hydroxy-butyrates	2	2.67	2 ^d	Furdui & Ragsdale (2000), Huber et al. (2008)
PCRC	2	3	0.05 – 0.3 ^f	Tcherkez et al. (2006), Raven et al. (2008a)

^aAssuming no ATP synthesis in the conversion of CO₂ + H₂ to acetate (see Müller 2003); ATP required in gluconeogenic pathway to produce free sugars

^bBased on rate of reverse reaction of formate dehydrogenase in the CO₂ range 0 to 14 mol m⁻³. Approximately 40 mol m⁻³ is the concentration in solution in equilibrium with 100 kPa CO₂ in the gas phase at the ionic strength of cytosol at 15°C

^cFor the enzyme with the lowest known affinity for CO₂ in the RTAC (Kanao et al. 2002, Lebedeva et al. 2002; the latter computed from the cited value for HCO₃⁻ assuming a pK_a' of 6.1 at the ionic strength of the assay medium and an assumed temperature of 25°C and the assay pH of 6.5)

^dThe pyruvate synthase needed to convert the acetyl CoA product of the cycles into 3C compounds for biosynthesis has a K_{1/2} for CO₂ of 2 mol m⁻³ in *Clostridium*

^eThe carboxylases in these 2 pathways (acetyl CoA carboxylase and propionyl CoA carboxylase) have HCO₃⁻ as the inorganic carbon substrate; the equivalent CO₂ concentration was calculated from the pK_a and pH values at which the enzyme is thought to function in the cytosol, probably 0.7 units higher than the assay pH of 6.5. This calculation assumes that the K_{1/2} for HCO₃⁻ is independent of pH

^fNADPH and ATP requirements assume saturation of the carboxylase function of Rubisco with CO₂, completely suppressing the oxygenase activity. Additional ATP is used if this high intracellular CO₂ concentration is attained by a CCM rather than a very high external CO₂ with diffusive CO₂ entry. With lower intracellular CO₂ concentrations and in the presence of O₂, additional NADP and ATP are needed for the net fixation of CO₂ to allow for the cofactors required in the synthesis of 2-phosphoglycolate with subsequent excretion of glycolate and/or operation of the PCOC or its equivalent to convert glycolate into triose

Table 2. Summary of the aquatic phototrophic and chemolithotrophic micro-organisms and some of their metabolic characteristics. See text for details and references

Metabolism	Representative organisms	Electron donor	Electron acceptor	Photo-chemistry	Autotrophic CO ₂ fixation pathway
Chemolithotrophs					
Anammox	<i>Bacteria</i>	NH ₄ ⁺	NO ₂ ⁻ , CO ₂	None	Acetyl CoA pathway
Fe ²⁺ oxidizers, nitrifiers, S ²⁻ oxidisers	<i>Archaea, Bacteria</i>	Fe ²⁺ , NH ₄ ⁺ , NO ₂ ⁻ , S ²⁻	O ₂ , CO ₂	None obligatory	Rubisco/PCRC, 3-HO-propionate, 3-HO-propionate/4-HO-butyrate, dicarboxylate/4-HO-butyrate
Non-photolithotrophic anoxygenic phototrophs					
Bacterio-rhodopsin-like pigments	<i>Archaea, Proteobacteria</i>	Organic	Organic	Actino-, bacterio-, halo-, proteo-rhodopsin	None
Anoxic, anoxygenic, bacterio-chlorophyll	<i>Heliobacteria</i>	Organic	Organic	Bacterio-chlororophyll, PSI-like	None
Aerobic, anoxygenic bacterio-chlorophyll	<i>Proteobacteria</i> The acidobacterium 'Candidatus Chloracidobacterium thermophilum'	Organic	Organic	Bacterio-chlorophyll, PSII-like	None
Aerobic anoxygenic chlorophyll	The cyanobacterium UCYN-A	Organic	Organic	Chlorophyll, PSI	None
Photolithotrophic anoxygenic phototrophs					
Direct photo-chemical reduction of NAD ⁺	<i>Chlorobi</i> (obligate anaerobes)	S ²⁻ , Fe ²⁺	CO ₂	Bacterio-chlorophyll, PSI-like	RTCAC
Indirect photo-reduction of NAD ⁺ , only phototrophic under anoxia	<i>Chloroflexi</i> and <i>Proteobacteria</i> (purple sulfur and non-sulfur bacteria)	Organic, S ²⁻	CO ₂	Bacteriochlorophyll, PSII-like	3-HO-propionate
		Organic, S ²⁻	CO ₂	Bacteriochlorophyll, PSII-like	Rubisco IA,IC,II/PCRC
Oxygenic photolithotrophs	<i>Cyanobacteria</i> (except UCYN-A) Algae (except) mixotrophic and obligately chemo-organotrophic species	H ₂ O	CO ₂	Chlorophyll, PSI + PSII	Rubisco IA,IB/PCRC
		H ₂ O	CO ₂	Chlorophyll, PSI + PSII	Rubisco IB,ID,II/PCRC

autotrophs sensu lato. In view of the importance of oxygenic photolithotrophs in most extant aquatic environments, this paper starts with a brief summary of these organisms and their roles in the ecology of other autotrophs, even though representatives of the other categories of autotrophs preceded oxygenic photolithotrophs in the sequence of metabolic types on Earth.

METABOLIC STRATEGIES OF AQUATIC MICROBES

Oxygenic photolithotrophs

The group of oxygenic photolithotrophs includes the cyanobacteria and photolithotrophic eukaryotes. Their photochemistry involves chlorophyll-based Photosystems I and II (PSI and PSII) acting in series (Falkowski & Raven 2007; Table 2). PSI and PSII and the bacteriochlorophyll-based photosystems in anoxygenic photo-

synthetic bacteria are all derived from a common ancestral photosystem (Raymond et al. 2003, Sadekar et al. 2006).

While oxygenic photolithotrophs typically occur in oxygenated environments, anoxygenic autotrophic photosynthetic bacteria can only grow photolithotrophically under anoxic or very hypoxic conditions (considered below). Conversely, oxygenic photolithotrophs typically grow in inorganic carbon concentrations that are much closer to air equilibrium with respect to CO₂ than is the case for the anoxygenic autotrophic photosynthetic bacteria. This is because the anoxic habitats are generated by sedimentation of organic matter in an environment in which oxygen is consumed in respiration faster than it can be supplied from more oxygenated waters. This relative isolation causes a buildup of the CO₂ generated by the predominant chemo-organotrophic metabolism. Oxygenic photolithotrophs invariably use the photosynthetic carbon reduction cycle (PCRC) (Table 1), a pathway that

was discovered in green microalgae, and even those Rubiscos which have the highest affinity for CO₂, highest CO₂/O₂ selectivity and consequent lowest CO₂-saturated specific reaction rate (Tcherkez et al. 2006), are scarcely CO₂-saturated in the air-equilibrium CO₂ concentrations at the temperatures at which they live. In the water, then, slower diffusion of CO₂ and O₂ through water than through air implies lower CO₂/O₂ ratios at the Rubisco active site and, hence, lower carboxylation rates and higher oxygenation rates than in air for the same enzyme and cellular structure.

