The evolution of autotrophy in relation to phosphorus requirement

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Abstract

The evolution of autotrophy is considered in relation to the availability of phosphorus (P), the ultimate elemental resource limiting biological productivity through Earth’s history. Work on microbes and plants is emphasized, dealing in turn with the main uses for P in cells, namely nucleic acids, phospholipids, and water-soluble low molecular mass phosphate esters plus metabolically active inorganic orthophosphate. There is a greater minimum gene number and minimum DNA content in autotrophic than in osmochemoorganotrophic archaea and bacteria, as well as a lower rate of biomass increase per unit P (P-use efficiency) in autotrophs than in osmochemoorganotrophs, in eukaryotes as well as bacteria. This may be due to the diversion of rRNA from producing proteins common to all organisms to producing highly expressed proteins specific to autotrophs. The P requirement for phospholipids is decreased in oxygenic photolithotrophs, and some anoxygenic photolithotrophs, by substituting galactolipids and sulpholipids for phospholipids in the photosynthetic, and some other, membranes. The six different autotrophic inorganic carbon assimilation pathways have varying requirements for low molecular mass water-soluble phosphate esters. In oxygenic photolithotrophs, there is no clear evidence of a different P requirement for growth in the absence (diffusive CO₂ entry) relative to the presence of CO₂-concentrating mechanisms (CCMs). P limitation increases the expression of crassulacean acid metabolism (CAM) in facultative CAM plants, decreases the extent of inorganic carbon accumulation in algae with CCMs, and (usually) their inorganic carbon affinity and the water-use efficiency of growth of terrestrial plants, and the light-use efficiency of photolithotrophs.

Key words: Anoxygenic photosynthesis, chemolithotrophs, CO₂-concentrating mechanisms, oxygenic photosynthesis, phospholipids, rRNA, Rubisco.

Introduction

Phosphorus (P) is essential for all forms of life, and is widely perceived by geochemists as the element ultimately limiting global primary productivity (Berner and Berner, 2012; Williams and Rickaby, 2012). Despite this, P has been considered less than have carbon (C), nitrogen (N), and iron (Fe) in the evolution of autotrophy in relation to environmental changes of the last 4 billion years. This could be because one (or more) of C, N, and Fe is the proximate resource limiting local productivity. In attempting to address this lack of consideration of P, the paper begins with a consideration of the origin of life and whether autotrophy is an ancestral trait. There is then a consideration of the roles of P in organisms, and of elemental stoichiometries in food webs, starting from autotrophs and proceeding to phago- and osmchemoorganotrophs. P-use efficiency (PUE), measured as the rate of dry matter (DM) gain per unit P in the organism, or rate of increase of organism C per unit of organism P, is then compared for autotrophs and chemoorganotrophs. The mechanistic reasons for differences in PUE are then related to P allocation among nucleic acids, phospholipids, and low
molecular mass water-soluble phosphate esters in chemoorganotrophs and autotrophs, and among variants on photolithotrophy. Finally, there is consideration of the effects of limitation of growth by P availability on the water-use efficiency of the growth of terrestrial plants, and on acclimatory variations in the expression of inorganic carbon-concentrating mechanisms in photolithotrophs.

**Origin and early evolution of life: autotrophy and chemoorganotrophy**

After a long reign for the hypothesis of a ‘primordial soup’, involving an abiological origin of organic precursors and chemoorganotrophy as the earliest trophic mechanism of organism (Haldane, 1929), there are strong arguments for an autotrophic, and specifically a chemolithotrophic (see Glossary), origin of life (Lane et al., 2010; Table 1). Accepting this view, the last universal common ancestor of all extant life on Earth was a chemolithotrophic autotroph (Lane et al., 2010), so that these organisms form the baseline for considerations of P requirements. Derived trophic modes were osmochemoorganotrophic consumers and photolithotrophs such as ferrous iron-oxidizing anoxygenic photosynthetic bacteria in the ferruginous (dissolved Fe^{2+} exceeds dissolved S^{2–} in the anoxic environment) Archean (Table 1) ocean (Crowe et al., 2008).

Chemoorganotrophy, and especially phagotrophy, which involves the consumption of particulate organic matter (see Glossary), requires that some of the organic matter ingested is metabolized in exergonic reactions, which provide the energy

### Table 1. Geological time scale, with some key events

<table>
<thead>
<tr>
<th>Eon</th>
<th>Era</th>
<th>Period</th>
<th>Date (Ma)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phanerozoic</td>
<td>Coenozoic</td>
<td>Quaternary</td>
<td>2.6–today</td>
<td>Spread of C_{4} grasslands</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Coenozoic</td>
<td>Neogene</td>
<td>23–2.6</td>
<td>First C_{4} plants</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Coenozoic</td>
<td>Palaeogene</td>
<td>66–23</td>
<td>First fossil evidence of angiosperms involved in organic N, and also P nutrition</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Mesozoic</td>
<td>Cretaceous</td>
<td>146–66</td>
<td>First fossil evidence of angiosperms whose extant relatives have cluster roots involved in P acquisition</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Mesozoic</td>
<td>Jurassic</td>
<td>200–146</td>
<td>First fossil evidence of (sulfuric) diatoms</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Mesozoic</td>
<td>Triassic</td>
<td>251–200</td>
<td>Earliest marine planktonic coccolithophores; some extant coccolithophores have a high affinity for phosphate</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Palaeozoic</td>
<td>Permian</td>
<td>299–251</td>
<td>Earliest fossil evidence of cyads whose extant members are all symbiotic diazotrophs which may increase P requirement</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Palaeozoic</td>
<td>Carboniferous</td>
<td>359–299</td>
<td>Early members of the clade which gave rise to extant aquatic and ambilious CAM lycophyte Isoetes</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Palaeozoic</td>
<td>Devonian</td>
<td>416–359</td>
<td>First evidence of roots and of arbuscular mycorrhizas involved in P acquisition</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Palaeozoic</td>
<td>Silurian</td>
<td>444–416</td>
<td>First fossil evidence of eukaryotic plants</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Palaeozoic</td>
<td>Ordovician</td>
<td>488–444</td>
<td>First fossil evidence of homoiophydic plants</td>
</tr>
<tr>
<td>Phanerozoic</td>
<td>Palaeozoic</td>
<td>Cambrian</td>
<td>542–488</td>
<td>First fossils of silicified radiolarians</td>
</tr>
<tr>
<td>Proterozoic</td>
<td>Neoproterozoic</td>
<td>Ediacaran</td>
<td>635–542</td>
<td>First fossils of marine silicified sponges</td>
</tr>
<tr>
<td>Proterozoic</td>
<td>Neoproterozoic</td>
<td>Cryogenian</td>
<td>850–635</td>
<td>Deep ocean oxygenated from Ediacaran till today</td>
</tr>
<tr>
<td>Proterozoic</td>
<td>Neoproterozoic</td>
<td>Turonian</td>
<td>1000–850</td>
<td>Three global glaciations: Kaigas, Sturtian, then Marnonan</td>
</tr>
<tr>
<td>Proterozoic</td>
<td>Neoproterozoic</td>
<td>Ediacaran</td>
<td>1600–1000</td>
<td>Ocean ferruginous with oxygenated surface layer</td>
</tr>
<tr>
<td>Proterozoic</td>
<td>Palaeoproterozoic</td>
<td>Turonian</td>
<td>2500–1600</td>
<td>Ocean sulphidic with oxygenated surface area</td>
</tr>
</tbody>
</table>