In all cyanobacteria and most aquatic photosynthetic eukaryotes, inorganic carbon concentrating mechanisms (CCMs) increase the CO₂ concentration around Rubisco during steady-state photosynthesis to concentrations higher than those in the bulk medium (Giordano et al. 2005, Raven et al. 2008a,b). Analysis of the gene sequence of Rubiscos shows that there are 4 main groups, denoted Forms I, II, III and IV. Of these, the main forms involved in carboxylation in nature are Form I, with 8 large (catalytic) and 8 small subunits, and Form II, with 2 large subunits (Badger & Bek 2008, Tabita et al. 2008). Form I Rubiscos are in turn subdivided into Forms IA, IB, IC and ID that, with Form II, have characteristic phylogenetic distributions and ranges of kinetic properties (Badger & Bek 2008). Most cyanobacteria, like glaucocystophyte and chlorachniophyte, chlorophyte and euglenophyte algae, as well as embryophytic plants, have Form IB Rubiscos. However, Form IA Rubisco, and fewer CCM components, exist in the open-ocean *Prochlorococcus* and *Synechococcus* strains (α -cyanobacteria) than in coastal and freshwater β -cyanobacteria with Form IB Rubiscos. The β -cyanobacteria represent the ancestral condition of inorganic carbon assimilation (Price et al. 2008). Form ID Rubiscos occur in red algae and the algae that gained photosynthesis from red algae by secondary endosymbiosis; most eukaryotic marine phytoplankton today have Form ID Rubiscos. The exception are dinoflagellates, which almost all have a Form II Rubisco. Nevertheless, even the phytoplankton with Rubiscos that have kinetic properties most conducive to effective inorganic carbon assimilation using diffusive CO₂ entry have CCMs (Giordano et al. 2005, Raven et al. 2008a,b).

CCMs in cyanobacteria are all based on active transport of HCO₃⁻ or entry of CO₂ followed by energized conversion to HCO₃⁻ in the cytosol, followed by carbonic anhydrase-catalysed conversion of HCO₃⁻ to CO₂ in carboxysomes, which house all of the Rubisco (Price et al. 2008). The occurrence of Rubisco and the PCRC is a necessary, but not sufficient, condition for the occurrence of carboxysomes in other autotrophic bacteria. Carboxysomes occur in several chemolitho-

trophs, but very rarely in anoxygenic phototrophs, which use the PCRC (Codd & Marsden 1984, Cannon et al. 2001). The relation between the occurrence of carboxysomes, the kinetics of Rubisco, and the inorganic carbon availability in the environment of autotrophic *Bacteria* other than cyanobacteria, has not yet been adequately explored (Badger & Bek 2008).

Less is known about the CCMs of eukaryotic algae, but in most organisms they are thought to involve active entry of one or more inorganic carbon species, often with pyrenoids in the plastids as the analogues of carboxysomes. C₄-like inorganic carbon assimilation, presumably with a contribution of this pathway to the CCM, has been suggested in the diatom *Thalassiosira weissflogii* on the basis of enzyme localization and short-term labelling with inorganic ¹⁴C (Reinfelder et al. 2000). Subsequent labelling studies showed that the pattern in this algae was more like that of a C₃-C₄ intermediate flowering plant, with C₃ biochemistry following the action of a CCM in *T. pseudonana* (Roberts et al. 2007a,b). The complete genome sequence of *T. pseudonana* (Armbrust et al. 2004) shows the presence of the genes encoding the enzymes needed for C₄ metabolism. However, these genes are widespread among oxygenic eukaryotes and have a number of metabolic roles, and cannot be taken to indicate C₄ metabolism. Transcriptome studies are in general agreement with the labelling studies on *T. pseudonana* in not supporting the occurrence of C₄ metabolism (Roberts et al. 2007a,b, McGinn & Morel 2008); however, inhibitor studies have been used to support the presence of C₄ photosynthesis in the 2 *Thalassiosira* species and the diatom *Phaeodactylum tricornutum*. Kroth et al. (2008, see also Bowler et al. 2008) show from genomic studies that C₄ photosynthesis is unlikely to occur in the diatom *P. tricornutum* despite the occurrence of the appropriate genes in the completely sequenced genome, which is in agreement with Cassar & Laws (2007). These results show that genomic data must be used with caution in drawing conclusions about metabolic pathways when the genes involved are components of more than one pathway.

The CCMs are one sort of carbon pump; ecologically, there is an ecosystem-scale biological carbon pump resulting from sedimentation of particulate organic carbon from oxygenic primary production down the water column. In the context of the present paper, this is important as a factor in providing habitats for other kinds of autotrophs. When the oxygen requirement for the aerobic microbial oxidation of the non-refractory component of the organic carbon is greater than the rate at which oxygen is supplied advectively in solution, microbial metabolism gives hypoxic, then anoxic conditions, with the sequential use of nitrate (by means of ammonification, denitrification, anaerobic ammo-

mium oxidation or anammox), sulfate (by means of dissimilatory sulfate reduction) and carbon dioxide (by means of methanogenesis) as electron acceptors. As will be seen below in 'Chemolithotrophs', the anammox reaction supports chemolithotrophy, and methanogenesis from carbon dioxide is a reductive process akin to autotrophy, while the sulfide resulting from sulfate reduction provides the substrate for photolithotrophic sulfide oxidation in illuminated anoxic habitats and for sulfide-based chemolithotrophy at the oxycline. Chemolithotrophic sulfide oxidation involves oxygen, nitrate or nitrite as an electron acceptor, while the anammox reaction uses nitrite in oxidising ammonium. Biologically (i.e. in addition to lightning, Raven & Yin 1998), nitrate and nitrite result from nitrification in the water body; this depends on oxygen and also involves chemolithotrophic inorganic carbon assimilation. Dissolved organic carbon, ultimately from oxygenic photolithotrophy, supports the autotroph-like light-stimulated growth of aerobic anoxygenic photosynthetic *Bacteria*, as well as *Bacteria* and *Archaea* with rhodopsin-like energy-converting pigments. These too are discussed in the next section.

Another important influence of oxygenic photolithotrophs on anoxygenic photolithotrophs is shading. Stomp et al. (2007b) have reinvigorated the longstanding debate on 'complementary chromatic adaptation' of oxygenic photolithotrophs in relation to the predominant wavelengths in their natural underwater environment as a function of lake or ocean chemistry, and depth. Raven & Wolstencroft (2004), Kiang et al. (2007a,b) and Stomp et al. (2007a) also revisit the extent to which absorption of electromagnetic radiation by water and by oxygenic photolithotrophs affects the wavelengths available to anoxygenic photolithotrophs. This is dealt with in more detail in the next 2 sections when the various anoxygenic photosynthetic bacteria are considered.