Based on Raven and Edwards (2001, 2013); Taylor et al. (2009); Planavsky et al. (2010); and Raven and Andrews (2010).
Phosphorus availability in the biosphere over the last 3.5 billion years

The P biogeochemical cycle over the last 3.5 billion years (Table 1) involves physical and chemical weathering of rocks on land (when continental crust became exposed to the atmosphere), with increasing biological contributions as the land surface was colonized and biological activity intensified (Lenton and Watson, 2000; Raven and Edwards, 2001, 2013; Saltzman, 2005; Lambers et al., 2008; Raven and Andrews, 2010; Berner and Berner, 2012; Williams and Rickaby, 2012; Table 1). After a variable number of cycles through biota on land and in inland waters exposed to the atmosphere, phosphate entered the ocean through rivers and, to a smaller extent, as dust. P is essential for all forms of life on Earth, and an understanding of the factors altering its availability over time is important in understanding the quantitative role of P in organisms and in the evolution of life. After the chemolithotrophic last universal common ancestor (Lane et al., 2010), Blake et al. (2010) argue for a well-developed biogeochemical P cycle in the ferruginous Archean ocean 3.2–3.5 billion years ago (Table 1). Planavsky et al. (2010) suggest, on the basis of P:Fe ratios in non-silicoclastic (deposits of broken rocks dominated by silica) iron deposits, corrected for silicic acid:phosphate competition in the relatively silicic acid-rich ocean, that P was relatively available in the generally ferruginous Archean and early Palaeoproterozoic ocean. This contrasts with earlier work on banded iron formations, uncorrected for silicic acid competition, suggesting that P scavenging by ferric oxide meant that the ocean phosphate concentration in the Archean and early Proterozoic ferruginous ocean (3.5–1.9 billion years ago, with a possible sulphidic phase around the Global Oxygenation Event (GOE) ~2.32 Ga; Bekker et al., 2004; Planavsky et al., 2010) was only 10–25% of that in present oceans (Bjerrum and Canfield, 2002) (Table 1). The values of Planavsky et al. (2010) are preferable, because they are corrected for silicic acid competition in phosphate binding to ferric oxide.

Blank and Sanchez-Baracaldo (2010) showed that cyanobacteria evolved in freshwater, based on the the habitat requirements of the extant organisms at the base of molecular reconstructions of cyanobacterial phylogeny. This means that the GOE ~2.32 billion years ago (Bekker et al., 2004) was permitted by the escape of these first oxygenic photolithotrophs into the ocean, thus allowing them a global biogeochemical role (Blank and Sanchez-Baracaldo, 2010) (Table 1). This scenario means that the origins of oxygenic photolithotrophy would not have been constrained by oceanic P availability, but could have been restricted by whatever the (unknown) local freshwater constraints there were on P supply. Bjerrum and Canfield (2002; but see Planavsky et al., 2010) suggest that an increase in ocean sulphate concentration from the time of the GOE ~2.32 Ga ago led to increased sulphate reduction and hence increased phosphate availability, since ferrous sulphide deposits do not sequester phosphate. This would be to some extent offset by phosphate binding to ferric oxides deposited in the oxygenated surface ocean, though this would have been reversed as the ferric iron was reduced in the deeper sulphidic waters, allowing phosphate to return to the surface ocean through the thermocline separating cold dark deep water from the generally illuminated surface waters. Such a relatively constant surface ocean phosphate concentration agrees with the later analysis by Planavsky et al. (2010) and suggests little change in phosphate availability in the surface ocean with the GOE and then until the Snowball Earth (Cryogenian) episode 730–650 Ma ago when the ocean was ferruginous, with a possible contemporary model of how the ferruginous ocean arose and persisted (Mickuchi et al., 2009) (Table 1). The three widespread glaciations presumably arose by weathering related to tectonic activity drawing down greenhouse gases, predominantly CO₂, combined with the lower radiant energy output from the sun earlier in its existence. These widespread glaciations were presumably terminated by the build up of CO₂ from vulcanism in the absence of drawdown by biology and solution in seawater in the very cold Earth overcoming the high albedo of snow and ice.

Oxygenation of the deep ocean probably did not occur until the late Proterozoic in the wake of the Marinoan glaciation at the end of the Snowball Earth (Table 1), with agreement that there were high phosphate concentrations in the surface ocean during the Snowball Earth (Shen et al., 2000; Planavsky et al., 2010; Johnston et al., 2012; Sahoo et al., 2012; see also Papineau, 2010). After Snowball Earth, the evolution of silicified marine organisms such as benthic sponges from the late Ediacaran (550 Ma) and planktonic radiolarians from the earliest Cambrian (540 Ma), and then planktonic and benthic diamicta ~120 Ma ago, decreased the silicic acid concentration in the surface ocean and decreased competition with phosphate for scavenging by ferric oxide (Planavsky et al., 2010) (Table 1). This increased scavenging, decreasing the phosphate concentration in the surface ocean (Planavsky et al., 2010). Fluctuation in redox state phosphate availability in the oceans occurred through the Palaeozoic, with more stability in the Mesozoic (Lenton and Watson, 2000; Saltzman, 2005). Cadmium in sediments has been used as a proxy for phosphate in Phanerozoic seawater (Finkel et al., 2007; Horner et al., 2013) but, in the absence of proxies for other nutrients, cadmium cannot indicate which is the productivity-limiting resource.
An unanswered question is, accepting that P availability in the ocean declined with the evolution of silicified marine organisms, was P then the ultimate resource limiting global productivity? A personal view is that it probably was, but much more consideration and evidence is needed.