Some properties of oxygenic photolithotrophs are outlined in Table 2.

Organisms with energy-transforming photochemistry but not capable of autotrophic CO₂ assimilation

Organisms considered under this heading are *Archaea* and *Bacteria* with rhodopsin-like pigment-protein complexes whose photochemistry results in the generation of an ion electrochemical potential difference across the plasma membrane but not in oxidised and reduced products (see Table 2). Also considered are those anoxygenic bacteria that grow chemo-organotrophically or photo-organotrophically and with, at most, a facultative and limited capacity for autotrophic inorganic carbon assimilation (see Table 2).

The occurrence of the rhodopsin-like photochemical energy conversion systems (bacteriorhodopsin and halorhodopsin) in *Archaea* from hypersaline environments has been known for over 3 decades (Oesterhelt & Stoeckenius 1973). Even before the advent of genomic techniques, the ecology, photochemistry and physiology of these organisms was well understood. The 2 rhodopsin-like pigments were expressed mainly under anoxic conditions, where the input of photochemical energy enabled the production of more biomass per unit organic substrate consumed than if fermentation was the only energy source for growth. The contribution of photochemical energy to organotrophic growth under aerobic conditions was minimal, and there was little evidence for autotrophic inorganic carbon assimilation under any condition (Danon & Caplan 1977, Danon 1983, Moran & Miller 2007).

Genomic studies were instrumental in showing that these rhodopsin-like photochemical energy transducers occurred in *Bacteria* (as proteorhodopsin in marine forms and as actinorhodopsins in freshwater and estuarine forms) and (as proteorhodopsins) in a range of *Archaea* (Ng et al. 2000, Bèjà et al. 2001, Baliga et al. 2004, Giovannoni et al. 2005, Mongodin et al. 2005, Atamna-Ismaeel et al. 2008, Sharma et al. 2008). There is evidence of genotypic difference in the absorption spectra of proteorhodopsins from marine environmental samples that may be related to the predominant wavelengths incident on the organisms in their natural environment (Man et al. 2003). Only sensory rhodopsins occur in oxygenic photolithotrophs (e.g. Merchant et al. 2007).

The global significance of the energy-transducing rhodopsins in energy flow and element cycling in aquatic ecosystems is unclear. References in the previous paragraph show the general absence from their genomes of mechanisms of autotrophic inorganic carbon assimilation. Such stimulation by light of chemo-organotrophic growth in principle can be attributed to the lower respiratory loss of organic substrate in growth and maintenance when there is the possibility of supplementing the respiratory or fermentative energy supply with photochemical energy.

A number of bacteriochlorophyll-containing *Bacteria* can grow chemo-organotrophically or photo-organotrophically, with no supplementation by autotrophic inorganic carbon fixation. *Heliobacteriaceae* with PSI-type reaction centres are strict anaerobes that can grow photo-organotrophically in the light and fermentatively in the dark (Gest & Blankenship 2004, Heinzel & Golbeck 2007, Sattley et al. 2008). Their inability to bring about autotrophic inorganic carbon assimilation is attributable to the absence of ATP-citrate lyase; the other reactions of the reductive tricar-

boxylic acid cycle (RTCAC) occur in these organisms (Pickett et al. 1994, Sattley et al. 2008). These organisms are found mainly in soil, and their occurrence in aquatic environments is apparently restricted to rice paddy soils and certain hot springs.

This restricted range of aquatic habitats occupied by *Heliobacteriaceae* contrasts with the habitat area available to the other category of non-autotrophic bacteriochlorophyll-containing organisms, i.e. the aerobic anoxygenic photosynthetic bacteria: they are found throughout the surface ocean and also occur in fresh water (Yurkov & Beatty 1998, Gest & Blankenship 2004, Moran & Miller 2007, Sattley et al. 2008). These organisms express their photosynthetic apparatus, with a Photosystem II (PSII)-like reaction centre, under aerobic conditions and carry out photochemistry and photo-organotrophy if there is sufficient high irradiation at the appropriate wavelengths. The light-harvesting pigments of these organisms are similar to those of the proteobacterial non-sulfur photosynthetic bacteria, even though they can live much closer to the surface than is true for most of their relatives that cannot carry out photosynthetic reactions under aerobic conditions. This means that the selective pressures on the wavelengths at which the photosynthetic apparatus absorbs (Kiang et al. 2007a,b, Stomp et al. 2007a,b) may be somewhat relaxed. The fully sequenced genomes of these organisms lack genes that would allow any of the known pathways of autotrophic inorganic carbon assimilation, which is in agreement with the absence of growth when inorganic carbon is the only carbon source (Fuchs et al. 2007, Moran & Miller 2007, Swingley et al. 2007). While these organisms do not seem, on the basis of genome studies, to be diazotrophs, some legumes have stem nodules with diazotrophic symbiotic rhizobia carrying out aerobic anoxygenic photosynthesis (Giraud & Fleischman 2004).

These organisms can grow chemo-organotrophically or photo-organotrophically. Bacteriochlorophyll synthesis occurs in the dark, and relatively low irradiances suffice to substantially inhibit this synthesis (reviewed by Biebl & Wagner-Döbler 2006). However, natural diel light–dark alternations are sufficient to maintain photochemically significant levels of photosynthetic apparatus in at least some strains (Yurkov & van Gemerden 1993, Kolber et al. 2001, Biebl & Wagner-Döbler 2006), and pigment synthesis can also be stimulated by low levels of organic substrates (Biebl & Wagner-Döbler 2006).

Also belonging in this ecological group, but not yet examined for the possibility of photolithotrophic growth, is a member of the *Acidobacteria*, '*Candidatus Chloracidobacterium thermophilum*' (Bryant et al. 2007). This organism, from phototrophic microbial mats in a hot spring, was characterised using an exem-

plary synergistic combination of metagenomic and classical microbiological enrichment culture techniques. These methods showed the occurrence of a PSII-like reaction centre and light stimulation of chemo-organotrophic growth (Bryant et al. 2007).

Further aerobic anoxygenic phototrophs are the globally distributed diazotrophic cyanobacteria, termed UCYN-A (Zehr et al. 2008). These uncultured organisms have been characterised using genomic techniques from environmental samples highly enriched in the diazotroph by cell sorting based on fluorescence, using quantitative fluorescence real-time PCR of a nitrogenase gene. The cells lacked the genes for PSII and for autotrophic inorganic carbon assimilation, and presumably bring about photo-organotrophic and/or chemo-organotrophic aerobic N₂ fixation (cf. Giraud & Fleischman 2004).

Light stimulation of chemo-organotrophic growth is, as in the organisms with energy-transducing rhodopsins, presumably a result of increased conversion of the dissolved organic matter consumed into cell material rather than CO₂. This is a result of the supplementation, and partial replacement, of light-independent bioenergetic processes with those driven by photochemistry (Kolber et al. 2001, Goericke 2002, Moran & Miller 2007). There is still uncertainty as to the effect of bacteriochlorophyll-based photo-organotrophy on the overall bacterial organotrophy in the surface ocean: a value on the order of a 1% enhancement of overall organotrophic bacterial growth (i.e. including bacteria that lack energy-transforming photochemistry) seems appropriate on the basis of present knowledge (Goericke 2002, Moran & Miller 2007). The increase for the bacteriochlorophyll-containing bacteria is, of course, larger, and could well be evolutionarily significant for them. How important this enhancement is for food web function awaits further research. Even in the hypothetical case of bacterial organotrophic growth in the surface ocean (units of bacterial C per unit area and time) consuming all the net primary productivity (in the same units) in the surface ocean (Hoppe et al. 2002), the overall light stimulation of bacterial chemo-organotrophic productivity is only 1% of net primary productivity. This is smaller than their contribution to overall primary photochemistry in the ocean as estimated from fast repetition rate (i.e. bacterio-) chlorophyll fluorescence measurement (Kolber et al. 2001). Even a rough estimate does not yet seem possible for the global contribution of organisms containing energy-transforming rhodopsins to biogeochemical cycles and energy flow (Moran & Miller 2007).

These organisms cannot be legitimately regarded as primary producers since, although they use light energy, all of their carbon other than the inorganic carbon used in anaplerotic pathways ultimately comes

from (almost always oxygenic) photolithotrophs. The question of whether these organisms could confound measurements of primary productivity in environmental samples is discussed later with a similar discussion of the possible influences of anoxygenic photolithotrophs and chemolithotrophs.

Anoxygenic photosynthetic organisms with autotrophic inorganic carbon assimilation

All anoxygenic photosynthetic *Bacteria* with the potential for autotrophic inorganic carbon assimilation can only photosynthesise under anoxic conditions (Table 2). Of these, the green sulfur bacteria (*Chlorobi*) are obligate anaerobes and obligate photolithotrophs, and they have PSI-like reaction centres (Frigaard et al. 2003, Bryant & Frigaard 2006) (Table 2). The *Chlorobi* use sulfide and ferrous iron as their source of reductant (Crowe et al. 2008). *Chloroflexi*, and potentially phototrophic members of the *Proteobacteria*, are all capable of aerobic, or at least microaerophilic, organotrophy in the dark. Anoxic conditions are required for photolithotrophic or photo-organotrophic growth in the light by *Chloroflexi* and *Proteobacteria*, using PSII-like reaction centres (Bryant & Frigaard 2006) (Table 2).

The *Chlorobi* are the organisms in which the RTCAC (Tables 1 & 2) autotrophic pathway of inorganic carbon assimilation was characterised using tracer labelling and enzymology (Sirevåg 1995). The complete gene sequence of *Chlorobium tepidum* showed that there were no pathways of autotrophic inorganic carbon assimilation other than the RTCAC in this organism, although there was a gene for a Form IV Rubisco that does not function as a carboxylase (Eisen et al. 2002). The low inorganic carbon affinity of the RTCAC (Table 1) is presumably not a problem in the anoxic and, hence, relatively high CO₂ environments of the *Chlorobi*. More work is needed on the inorganic carbon availability to chemolithotrophs with this pathway (see 'Chemolithotrophs' for further details on the occurrence of the RTCAC).

Pre-genomic work using tracer labelling and enzymology showed that the purple sulfur (and purple non-sulfur) *Proteobacteria* used the PCRC for autotrophic inorganic carbon assimilation (Tabita 1995) (Table 2). Complete genome sequences of purple photosynthetic bacteria showed no autotrophic pathways other than the PCRC (e.g. Larimer et al. 2004, Tabita et al. 2008) (Tables 1 & 2). Metagenomics showed the distribution of Forms IA, IC and II among the purple bacteria, often with more than one form in a single organism (Tabita et al. 2008). There are plausible ecological rationalisations for these distributions; however, while these rela-

tionships to the ecology of the organisms rely on kinetic studies of Rubisco from individual organisms, these studies do not give sufficiently precise kinetic data to relate the Rubisco form to ecological interactions (Badger & Bek 2008).

The 3-hydroxypropionate pathway in *Chloroflexus* has been almost completely elucidated. It is now known to function as a bi-cycle involving 2 carboxylations by acetyl-CoA-carboxylase for every 1 by propionyl-CoA-carboxylase, and the product is a 3-carbon compound (Herter et al. 2002, Friedmann et al. 2006, Klatt et al. 2007), so the previously suggested involvement of the photorespiratory carbon oxidation cycle (PCOC) (see Sirevåg 1995) is not needed (Tables 1 & 2). This pathway has a relatively high affinity for inorganic carbon (Table 1), despite occurring mainly in organisms (*Chloroflexi*, some chemolithotrophs) living in environments with relatively high inorganic carbon availability (see 'Chemolithotrophs' for further details of the occurrence of the 3-hydroxypropionate pathway).

Photosynthetic growth of these organisms occurs just below the oxycline in illuminated environments. The anoxic zone is generated by a gravitational influx of particulate organic matter from (usually) oxygenic primary productivity that stoichiometrically exceeds the advective and diffusive influxes of oxygen for oxidative conversion to CO₂. The depth of the oxycline varies, so there can be large variations in irradiance and its spectral distribution, since there is very significant absorption by water, especially in the infrared, as well as absorption by pigments of oxygenic photolithotrophs (Stomp et al. 2007a). This helps to rationalise the absorption properties of photosynthetic pigments in anoxygenic photosynthetic bacteria, regardless of whether they are predominantly or entirely autotrophic or are at least facultatively photo-organotrophic (Stomp et al. 2007a).

There are limits on the wavelength in the infra-red at which absorption of radiation can be used in photosynthesis, resulting from the lower energy per photon at longer wavelengths. These constrain the redox span of the photochemistry that can be performed at longer wavelengths, so that it might require the use of 2 photons to carry out the same overall metabolism as is achieved by 1 photon at shorter wavelengths (Wolstencroft & Raven 2002, Raven & Wolstencroft 2004, Kiang et al. 2007a,b, Raven 2007, Stomp et al. 2007a,b). Thus, direct use of a single photochemical system to reduce NAD⁺ using sulfide as the electron donor only occurs in the *Chlorobi*, where the excited state bringing about the photochemistry corresponds to a wavelength of 840 nm, although the long wavelength absorption maximum of the major light-harvesting bacteriochlorophyll is at 760 nm (see Falkowski & Raven 2007).