On land, the increase in area of land vegetated, and in the depth of rooting (roots evolved in the lower Devonian) with increasing plant height, as well as arbuscular mycorrhizas involved in P acquisition, through the Devonian and into the Carboniferous would have increased biological weathering (Raven and Edwards, 2001, 2013; Smith and Read, 2008; Raven and Andrews, 2010). This, increased the quantity of P used by the terrestrial biosphere and increasing runoff to the oceans until a new steady state related to the quantity of weatherable surface-exposed rock (Raven and Edwards, 2001, 2013; Smith and Read, 2008; Raven and Andrews, 2010). Additional characteristics of vascular land plants which can enhance P acquisition (ectomycorrhizas and taxa which today have representatives with clusters of roots) are known from the Cretaceous (Table 1; Raven and Andrews, 2010). Long periods without tectonic or glacial disturbance can lead to P leaching from terrestrial biomes, so that P becomes the element limiting terrestrial primary productivity and with a major impact on plant diversity (Lambers et al., 2008, 2010; Menge et al., 2012; Laliberté et al., 2013).

The roles of phosphorus in cells

In considering the categories of P and its compounds in the structures and catalysis of cells and organisms, I exclude P stored as metabolically inactive dissolved inorganic orthophosphate in vacuoles, or as polyphosphate or as phytate. The major chemically distinguishable pools of P in P-replete unicellular and multicellular oxygenic photolithotrophs are, in decreasing order of abundance (i) RNA (predominantly) and DNA (and a very small quantity of phosphorylated proteins); (ii) membrane phospholipids; (iii) low molecular mass water-soluble phosphate esters; and (iv) metabolically active inorganic orthophosphate (Bieleski, 1968a, 1968b, 1973; Chapin and Bieleski, 1982; Chapin et al., 1982; Raven, 2012; Veneklaas et al., 2012). These pools are involved in (i) the storage, replication, and conversion into catalysts and structural material of genetic material; (ii) the formation of barriers to free diffusion of most solutes and a structure into which protein catalysts of transport and other processes are housed; and (iii) and (iv) intermediate metabolites in energy transduction and in biosynthesis.

Is there a difference in C:N:P ratio and in P-use efficiency between autotrophs and chemoorganotrophs?

Background

P-use efficiency (PUE) is defined here as the rate of dry mass increase per unit P in the organism. Values for PUE can be calculated by multiplying the specific (= relative) growth rate (g DM increase per organism g DM content d−1) by the organism DM mol−1 of plant P (Berendse and Aerts, 1987; Raven, 2011, 2012). This latter term, organism DM mol−1 of plant P, is also used as a measure of PUE (e.g. Veneklaas et al., 2012), and is appropriate for comparisons among determinate annual species where agriculturally and ecologically important outcome is biomass, harvestable product, or viable seed produced in the growing season, but not for work on microorganisms or comparisons among organisms of different sizes (see below). Since many of the data sets involve specific growth rates of smaller organisms on a per cell or a per organism carbon basis, it is also convenient in these cases to work on an organism C basis, and the atomic C:N:P ratio. In comparing autotrophs and chemoorganotrophs, it is important to compare organisms of the same size, since there are differences with organism size in both specific growth rate and in P per unit C or per unit DM. Larger organisms have a lower PUE than smaller ones: the decrease in growth rate with size between cyanobacteria and flowering plants much more than offsets the slightly lower P content of biomass in the larger organisms.

The effect of organism size on PUE was shown in the analysis by Raven (2012), who calculated PUE of 250–750 g DM increase d−1 mol−1 P in the organism for terrestrial leguminous flowering plants and 1740–10 150 of those units for heterocystous cyanobacteria, with the general trend of increasing values with N source of N2 lowest, then NO3, and NH4/urea highest. The work of Sanginga et al. (1989) extends the data set for flowering plants, limited to legumes in Raven (2012), to the actinorhizal Casuarina equisetifolia, with PUEs of 178 and 156 g DM increase d−1 mol−1 organism P for P-replete plants with nitrate and dinitrogen as N sources, respectively, and values of 400 and 210, respectively, with P limitation. These data extend the range (250–750) for legumes alone to 156–750 g DM d−1 mol−1 plant P for legumes plus Casuarina.

C:N:P and PUE in autotrophic micrororganisms

The Redfield Ratio of C 106:N 16:P 1 (by atoms) in marine phytoplankton is a time-averaged mean value for the world ocean. This ratio is a very useful baseline (Falkowski, 2000; Geider and La Roche, 2002) for which a mechanistic basis has been suggested (Loladze and Elser, 2011). The mean ratio for 29 species of nutrient-replete organisms from a wide phylogenetic range of eukaryotic microalgae and cyanobacteria is C 132:N 18:P 1; that is, with slightly more C and N relative to P than in the Redfield Ratio (Ho et al., 2003; Quigg et al., 2003, 2011; Falkowski et al., 2004). There is phylogenetic variation in the ratio, although the number of strains tested in each clade was necessarily small. Table 2 shows the PUE calculated from the Redfield Ratio of C:P, and the maximum reported specific growth for a range of autotrophic microorganism adjusted to 20 °C assuming a Q10 of 2.

C:N:P and PUE of chemoorganotrophic microorganisms and metazoan microzooplankton

Fagerbakke et al. (1996) and Cotner et al. (2010) show that native aquatic and cultured osmoocheroorganotrophic bacteria maintain a C:N:P ratio of 50:10:1, although other work
Table 2. Specific growth rates, atomic C:N:P ratios, and PUE values for growth for Archea, Bacteria, and Eukarya of a range of trophic modes

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cell diameter (µm)</th>
<th>Specific growth rate, mol C increase mot⁻¹ cell C s⁻¹</th>
<th>C:N:P</th>
<th>PUE, mol C increase mot⁻¹ cell P s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic osmochemoorganotrophic bacterium <em>Escherichia coli</em></td>
<td>1.5</td>
<td>283 × 10⁻⁶</td>
<td>50:10:1</td>
<td>14.2 × 10⁻⁵</td>
</tr>
<tr>
<td>Aerobic osmochemoorganotrophic bacterium <em>Escherichia coli</em></td>
<td>1.5</td>
<td>146 × 10⁻⁶</td>
<td>41:11:1</td>
<td>5.99 × 10⁻⁵</td>
</tr>
<tr>
<td>Aerobic osmochemoorganotrophic bacteria from a lake²</td>
<td>7</td>
<td>138 × 10⁻⁶</td>
<td>95:22:1</td>
<td>13.1 × 10⁻³</td>
</tr>
<tr>
<td>Aerobic chemolithotrophic bacterium</td>
<td>1</td>
<td>48 × 10⁻⁶</td>
<td>50:10:1</td>
<td>2.40 × 10⁻³</td>
</tr>
<tr>
<td><em>Alcaligenes eutrophus</em></td>
<td>2</td>
<td>15 × 10⁻⁶</td>
<td>50:10:1</td>
<td>0.75 × 10⁻³</td>
</tr>
<tr>
<td>Anaerobic chemolithotrophic archaean</td>
<td>2</td>
<td>27 × 10⁻⁶</td>
<td>106:16:1</td>
<td>2.86 × 10⁻³</td>
</tr>
<tr>
<td><em>Methanococcus vannieli</em></td>
<td>2</td>
<td>27 × 10⁻⁶</td>
<td>106:16:1</td>
<td>18.0 × 10⁻³</td>
</tr>
<tr>
<td><em>Anoxygenic photolithotrophic bacterium Chlorobium thiosulphatophilum</em></td>
<td>1</td>
<td>24 × 10⁻⁶</td>
<td>106:16:1</td>
<td>2.54 × 10⁻³</td>
</tr>
<tr>
<td><em>Oxygenic photolithotrophic cyanobacterium</em></td>
<td>1</td>
<td>75 × 10⁻⁶</td>
<td>106:16:1</td>
<td>7.95 × 10⁻³</td>
</tr>
<tr>
<td><em>Synechococcus leopoldianum</em> (as <em>Anacystis nidulans</em>)</td>
<td>5</td>
<td>27 × 10⁻⁶</td>
<td>106:16:1</td>
<td>2.86 × 10⁻³</td>
</tr>
<tr>
<td><em>Oxygenic photolithotrophic eukaryote</em></td>
<td>5</td>
<td>11.4 × 10⁻⁶</td>
<td>665:61:1</td>
<td>2.50 × 10⁻³</td>
</tr>
<tr>
<td><em>Thalassiosira pseudonana</em></td>
<td>5</td>
<td>4.72 × 10⁻⁶</td>
<td>220:37:1</td>
<td>3.14 × 10⁻³</td>
</tr>
</tbody>
</table>