Indirect means of reducing NAD^+ using sulfide as an electron acceptor, involving proton cycling across membranes and with a photochemically generated proton-free energy difference coupled to movement of electrons from a higher to a lower redox potential, occurs in the *Chromatiaceae* (purple sulphur bacteria) where the effective wavelength for photochemistry is 890 nm. However, the direct versus indirect use of photochemistry to reduce NAD^+ is probably a result of the less negative redox potential of the first stable reduced product of photochemistry in the purple as compared with the green sulfur bacteria, rather than the smaller redox span covered by the excitation energy in the reaction centre of the purple sulfur bacteria (Falkowski & Raven 2007). It is not clear whether the indirect pathway of energizing NAD^+ reduction and sulfide oxidation in purple sulfur bacteria requires more photons absorbed per electron transferred than the direct pathway of *Chlorobi*, although it is known that *Chlorobi* typically grow deeper in the water column than purple sulfur bacteria and generally have lower maintenance costs (van Germerden & Mas 1995).

The habitat area available for these organisms is relatively restricted; the continental shelves are only about 7% of the ocean's surface area and have a mean depth of less than about 100 m, although very little of this provides an appropriate habitat. The Black Sea, the largest anoxic basin in the world, provides a large area of illuminated anoxic benthic habitat in its shallower regions and an area of illuminated oxycline at 85 to 120 m depth where the water is deeper (Gorlenko et al. 2005, Manske et al. 2005). *Chlorobi* are essentially the only photosynthetic primary producers at the oxycline, but their primary productivity is low because of the very low irradiance at even the top of the green sulfur bacteria layer, i.e. a maximum of 0.75 to 2.2 nmol photon $\text{m}^{-2} \text{s}^{-1}$ (presumably 400 to 700 nm). The maximum rate of inorganic C assimilation into organic matter under these circumstances can be evaluated by assuming that all of the photons are absorbed by photosynthetic pigments, with a requirement of 8 mol photons per mol inorganic C assimilated (i.e. the minimum observed). This gives an area-based photosynthetic rate of 0.094 to 0.28 nmol C $\text{m}^{-2} \text{s}^{-1}$ or 3 to 9 mmol C $\text{m}^{-2} \text{yr}^{-1}$ (36 to 108 g C $\text{m}^{-2} \text{yr}^{-1}$). This neglects any organic carbon use in maintenance, or the lower irradiance over part of the average 12 h day and over the year. Even so, the productivity of the *Chlorobi* in the Black Sea, which probably represents their largest single habitat, is less than 1/1000 of the area-based productivity of the ocean, which has at least 115 g C $\text{m}^{-2} \text{yr}^{-1}$ (at least 50×10^{15} g C yr^{-1} net productivity in the world ocean over $361 \times 10^{12} \text{m}^2$ of the ocean). Some *Chlorobi* habitats, and especially those of purple sulphur bacteria, have significantly higher solar irradiance levels

(Van Germerden & Mas 1995). A much smaller, but interesting, habitat for *Chlorobi* are deep-sea hydrothermal vents (Beatty et al. 2005), where the only electromagnetic radiation available is the infra-red radiation from the hot vents. While not contributing significantly to global primary production, the ability of these *Chlorobi* to grow at such low irradiances raises interesting questions in cell physiology. The recent finding of a substantially increased ^{14}C -inorganic carbon assimilation after light exposure of *Chlorobi* (Casamayor et al. 2008) requires further investigation, both with respect to the mechanism and to the implications for measurements of *in situ* contributions to primary productivity.

Sulfide-oxidising photolithotrophs can be in competition for reductant with sulfide-oxidising chemolithotrophs. The photolithotrophic sulfide oxidation by anoxygenic bacteria only occurs in the absence of O_2 and would be expected to occur lower in the oxycline. In contrast, the chemolithotrophs need oxidants such as O_2 and NO_3^- and would be expected to occur in the upper part of the oxycline; consequently, they would not cause substantial attenuation of photosynthetic radiation.

Chemolithotrophs

Chemolithotrophs oxidise NH_4^+ , NO_2^- , Fe^{2+} and S^{2-} using O_2 as oxidant; S^{2-} can also be oxidised using NO_3^- as oxidant, with an increasing role found for *Archaea* in addition to *Bacteria* (Bach & Edwards 2003, Francis et al. 2007, Agogu e et al. 2008) (Table 2). There are also the relatively recently discovered (as a biological reality rather than a theoretical possibility) anammox bacteria that live by the oxidation of NH_4^+ by NO_2^- in anaerobic or suboxic, but not sulfidic, habitats (Schouten et al. 2004, Strous et al. 2006, Francis et al. 2007, Jensen et al. 2008) (Table 2).

Genomic and metagenomic studies have shown that the PCRC is widespread among chemolithotrophic *Bacteria*, and have defined the form of Rubisco involved in specific cases (Badger & Bek 2008) (Tables 1 & 2). Also, metagenomic studies have increased the known occurrence of the RTCAC among chemolithotrophic *Bacteria* (Campbell & Cary 2004, H ugler et al. 2005), demonstrated this pathway in chemolithotrophic *Archaea*, and helped in demonstrating that there are variations in the enzymes involved in the pathway. Variants of the 3-hydroxypropionate pathway also occur in chemolithotrophic *Archaea* (Alber et al. 2006, Hallam et al. 2006) (Tables 1 & 2) as well in as in the *Chloroflexaceae*. Berg et al. (2007) found a novel 3-hydroxypropionate/4-hydroxybutyrate pathway in the archaean

chemolithotroph *Metallosphaera*, using mainly classical rather than genomic methodology (Tables 1 & 2). Huber et al. (2008) discovered a dicarboxylate/4-hydroxybutyrate cycle in the crenarcheote *Ignicoccus hospitalis* (Tables 1 & 2), also using mainly classical methodology. These 2 recently characterised pathways (Berger et al. 2007, Huber et al. 2008) have, like the RTCAC, relatively low affinities for inorganic carbon (Table 1).

A final pathway of autotrophic inorganic carbon assimilation in chemolithotrophs is the occurrence in an anammox bacterium of all the genes necessary for the acetyl-CoA pathway for the total synthesis of acetate (Table 1) using the reductant at a very negative redox potential generated in the conversion of the intermediate hydrazine to N_2 (Strous et al. 2006) (Tables 1 & 2). The acetyl-CoA pathway had previously been suggested to occur in anammox organisms on the basis of the very low natural ^{13}C level in anammox-specific lipids relative to that of CO_2 in the medium (Schouten et al. 2004). This pathway seems to have a very low affinity for inorganic carbon (Table 1).

While perhaps anaplerotic rather than strictly autotrophic, the synthesis of methane from CO_2 and H_2 , which probably accounts for up to half of the total global methanogenesis, is certainly reductive (Liu & Whitman 2008). Furthermore, methanogens (all of

which are members of the *Archaea*) contribute a very simple organic compound to food webs, i.e. for methanotrophs. CO_2 is the inorganic carbon substrate for methanogenesis from inorganic carbon and H_2 . Based on the kinetics of an exchange reaction using $^{14}CO_2$, the half-saturation concentration for CO_2 is 0.7 mol m^{-3} (Vorholt & Thauer 1997), which is an intermediate value compared with the range for the carboxylases involved in autotrophic inorganic carbon assimilation (Table 1).

SIGNIFICANCE OF CHEMOLITHOTROPHS AND ANOXYGENIC PHOTOLITHOTROPHS IN GLOBAL PRIMARY PRODUCTIVITY

Contribution of chemolithotrophs and anoxygenic photolithotrophs today

The global biogeochemical significance of chemolithotrophs (Tables 2 & 3) in autotrophic inorganic carbon assimilation is not well constrained, although data are available from which some estimates can be made. Raven (1996) estimated the maximum inorganic carbon assimilation that could accompany nitrification in the ocean, assuming that all nitrification was chemolithotrophic and that the inorganic carbon assimilated per inorganic nitrogen oxidised from NH_4^+

Table 3. Comparison of global rates of autotrophic inorganic carbon assimilation processes that occur in aquatic environments. For additional details of the data sources and the calculations. See 'Significance of chemolithotrophs and anoxygenic photolithotrophs in global primary productivity'

Process	Flux from inorganic carbon (Pg C yr^{-1})	Source
Present nitrification	0.19 – 0.4 (mean 0.3)	Raven (1996), Wuchter et al. (2006)
Present anammox	0.00017 – 0.0035 (mean 0.0018)	Strous et al. (1998), Francis et al. (2007)
Present day oxidation of biogenic sulfides by chemolithotrophs and anoxygenic photolithotrophs	0.022 – 0.043 (mean 0.033)	Mandernack & Tebo (1999), Turchyn & Schrag (2004)
Present day oxidation of hydrothermal vent sulfides	0.002	Mandernack & Tebo (1999), Turchyn & Schrag (2004)
Present day oxidation of sulfide from basalt-hosted ocean aquifers	0.001	Bach & Edwards (2003)
Present day methanogenesis from CO_2 in wetlands and the ocean	0.039 – 0.095 (mean 0.067)	Liu & Whitman (2008)
Present day total from chemolithotrophy and anoxygenic photolithotrophy	0.26 – 0.54 (mean 0.40)	
Present day net primary productivity by marine and inland waters oxygenic photolithotrophs (99% from phytoplankton)	50	Field et al. (1998), Raven & Maberly (2005), del Giorgio & Williams (2005)
Pre-photosynthetic (Hadean–Archaean, <4 giga-annum [Ga] ago) chemolithotrophy	0.00074	Sleep & Bird (2007, 2008)
Pre-oxygenic phototynthesis (Archaean, 3.8 Ga ago) primary production by chemolithotrophs and anoxygenic photolithotrophs	<1.2 – <5	Kharecha et al. (2005), Canfield et al. (2006)

to NO_3^- was the highest found in laboratory cultures of nitrifying bacteria, and suggested a value of $0.19 \text{ Pg C yr}^{-1}$. It is now believed that the *Crenarcheota* are probably the dominant marine nitrifiers, and Wuchter et al. (2006) have estimated that inorganic carbon assimilation in the ocean associated with nitrification by these organisms as 0.4 Pg C yr^{-1} (see also Agogué et al. 2008).

The other aspect of the nitrogen cycle that involves chemolithotrophy is in the anammox contribution to denitrification (Tables 2 & 3). Unlike the previously recognised chemo-organotrophic denitrification, which involved organic carbon oxidation with nitrate or nitrite as electron acceptors and the production of CO_2 , the anammox reaction involves autotrophic inorganic carbon assimilation with 0.065 mol CO_2 fixed per mol N_2 released (Strous et al. 1998), i.e. 0.028 g C assimilated from CO_2 for each gram of N released as N_2 . The total N_2 released in marine denitrification is poorly constrained, with values ranging from 20 to 250 Tg N yr^{-1} (see Tyrrell 1999, Box 1 on p. 527); the fraction of this total N_2 release that involves the anammox bacteria is probably in the range 30 to 50% (Francis et al. 2007), i.e. 6 to 125 Tg N corresponding to 0.00017 to 0.0035 Tg C or 0.00000017 to 0.0000035 Pg C assimilated from CO_2 each year.

Smaller values for autotrophic inorganic carbon assimilation have been suggested for organisms bringing about chemolithotrophic oxidation of sulfide to sulfate (Tables 2 & 3). Turchyn & Schrag (2004) cite values for sulfide oxidation in ocean sediments of 6 to $12 \text{ Tmol S yr}^{-1}$. Assuming that all oxidation is biologically mediated, with the highest reported laboratory ratios of chemolithotrophic inorganic carbon assimilation to sulfide oxidation to sulfate cited by Manderack & Tebo (1999), the rates of global sulfide oxidation correspond to inorganic carbon assimilation rates of 0.022 to $0.043 \text{ Pg C yr}^{-1}$. These values include any contribution from photolithotrophic sulfide-oxidisers (Tables 2 & 3), which on grounds of available habitat could only make a small contribution even though their inorganic carbon assimilation per sulfide oxidised could be higher than that for chemolithotrophs if oxidation went all the way to sulfate. Turchyn & Schrag (2004) cite values of sulfide oxidation from hydrothermal vents of up to 0.5 Tmol yr^{-1} ; if all of this is oxidised by chemolithotrophs there could be 0.002 Pg inorganic carbon assimilated per year at the vents. Bach & Edwards (2003, see also Santelli et al. 2008) considered that basalt hosted ocean aquifers, and suggested that chemolithotrophic oxidation of sulfide and ferrous iron from this source could account for $0.001 \text{ Pg C yr}^{-1}$.

The methanogens were considered above as 'honorary autotrophs,' at least in the case of those in which

the carbon source is CO_2 and the reductant is H_2 . Liu & Whitman (2008) give marine methane production as 5 to 10 Tg yr^{-1} as CH_4 . Making the poorly substantiated assumption that half of this methane comes from CO_2 rather than compounds containing methyl groups such as methanol or acetate, 0.004 to $0.006 \text{ Pg C yr}^{-1}$ is consumed as CO_2 . If wetlands are considered as an aquatic habitat, then the 92 to $237 \text{ Tg CH}_4 \text{ yr}^{-1}$ corresponds to 0.035 to $0.089 \text{ Pg C yr}^{-1}$ consumed as CO_2 . This gives a total for methanogenesis of 0.039 to $0.095 \text{ Pg C yr}^{-1}$ consumed as CO_2 (Table 3).

Summing these estimates yields global net chemolithotrophic and sulfide-oxidising anoxygenic photolithotrophic inorganic carbon assimilation rates of 0.26 to $0.54 \text{ Pg C yr}^{-1}$, with a mean of $0.40 \text{ Pg C yr}^{-1}$ (Table 3). This is 0.8% of the 50 Pg C yr^{-1} of global aquatic photolithotrophic oxygenic net primary productivity (Field et al. 1998) (Table 3), but is only about 0.5% of the value suggested from measurements of respiration (del Giorgio & Williams 2005).