Growth rates are from Raven et al. (2013), except where indicated by superscript numbers: †Makino et al. (2003); ‡Makino and Cotner (2004); *Ishimi et al. (2012); †Perry (1976). C:N:P for bacteria other than oxygenic photolithotrophic cyanobacteria from Fagerbakke et al. (1996) and Cotner et al. (2010), except where indicated for cases in which growth rate and C:N:P are available for the same culture in the work of 1Makino et al. (2003) and 2Makino and Cotner (2004). C:N:P for oxygenic photolithotrophic cyanobacteria, and for eukaryotes, are assumed to be the Redfield Ratio, except where indicated: 4Perry (1976). As in Raven et al. (2013), specific growth rates ae normalized to 20 °C assuming a Q₁₀ of 2. Cell diameter is the equivalent spherical diameter, except for *Achlya bisexualis* where the hyphal diameter is given.

gives a more Redfield-like ratio of 94:17:1 (Keiblinger et al., 2010), and large variations in C:N:P are seen under extremes of limitation by the C, the N, or the P substrates for growth (Chan et al., 2012). For soil microbes as a whole (Archaea, Bacteria, Eukarya), the C:N:P ratio is 77:7:1, with the usual large variance (Cleveland and Liptzin, 2007). The bacterial C:P corresponds to one term in the time-related PUE, namely organism DM per unit organism P which is about twice as high for the bacteria as for the algae, so that the PUE defined as the reciprocal of P content in DM is higher for photolithotrophs than for osmochemoorganotrophs. The maximum specific growth rate of osmochemoorganotrophic bacteria is well over twice that of oxygenic cyanobacteria and microalgae, when the rates are normalized to a temperature of 20 °C assuming a Q₁₀ of 2 (Raven et al., 2013). Thus, for the Archaea and Bacteria, the fastest-growing aerobic osmochemoorganotroph has about six times the specific growth rate of the fastest-growing aerobic chemolithotroph (Table 2), and 10 times that of the fastest-growing oxygenic photolithotroph (Table 2), using growth rate data from Raven et al. (2013) and additional data from Makino et al. (2004) and Makino and Cotner (2004). Combining these specific growth rates with the C content per unit organism P suggests that time-based PUE is five times higher for chemoorganotrophs than for oxygenic photolithotrophs (Table 2).

For eukaryotic microorganisms, the fastest-growing aerobic osmochemoorganotrophic organism has more than twice the specific growth rate of the fastest-growing oxygenic photolithotroph; for an aerobic phagochemoorganotroph, the rate is only slightly higher than for the fastest-growing photolithotroph (Table 2), using growth rate data from Raven et al. (2013) and additional data from Perry (1976) and Ishimi et al. (2012). The C:N and C:P of fungal osmochemoorganotrophs and of unicellular phagochemoorganotrophs is similar to that of photolithotrophs (Ho et al., 2003; Quigg et al., 2003, 2011; Gruber et al., 2009; Keiblinger et al., 2010). The PUE of growth of the fastest-growing (Table 1) osmochemoorganotrophs and, by a much smaller margin and probably insignificantly, phagochemoorganotrophs, is higher than that of the fastest-growing oxygenic photolithotrophs (Table 2).

There are also C:N:P data for metazoan phagochemoorganotrophs (e.g. Beers, 1966; Gismervik, 1997). Beers (1966)
examined the elemental composition of nine taxonomically defined groups of metazoan zooplankton from the Sargasso Sea off Bermuda, and found that the range of C:P atomic ratios was 70.8:1 and 195.47:1 (i.e. ranging from below to above the Redfield Ratio value of 106:16:1 of their ultimate food source). It should be noted that the Sargasso Sea is very oligotrophic which may have altered the C:N:P of phytoplankton and hence of zooplankton. Gismervik (1997) examined marine planktonic crustaceans from the Oslofjord and found atomic ratios for C:N:P of between 34.8:1 and 188.40:1. Again, the values range from below to above the Redfield Ratio, and in this case the environment is less oligotrophic. For planktonic crustaceans in the Baltic Sea, Walve and Larsson (1999) found C:N:P ratios varying between 84.14:1 and 289.27:1, again spanning the Redfield Ratio for a more nutrient-rich habitat than the Sargasso Sea. The conclusion is that the C:N:P ratios of marine metazooplankton can be rather below to rather above the Redfield Ratio for a range of availabilities of nutrients to phytoplankton in the environment.

The specific growth rates for metazoan zooplankton, adjusted to a standard temperature of 20 °C using the appropriate Q10 value (Hansen et al., 1997; see also Hirst et al., 2003; DeLong et al., 2010), range from 16 × 10^-6 mol C increase mol^-1 organisms P s^-1 for volumes of 10^5 μm^3 to 3 × 10^-6 s^-1 for organism volumes of 10^8 μm^3 (metazoan entries in fig. 2B of Hansen et al., 1997). Assuming a C:P Redfield Ratio of 106:1, this gives PUEs of 1.7 × 10^-3 mol C increase mol^-1 organisms P s^-1 for a volume of 10^5 μm^3 and 0.32 × 10^-3 mol C increase mol^-1 organisms P s^-1 for a volume of 10^8 μm^3. These PUEs for phagocytizing organotrophs are less than the values for oxygenic photolithotrophs in Table 2. However, the organism volumes for these metazoans are 10^5–10^8 μm^3, while the organism (cell) volumes for the photolithotrophs in Table 2 are <70 μm^3, once more emphasizing the significance of the size of organisms for PUEs. However, for algal cells with a volume of 10^8 μm^3, the growth rate is 4.6 × 10^-6 s^-1 (Finkel et al., 2010) and, assuming a Redfield Ratio of C:P, a PUE of 0.49 × 10^-3 mol C increase mol^-1 organism P s^-1, which is closely similar to that of the metazoan grazer of the same organism volume, namely 0.32 × 10^-3 mol C increase mol^-1 organism P s^-1, perhaps reflecting in PUE the discontinuity noted between protists and metazoans in the size scaling of metabolic rates by DeLong et al. (2010).