Two points must be emphasised. One is that even the mean of these estimates of the contribution of chemolithotrophs and anoxygenic autotrophs is an upper limit. These estimates neglect abiological oxidations of sulfide and ferrous iron, and use the highest known stoichiometries of inorganic carbon assimilation to inorganic material oxidised.

The other point is that, if these autotrophic carbon assimilation mechanisms are considered to be additional to primary production by oxygenic photolithotrophs, the source of the reduced inorganic material and oxidant must be carefully considered. The production of the former may involve oxidation of organic matter produced by previous oxygenic photolithotrophy, and the oxidant is almost invariably oxygen from oxygenic photolithotrophy, or an oxidant such as nitrate whose presence is contingent on oxygenic photosynthesis. Even the sulfide and ferrous iron from hydrothermal vents and basalt-hosted ocean aquifers are oxidised by oxygen, and these reduced species may have originated in part from subducted sediments containing biogenic sulfide and ferrous iron. For the sedimentary sulfide and the meso- and bathypelagic ammonium, there is a clear biological origin that can ultimately be traced back to oxygenic primary producers. The sulfide comes from the oxidation by sulfate of sedimented organic material, ultimately from oxygenic photolithotrophy, after the local supply of oxygen, and then nitrate, is exhausted; even the presence of sulfate in the biosphere is ultimately a result of oxidation of sulfide following the advent of oxygenic photosynthesis. The ammonium, other than that derived from combined nitrogen from fertilizers, is ultimately a result of diazotrophy, which relies directly or indirectly on oxygenic photosynthesis, as is the oxidant

used in ammonium and nitrite nitrification. Nitrogenous fertilizers themselves depend on previous oxygenic photolithotrophy, either to generate the hydrogen used in the Haber-Bosch process from biogenic methane in making ammonia, or to supply the oxygen consumed in the Birkeland-Eyde process in making nitric acid. Of course, there are limits on how far back in palaeobiogeochemistry it is useful to go, and this will determine the extent to which different kinds of chemolithotrophy today can be said to depend on oxygenic photolithotrophy.

To put some of these arguments in quantitative terms, the first case considered is the photolithotrophic and chemolithotrophic oxidation of sulfide to sulfate. This uses sulphide generated by dissimilatory sulfate reduction. There is a net long-term burial of sulfide as pyrites of some $0.039 \text{ Pg S yr}^{-1}$ (Bernier 1982), corresponding to the production of 0.014 Pg C as carbon dioxide (2 mol carbon as CO_2 per 1 mol sulfate reduced to sulfide). To this production of CO_2 must be added the CO_2 released in the production of the sulfide, which is subsequently re-oxidised. With the light energy input to photolithotrophic oxidation of sulfide, the CO_2 taken up in photosynthesis is equal to that released in dissimilatory sulfate reduction in producing the sulphide. For chemolithotrophic sulfide oxidation, the CO_2 assimilated is less than the CO_2 produced in dissimilatory production of the sulfide, so there must be more than the global net 0.014 Pg CO_2 production per year associated with pyrites burial that is associated with the overall process of sulfate reduction to sulfide and reoxidation of some of it back to sulfate.

For the case of chemolithotrophic nitrification, oxygenic photolithotrophs growing with a given quantity of nitrogen use more energy when nitrate is the nitrogen source than when ammonium is the nitrogen source. Equating this extra energy to CO_2 assimilation, the inorganic carbon assimilation that is forgone by energy-limited photolithotrophic organisms using nitrate rather than ammonium as the nitrogen source exceeds the inorganic carbon assimilated in chemolithotrophy in recycling the nitrogen excreted as ammonium to nitrate (Raven 1996). In other words, the predicted primary productivity (photolithotrophy plus chemolithotrophy) with nitrogen cycling entirely at the ammonium level is predicted to exceed that with nitrogen cycling that also involves nitrate.

These arguments show that the chemolithotrophy and non-oxygenic photolithotrophy in extant aquatic habitats are all ultimately dependent on antecedent oxygenic photolithotrophy, and that chemolithotrophy and sulfide-based photolithotrophy only serve to partially decrease the carbon loss from the processes producing the inorganic reductant.

Contributions of chemolithotrophs and anoxygenic photolithotrophs in the past

It is widely believed that chemolithotrophy preceded phototrophy in the evolution of life on Earth. Estimates of the global primary productivity as long ago as 4.0 billion yr, which at that time was all aquatic, suggest that only 0.72 Tg of inorganic carbon was converted to organic carbon each year (Sleep & Bird 2007, 2008; Table 3). This annual production is only about $1/70\,000$ of annual aquatic production today, or about $1/555$ of annual aquatic chemolithotrophic and anoxygenic photolithotrophic production today (Table 3). The comparison between pre-photosynthetic and extant chemolithotrophic annual production shows, among other things, the role of recycling of photosynthesis and anaerobic respiration in recycling oxidants and reductants, which facilitate chemolithotrophy.

After the evolution of anoxygenic photosynthesis not earlier than 4 billion yr ago (Sleep & Bird 2007, 2008), there was significantly greater autotrophic inorganic carbon assimilation than before. Kharecha et al. (2005) suggest that global primary production by chemolithotrophs and anoxygenic photolithotrophs in the Archaean (3.8 billion yr ago) was not more than 1.2 Pg C yr^{-1} , while Canfield et al. (2006) suggest up to 3.4 Pg C yr^{-1} (Table 3). This Archaean productivity is not more than $1/15$ of the extant productivity based on oxygenic primary producers in the ocean and inland waters (Table 3), and about 6 times the extant productivity from chemolithotrophy and anoxygenic photolithotrophy, processes that today are largely coupled to oxygenic photolithotrophy-based food webs. However, the modelled aquatic productivity 3.8 billion yr ago was up to 5000 times the suggested chemolithotrophic productivity 4 billion yr ago (Table 3).

Since the onset of global oxygenation of the shallow ocean and the atmosphere about 2.4 billion yr ago (Sleep & Bird 2008), there have been times when the euxinic and sulfidic conditions in the ocean have included parts of the euphotic zone, much as in the present-day Black Sea and some Antarctic fjords. There is biomarker evidence for the occurrence of sulfide-oxidising anoxygenic *Bacteria* of the *Chlorobi* and the *Chromatiaceae* in the marine photic zone in the mid-Proterozoic oceanic some 1.64 billion yr ago (Brocks et al. 2005). In the Neoproterozoic low-latitude glaciation 740 to 700 million yr ago, there was significant marine primary productivity as recorded in the organic carbon content of sediments. Biomarkers show that cyanobacteria and green sulphur bacteria contributed to photolithotrophy at this time, so sea ice must have been absent or at least able to permit penetration of photosynthetically active radiation during

this 'Snowball Earth' episode (Olcott et al. 2005). There was a third time at which biomarker evidence suggests that green sulfur bacteria were important photolithotrophs in a euxinic marine photic zone, i.e. during the Permian–Triassic boundary mass extinction event some 250 million yr ago, and the 1 million yr or more thereafter (Grice et al. 2005).