Overall, for comparisons within a given range of sizes, microorganisms show higher PUE for chemooorganotrophs than for autotrophs (Table 2). It must, however, be emphasized that these calculations, apart from those based on Perry (1976), Makino et al. (2003), and Makino and Cotner (2004), involve different organisms for the P content (C:P) data and growth rate data within a trophic mode, and also assumptions about the C:N:P ratios for methanogens and for the anoxygenic photolithotroph for which no C:N:P data could be found (Table 2). Clearly, there is a need for measurements of the specific growth rate and C:P ratio for a range of organisms of each trophic mode, each under a range of P availabilities, to test the hypothesized higher PUE for chemooorganotrophs than for autotrophs.

Although there are values for cell size in Table 2, no corrections are made for cell size dependence of specific growth rate of the organisms. The available evidence suggests that a simple linear relationship of specific growth rate to log_{10} cell size with an exponent more negative than ~0.2 is not the rule (Raven, 1994; Bec et al., 2008; Chen and Liu, 2010, 2011; DeLong et al., 2010; Finkel et al., 2010; Sal and López, 2011; Kempes et al., 2012; Marañón et al., 2013). A further point is that the size range in the Bacteria and Archaea considered is rather small (Table 2); the eukaryote cells are larger and have a slightly larger size range.

### C:N:P and PUE in macrophytes

C:N:P ratios were found from analyses of published values for green, red, and brown marine macroalgae plus seagrasses (C 550:N 30:P 1) by Atkinson and Smith (1983), and for seagrasses (C 474:N 24:P 1) by Duarte (1990). In both data sets, the organisms were collected from the natural environment. The higher C:N and C:P ratios for the benthic macrophytes than for microalgae can be related to the consistent presence of cell walls in the macrophytes and, perhaps, to nutrient limitation in the natural environment. In no case do there seem to be have been measurements of C:N:P in the non-photosynthetic roots (seagrasses) or rhizoids (a few macroalgae such as Caulerpa: Raven, 1984b).

For terrestrial vascular plants, the possibility of separating the C:N:P ratio in photosynthetic from that in non-photosynthetic parts has been realized. This allows the estimation of the PUEs of the two parts. Assuming isometric growth (i.e. a constant ratio of contributions to total biomass of the photosynthetic and non-photosynthetic parts during growth; Raven, 2001), the ratio of the PUEs equals the C:P ratios. More data are available for shoots and leaves than for below-ground parts: it is more difficult to obtain complete below-ground structures for plants growing in soil than is the case for leaves, and ease of harvesting complete root systems from hydroponically growth plants must be set against the artificial nature of hydroponic growth for the great majority of terrestrial vascular plants.

Brodley et al. (2004) examined the elemental contents of the shoots of a range of flowering plants and found significant phylogenetic variation, and a mean ratio of C 144:N 17:P 1 (by atoms). While the N:P ratio is closely similar to the values for microalgae (Falkowski, 2000; Geider and LaRoche, 2002; Falkowski and Raven, 2007; Quigg et al., 2011), the C:N and C:P ratio is significantly higher than in the microalgae, presumably related to the presence of a cell wall of high C:N and C:P in all flowering plants, but not in many of the microalgae. Watanabe et al. (2007) found significant phylogenetic variation in the elemental composition of the leaves of flowering plants. These important data sets do not have comparative values for below-ground parts. Wang et al. (2010) present a compilation of meta-analyses of data on C:N:P ratios for leaves (Wright et al., 2004) and fine (<5 mm diameter) roots (Jackson et al., 1997; cf. Gordon and Jackson, 2000). The compilations used by Wang et al. (2010) involve 11 biomes for leaves, while for roots eight of the 11 biomes were lumped together. With this proviso, the mean leaf elemental ratio
is C 924:N 33.2:P 1, and the mean root elemental ratio is C 2229:N 33.2:P 1. The leaf ratios of C:P and N:P are higher than those found by Broadley et al. (2004) and Watanabe et al. (2007), presumably as a result of analysis of more sclerophyllous leaves than for solely mesophytic herbaceous leaves, and/or due to nutrient (N, P) limitation. Interpretation of the leaf and root C:N:P ratios requires that the root data contain meristematic tissues, while the leaf data are almost entirely for mature leaves. It would be expected that the C:N and C:P ratios would be lower in meristematic tissue where protein synthesis, and the necessary RNAs, predominate over the production of mechanical tissue. This means that the C:N and C:P ratios for leaves would be lower if expanding leaves were considered. Interestingly, the C:N:P of total fine roots (live plus dead) is closely similar to that of live fine roots (Jackson et al., 1997), suggesting negligible movement of N and P back to the main roots from senescent fine roots (see Robinson, 1990; Veneklaas et al., 2012) relative to the movement of C, despite the significantly greater fraction of C than of N and P in the unrecoverable cell wall material.

Other, more limited data sets include that of Zheng et al. (2012) on grasslands in Inner Mongolia. Leaves and roots samples from three communities, using only the ungrazed control treatment, show lower C:N:P ratios in the roots than in the leaves, in agreement with the outcome of the analysis by Wang et al. (2010). However, some data obtained using hydroponically grown plants give more mixed results (Allen and Raven, 1987; Allen et al., 1988).

An important paper (Yu et al., 2012) reports specific growth rates and C:N:P ratios for above- and below-ground structures of three species from the Inner Mongolian grasslands; these data, and the derived PUE values, are presented in Table 3. Despite their contrasting phylogenies and ecophysologies (a C₃ and a C₄ grass and a C₁ chenopod), the C:P ratio and the PUE values are much higher for below-ground than above-ground parts for all three species (Table 3). This is also in agreement with the higher PUE in chemoorganotrophic than photolithotrophic structures.

**Conclusion on differences in C:N:P and PUE among trophic modes**

There is some evidence of a lower C:P in chemorganotrophs than in otherwise comparable photolithotrophs, and rather stronger evidence for a higher PUE in osmochemorganotrophs than in photolithotrophs when organisms with the highest PUE in each trophic mode are compared.

**Are there differences in P pools between autotrophs and chemoorganotrophs?**

Is there a systematic difference in nucleic acids, phospholipids, and water-soluble low molecular mass phosphate esters plus metabolically active inorganic phosphate between autotrophs and chemoorganotrophs (Table 4)?

**Nucleic acids: DNA**

Genome sizes can be compared in terms of the number of base pairs and the number of genes. On both of these grounds, the smallest known genome of a chemoorganotrophic bacterium is smaller than the smallest known genome for an oxygenic photolithotrophic bacterium (Raven et al., 2013). However, there is a very large range of sizes of genomes for chemoorganotrophs and for photolithotrophs among Bacteria and among Eukarya, and no general conclusions can be drawn as to the genome sizes of chemoorganotrophs, chemolithotrophs, and photolithotrophs (Raven et al., 2013; see also Hodgson et al., 2010). As is discussed in more detail under differences in P pools among autotrophs, DNA can be <10% of the total nucleic acids.

**Nucleic acids: RNA**

Appealing to optimal allocation of P, Raven (2012) rationalized the lower PUE of some cyanobacteria and leguminous plants when growing on dinitrogen than when growing on

### Table 3. Specific growth rate, mol C: mol N: mol P, and PUE for above- and below-ground parts of an N- and of a P-limited C₃ grass, C₄ grass, and C₁ chenopod

<table>
<thead>
<tr>
<th>Plant and part</th>
<th>Specific growth rate, mol C* increase mol⁻¹ cell C s⁻¹</th>
<th>Mol C: mol N: mol P</th>
<th>PUE, mol C increase mol⁻¹ P s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leymus chinensis above-ground (perennial C₃, Poaceae)</td>
<td>0.66 × 10⁻⁶ (N lim)</td>
<td>258:22:1</td>
<td>0.17 × 10⁻³</td>
</tr>
<tr>
<td>Leymus chinensis below-ground (perennial C₃, Poaceae)</td>
<td>0.60 × 10⁻⁶ (P lim)</td>
<td>258:19:1</td>
<td>0.16 × 10⁻³</td>
</tr>
<tr>
<td>Cleistogenes squarrosa above-ground (perennial C₃, Poaceae)</td>
<td>0.86 × 10⁻⁶ (N lim)</td>
<td>1123:24:1</td>
<td>0.97 × 10⁻³</td>
</tr>
<tr>
<td>Cleistogenes squarrosa below-ground (perennial C₃, Poaceae)</td>
<td>0.79 × 10⁻⁶ (P lim)</td>
<td>698:16:1</td>
<td>0.55 × 10⁻³</td>
</tr>
<tr>
<td>Chenopodium glaucum above-ground (annual C₁, Chenopodiaceae)</td>
<td>0.61 × 10⁻³ (N lim)</td>
<td>185:17:1</td>
<td>0.11 × 10⁻³</td>
</tr>
<tr>
<td>Chenopodium glaucum below-ground (annual C₁, Chenopodiaceae)</td>
<td>0.64 × 10⁻³ (P lim)</td>
<td>152:9:1</td>
<td>0.097 × 10⁻³</td>
</tr>
<tr>
<td>Chenopodium glaucum (annual C₁, Chenopodiaceae)</td>
<td>0.84 × 10⁻⁶ (N lim)</td>
<td>1230:23:1</td>
<td>0.79 × 10⁻³</td>
</tr>
<tr>
<td>Chenopodium glaucum</td>
<td>0.58 × 10⁻⁶ (P lim)</td>
<td>994:16:1</td>
<td>0.58 × 10⁻³</td>
</tr>
</tbody>
</table>

Data are from fig. 5 of Yu et al. (2012).
combined N as related to the diversion of rRNA (and hence P) from other essential functions into synthesis of nitrogenase, including replacement of nitrogenase damaged by oxygen, and to the synthesis of catalysts and structures related to the protection of nitrogenase from oxygen. This effect would be particularly evident when the growth rate was limited by P supply; this is what was found for the effect of N sources in the cases examined by Raven (2012), and see also Sanginga et al. (1989; discussed above). Raven et al. (2013) extend this to the difference between autotrophs and chemooorganotrophs, arguing that more unique highly expressed proteins are involved in autotrophy than in chemooorganotrophy (see Ellis, 1979; Raven, 1984a, b, 1991, 2013a; Geider and La Roche, 2002; Losh et al., 2013). This would mean a lower PUE for autotrophs than for chemooorganotrophs, as was shown above for bacterial and archaean examples. However, rRNA measurements are needed to test the correlation further, and then to attempt to falsify the hypothesis. It is clear that optimal allocation of P in rRNA, at least as indicated by the growth rate hypothesis, is more readily seen in chemooorganotrophs than in photolithotrophs (Sterner and Elser, 2002; Vrede et al., 2002, 2004; Karpinets et al., 2004; Matzek and Vitousek, 2009; Nicklisch and Steinberg, 2009; Flynn et al., 2010; Reef et al., 2010, 2012; Loladze and Elser, 2011). rRNA requirements will be returned to below in the context of the possibility of variations with pathways among autotrophs.

Phospholipids

Phosphorylated lipids are very widespread among the polar lipids of biological bilayer membranes, regardless of whether the lipids have an ether (Archaea) or ester (Bacteria, Eukarya) bond between the fatty alcohol (Archaea) or fatty acid (Bacteria, Eukarya) and glycerol. However, there are a number of instances of dominance of glycolipids, for example in methanogenic Archaea (Koga et al. 1993), and of glycolipids, namely the galactolipids monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) and the sulpholipid sulphoneosyldiacylglycerol in photosynthetic membranes of some anoxygenic photosynthetic bacteria, and almost all oxygenic photolithotrophs (Raven, 1989; Imhoff and Bais-Imhoff, 1995; Selstam and Campbell, 1996; Rexroth et al., 2011; Yuzawa et al., 2012; Mizoguchi et al., 2013).

Under P deficiency, an anoxygenic photosynthetic bacterium (Benning et al., 1993, 1995), freshwater cyanobacteria and eukaryotic algae (Khozin-Goldberg and Cohen, 2006; Bellinger and van Mooy, 2012), some marine cyanobacterial and eukaryotic phytoplankton (Van Mooy et al., 2006, 2009;...