IMPLICATIONS FOR MEASUREMENTS *IN SITU* OF PRIMARY PRODUCTIVITY BY OXYGENIC PHOTOLITHOTROPHS

For phototrophs lacking autotrophic inorganic carbon assimilation, there are 2 sources of possible interference in the ^{14}C -based estimates of productivity that involve filtration and which include in the assayed particulate organic carbon organisms of the size of free-living *Archaea* and *Bacteria* and in which readings from dark are subtracted from light incubations. One is the occurrence of light stimulation of anaplerotic inorganic ^{14}C assimilation in photo-organotrophs with rhodopsin- or bacteriochlorophyll-based stimulation of growth. This is unlikely to be significant, since anaplerotic inorganic carbon assimilation contributes not more than 5% of the total net carbon use in growth, so that the light-stimulated component of anaplerotic inorganic carbon assimilation would only be 5% of 1% (see 'Organisms with energy-transforming photochemistry but not capable of autotrophic CO_2 assimilation) or 0.05% of photolithotrophic primary production. This would not be detected in ^{14}C estimates of primary production. The other is the occurrence of efflux of ^{14}C -labelled dissolved organic carbon from oxygenic photolithotrophs, followed by assimilation by the photo-organotrophs. This second possibility seems unlikely to be significant, because dilution with unlabelled dissolved organic carbon in seawater makes it unlikely that recently produced dissolved organic matter is more accessible to the photo-organotrophs than is the average of the predominantly refractory dissolved organic matter in natural waters.

A related question is the extent to which chemolithotrophy and anoxygenic photolithotrophy are experimentally separable from oxygenic photolithotrophy in field measurements of primary productivity that are attributed to oxygenic organisms. The answer is that, with the probable exception of nitrifiers, they are generally spatially separate from the oxygenic phytoplankton. This is clearly the case for autotrophs at hydrothermal vents and in basalt-hosted marine aquifers, as well as for sulfide oxidisers at the oxycline of sulfidic sediments and bulk water below the upper mixed layer or epilimnion. Similarly for the anammox

bacteria, their restriction to anoxic or suboxic habitats means ready separation from measurements on oxygenic photolithotrophs.

The situation for nitrifiers is less clear; it used to be thought that these organisms are subject to photoinhibition and so would not significantly affect measurements in the upper mixed layer or epilimnion. However, the analysis by Yool et al. (2007) on the specific rate of nitrification (i.e. normalised to ammonium concentration) shows no significant decrease with depth and suggests that half of ocean nitrification occurs above the permanent thermocline, i.e. with a significant amount going on in the euphotic zone. Since nitrification consumes ammonium, carbon dioxide and oxygen, its occurrence in the euphotic zone would (1) add a nitrification step between some of the disappearance of ammonium from solution and the appearance of organic particulate nitrogen, (2) contribute to ^{14}C inorganic carbon assimilation in 'dark' bottles and 'light' bottles, and (3) be a positive component of oxygen uptake under dark conditions and a negative component of net oxygen production in the light. The studies indicating photoinhibition of nitrification in the surface ocean were based on studies with nitrifying bacteria, rather than with the predominant *Crenarcheota* (Yool et al. 2007).

DISCUSSION AND CONCLUSIONS

There is a great diversity of autotrophs *sensu lato*, other than oxygenic photolithotrophs, in aquatic habitats today. The chemolithotrophs use a wide range of reductants and a number of oxidants other than oxygen. Many of the anoxygenic phototrophs have bacteriochlorophyll-based photochemistry and use photosystems resembling the PSI or PSII of oxygenic photolithotrophs. All of these photosystems have a common evolutionary origin (Raymond et al. 2003, Sadekar et al. 2006). The remaining phototrophs, none of which can carry out autotrophic inorganic carbon assimilation, have rhodopsin-like energy-conserving photochemistry.

The non-oxygenic organisms that carry out autotrophic inorganic carbon assimilation either have the PCRC (as do all oxygenic photolithotrophs) or 1 of 4 (5 if methanogens are included) other pathways of inorganic carbon assimilation (Table 1). The phylogenetic distribution of these pathways probably involves horizontal gene transfer. The properties of some of these pathways in terms of inorganic carbon affinity, absence of competition between oxygen and CO_2 , and lower ATP consumption per inorganic carbon assimilated, are apparently more appropriate for

photosynthesis in water in equilibrium with today's atmosphere than the PCRC using Rubisco (Table 1). The absence of any of the alternative pathways of autotrophic inorganic carbon assimilation from oxygenic photolithotrophs is presumably a result of non-competitive inhibition, or inactivation, of the other pathway by oxygen, and/or mechanistic problems with horizontal gene transfer and the replacement of the incumbent PCRC by an alternative pathway of autotrophic inorganic carbon assimilation (Raven et al. 2008a,b).

In today's aquatic habitats, the combined contribution of anoxygenic photolithotrophs and chemolithotrophs to autotrophic inorganic carbon assimilation is 0.5 to 1.0% of the net primary productivity attributable to oxygenic photolithotrophs (Table 2). The activities of anoxygenic phototrophs lacking autotrophic inorganic carbon assimilation using bacteriochlorophyll- or rhodopsin-like pigments probably increase, by 1% or less, the conversion of dissolved organic carbon into particulate organic carbon by euphotic zone chemoorganotrophic *Archaea* and *Bacteria*. These contributions to aquatic productivity today by non-oxygenic autotrophs and non-autotrophic phototrophs are all dependent indirectly on the activities of oxygenic photolithotrophs. Overall, the activities of non-oxygenic autotrophs do not balance the CO₂ production involved in generating the anoxic environment and biogenic sulfide used by anoxygenic photolithotrophs and the biogenic sulfide, ammonium, nitrite and oxygen (or other oxidants) used by chemolithotrophs. There is minimal interference of the activities of phototrophs lacking autotrophic inorganic carbon assimilation, non-oxygenic photolithotrophs or chemolithotrophs with measurement of *in situ* inorganic carbon assimilation by oxygenic photolithotrophs.

Before the advent of oxygenic photolithotrophy at least 2.32 to 2.45 billion yr ago (Rasmussen et al. 2008), the activity of anoxygenic photolithotrophs and of chemolithotrophs was greater than the present-day values, largely as a result of the availability of the entire aquatic euphotic zone to anoxygenic photolithotrophs. Before anoxygenic photolithotrophs evolved 4 billion yr ago, there was only limited productivity by chemolithotrophs. Even after oxygenic photolithotrophs evolved and the atmosphere became oxygenated, there was a long period in which the ocean was euxinic and sulfidic, giving some productivity by anoxygenic photolithotrophs at the base of the euphotic zone, as in the Black Sea and some Antarctic fjords today. Since oxygenation of essentially the whole ocean became the norm in the late Proterozoic, there have been periods in which the ocean was euxinic and sulfidic, e.g. at the Permian–Triassic boundary.

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