<table>
<thead>
<tr>
<th>Role of P</th>
<th>Relationship to C assimilation pathway</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>RNA</td>
<td>Organisms with C assimilation pathways with higher rates of CO₂ assimilation per unit protein committed to the pathway have less protein to allocate to other growth-related processes. Under P limitation, with protein synthesis limited by rRNA availability, the PUE of growth (rate of biomass gain per unit P in biomass) would decrease when the subsistence quota for P is approached. Savings of up to 10% are possible for CCM relative to CO₂ diffusion, or for antennae based on chlorophyll/carotenoid pigment–protein complexes relative to phycobiliproteins</td>
<td>Raven (1984a, b, 2011); Flynn et al. (2010); Bar-Even et al. (2010, 2012); Raven et al. (2012); Veneklaas et al. (2012)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Replacement of phospholipid by galactolipid and/or S-lipid might alter permeability to CO₂ with implications for diffusive CO₂ entry, in parallel with any aquaporin-like CO₂ channels, in C₃ plants and in CO₂ entry for cyanobacterial CCMs, and for leakage from CCMs. Not quantifiable, as there are no comparative data on the CO₂ permeability of galactolipid and/or S-lipid membranes and for phospholipid membrane.</td>
<td>Raven et al. (2012); Veneklaas et al. (2012, in supplementary information)</td>
</tr>
<tr>
<td>P-esters (low molecular mass water-soluble P esters)</td>
<td>Possible saving on P in organism with C assimilation pathways involving fewer P-esters could have a higher PUE of growth. There is little scope for economizing on P in P-deficient organisms by decreasing P-ester concentrations, since this would apparently require higher enzyme content, with a corresponding increase in the requirement for RNA for protein synthesis</td>
<td>Vaneklaas et al. (2012, in supplementary information)</td>
</tr>
<tr>
<td>Regulation of expression of C assimilation pathway</td>
<td>Possible decreased P requirement for growth if decreased P availability causes expression of a more P-efficient pathway, or variant on a pathway; not readily quantified</td>
<td>Raven et al. (2012) (only relates to the phenomenon, not the specific mechanism mentioned)</td>
</tr>
</tbody>
</table>

Table 4. Outline of possible interactions of P with the autotrophic CO₂ assimilation pathway
Martin et al., 2011; Popendorf, 2011a, b; Dyhrman et al., 2012), and flowering plants (Lambers et al., 2012; Veneklaas et al., 2012; Okazaki et al., 2013) replace phospholipids in non-photosynthetic membranes with the glycolipids common in photosynthetic membranes, namely sulpho- and galactolipids (Table 4). The functional results of such replacements, in, for example, the permeability of the lipid bilayer membrane, and the function of integral membrane proteins, are not yet clear (supplementary information in Veneklaas et al., 2012).

Such substitution of phospholipids is not limited to autotrophs, Minnikin et al. (1974) found that P limitation of the chemoorganotroph Pseudomonas diminuta led to the replacement of acidic phospholipid by acidic glycolipids, while the chemoorganotroph Bacillus acidocaldarius has constitutive replacement of phospholipid by sulpholipid (Langworthy et al., 1976), as does Bacillus coahuilensis from a very P-limited habitat (Souza et al., 2008). Sulpholipid has also been reported from the chemooorganotrophic bacterium Magiciculis spp. (Abraham et al., 1999). Furthermore, Popendorf et al. (2011a) showed a significant content of MGDG in chemooorganotrophic marine bacteria from the low-P Sargasso Sea, and suggested that the occurrence of MGDG in chemoorganotrophs is a function of P deficiency. Lópeze-Lara et al. (2005) and Geske et al. (2012) found similar results for the Proteobacteria Sinorhizobium meliloti and Agrobacterium melanoli, respectively, while sulpholipid occurs constitutively in some members of the proteobacterial Rhizobiaceae (Cederberg and Hollingsworth, 1994). The occurrence of substitutes for phospholipids in the Rhizobiaceae is, as pointed out by Benning (1998), perhaps not surprising since some members of the Rhizobiaceae are photosynthetic. It should be emphasized that the distinction between chemoorganotrophs and photolithotrophs is not absolute, with many photosynthetically competent organisms functioning as mixotrophs, for example the phagomixotrophs in aquatic habitats (Flynn et al., 2013) and a range of mixotrophs on land (Schmidt et al., 2013). In the context of P, plastic as well as aplastic small protists in the North Atlantic tropical gyre obtain much of their P by phagotrophy, rather than uptake of inorganic phosphate across the plasmalemma (Hartmann et al., 2011).

It would be particularly interesting in the context of economizing on P in autotrophs to know if there is any replacement of phospholipid by glycolipids (including sulpholipids) in mycorrhizal fungi, either constitutively or in response to P deficiency. This applies especially to arbuscular mycorrhizas which have a particular role in P acquisition (Smith and Read, 2008). However, there seem to be no relevant data for the extraradicular hyphae of mycorrhizas, although there are data for the glycolipid and phospholipid content of vesicles of an arbuscular mycorrhizal fungus (Jabaji-Hare et al., 1984). In soils of very low P availability, the relative utility of the alternatives of arbuscular mycorrhizas and of cluster roots (Lambers et al., 2008, 2010) might be influenced by differences in the capacity to recover P to the longer lived parts of the plant from senescent cluster roots or arbuscular mycorrhizas. Plasmalemma lipids would be particularly difficult to resorb since resorption requires maintenance of plasmalemma functions. An analogous, but better documented, situation is found with N acquisition by ectomycorrhizas (Smith and Read, 2008): here the non-recoverable N-containing component is chitin in the fungal cell walls with a C:N ratio of eight, while the equivalent structural element in the plant structures, cellulose, contains no N.

**Low molecular mass water-soluble phosphate esters**

There are at least five autotrophic CO₂ fixation pathways found in Archaea and Bacteria, in addition to the Benson–Calvin cycle (the C-reduction cycle) found in many bacterial autotrophs as well as in all photosynthetic eukaryotes (Raven, 2009; Bar-Even et al., 2010, 2012; Raven et al., 2012). These pathways have a variable number, sometimes zero, of low molecular mass water-soluble phosphate esters, which are unique to that pathway. Do these additional phosphate esters add significantly to the total content of these low molecular mass water-soluble phosphate esters per unit biomass? Data only seem to be available for organisms with the Benson–Calvin cycle. Bielecki (1968a, b; see also Chapin and Bielecki, 1982; Chapin et al., 1982) examined these phosphate esters in the duckweed Spirodela: his results showed that any contributions made by the specific phosphate esters ribulose-1,5-bisphosphate and sedoheptulose-1,7-bisphosphate, which are not involved in the oxidative pentose phosphate cycle or glycolysis, was very small, since they are included in the ‘other compounds’ not listed by name which amount to 7.5% of the total low molecular mass water-soluble phosphate esters. Despite this small contribution of ribulose-1,5-bisphosphate to the total pool of phosphate esters, it is worth pointing out the large range of K₉₅ values of Rubisco (ribulose bisphosphate carboxylase-oxygenase) for ribulose-1,5-bisphosphate of a wide range of green algae and aquatic and terrestrial embryophytes: the range is from 10 mmol m⁻³ for Clematis sp. to 136 mmol m⁻³ for Stipa mollis (Yeoh et al., 1981). This range of values relates to phylogeny rather than to the environment or the photosynthetic pathway. Regardless of the cause of the genetically determined variation, it is clear that some plants can potentially carry out photosynthesis at the same rate as others but with less than a tenth of the concentration of ribulose-1,5-bisphosphate, regardless of whether the photosynthetic apparatus usually functions with Rubisco-saturating concentrations of ribulose-1,5-bisphosphate or whether the concentration of this substrate is typically near the concentration corresponding to K₉₅. This argument requires that there is no trade-off between affinity and the substrate-saturating rate for Rubisco for ribulose-1,5-bisphosphate of the kind found for CO₂ (Tcherkez et al., 2006) (Table 4).

For observed K₉₅ values of phosphate esters for their enzymes, there is a potential trade-off in (optimal) P allocation between low molecular mass water-soluble phosphate esters and rRNA (Veneklaas et al., 2012). For those low molecular mass water-soluble phosphate esters that are present at concentrations which are subsaturating for the enzyme(s) for which they are substrate(s), maintenance of the metabolic flux through the pathway(s) involving the enzyme(s) if the concentration of the low molecular mass water-soluble...
phosphate ester substrate(s) is decreased would require a compensatory increase in the content of the enzyme(s) and hence (with optimal allocation) of the rRNA needed to synthesize the increased content of enzymes (Veneklaas et al., 2012). There is unlikely to be a decreased P requirement for metabolism from such a decrease in the concentration of low molecular mass water-soluble phosphate esters. If, however, the low molecular mass water-soluble phosphate esters are well above the saturating concentrations for the enzymes for which they are substrates, then the concentration of those particular low molecular mass water-soluble phosphate esters could be decreased without decreasing the flux through the pathway(s) or needing synthesis of additional enzyme protein and hence of the additional rRNA. Of course, it is possible that a certain concentration of low molecular mass water-soluble phosphate esters in compartments such as the cytosol, plastid stroma, and mitochondrial matrix might be needed for some reason apart from their role as enzyme substrates or activity modifiers, for example as acid–base buffers (Raven, 2013b) (Table 4).

Conclusions on differences in P pools between autotrophs and chemoorganotrophs

Osmochemorganotrophic bacteria have a smaller minimum genome size than do autotrophic bacteria, but with a large range of genome sizes in both trophic groups. RNA content is probably more closely related to growth rate than in photoheterotrophs. Replacement of phospholipids by glycolipids is more common in photoheterotrophs than in chemoorganotrophs. There is no evidence of differences in allocation to low molecular mass water-soluble phosphate esters in chemoorganotrophs relative to autotrophs.

Are there differences in phosphorus pools among autotrophs?

Is there a systematic difference in nucleic acid, phospholipids, and water-soluble low molecular mass phosphate esters plus metabolically active inorganic phosphate among autotrophs depending on the autotrophic pathway used?

Nucleic acids: DNA

The smallest known genome of oxygenic photoheterotrophs is for a strain of the very small (0.5 μm equivalent spherical diameter) marine planktonic cyanobacterium Prochlorococcus marina which expresses a CO₂-concentrating mechanism (CCM) and which occurs in very low-P habitats (Raven et al., 2013). For photoheterotrophic eukaryotes, the smallest gene number (but not the smallest genome) is for the acidophilic red microalga Cyanidiopsiszcyon merolae; rather smaller genomes, and slightly greater gene numbers are found in Ostreococcus spp., some strains of which grow in low-P environments (Raven et al., 2013).

Greilhuber et al. (2006) suggest that the smallest known genome size among flowering plants is that of Genlisea margaretae (Lentibulariaceae), a C₃ carnivorous plant from low-P environments, with 63 Mbp; although Soltis et al. (2003) report the lower value of 49 Mbp for Cardamine amara (Brassicaceae), Johnston et al. (2005) cite a value of 221 Mbp for this species. For another family tolerant of low P availability, the Proteaceae, genome sizes for six species range from 807 Mbp to 946 Mbp (Soltis et al., 2003; Morgan and Westoby, 2005), which is smaller than the largest genome of the carnivorous family Lentibulariaceae (1620 Mbp). Relating genome size to tolerance of low P availability is very much a ‘fits where it touches’ exercise.

For multicellular plants such as those considered in the previous paragraph, the positive correlation of genome size, and hence nucleus size, with cell size (see Soltis et al., 2003; Connolly et al., 2008) means that, for a plant of a given size, more P per nucleus does not necessarily mean more P in DNA per plant since there are fewer, larger cells. Zhu et al. (2005) showed that there was an increased copy number of rRNA genes with increasing cell size in phytoplankton, since larger cells need more copies of the genes for highly expressed proteins to supply protein in the larger growing cells which have a very small specific growth rate decrement with increasing cell size (see fig. 3 of Finkel et al., 2010). It would be of interest to see if there is a correlation of rRNA copy number in the genome with cell size. There are necessarily relatively few highly expressed proteins, since few proteins from a proteome of thousands of different proteins can be highly expressed, defining highly expressed proteins (or functionally related groups of a few proteins) as those contributing >1% of all proteins (Raven et al., 2013). This argument also applies to coenocytic organisms, such as the glomeromycote arbuscular mycorrhizal fungi, some of which have very small (for eukaryotes) genomes—as little as 15 Mbp (Hijri and Sanders, 2004a, b). This cannot necessarily be used to indicate that arbuscular mycorrhizas represent a means of P acquisition with minimal P use in DNA, since the small genome size may well be offset by a greater number of nuclei per unit of fungal biomass.

Soltis et al. (2003) point out that basal angiosperms, and many of the basal members of major flowering plant clades, have small genomes with a general increase through polyploidy and, especially, proliferation of retrotransposons, although there are examples of decreasing genome size in several clades. As well as these phylogenetic signatures in genome size, there are ecophysiological considerations other than P limitation related to small genome sizes; an example is the smaller size of stomatal guard cells with smaller genomes, permitting more rapid opening and closing even if the change from Fick’s law diffusion to Knudsen diffusion (where the width of stomatal pores is less than the mean free path of gas molecules) requires smaller guard cells than are likely from the smallest known plant genomes (Hodgson et al., 2010). The conclusion on gene number and genome size is that there is little evidence that economizing on P is a significant selective pressure in minimizing genome size, perhaps because of the generally small fraction (<5%) of non-storage P which is in DNA (see Raven et al., 2013, the section ‘Differences among P pools in autotrophs and chemoorganotrophs?’, and the following paragraph).
As a link to the discussion below on RNA, it should be pointed out that RNA is typically thought to account for most of the total nucleic acid pool. Bieleski (1973) gives a typical RNA:DNA ratio of 10 in young flowering plant leaves. However, there are reports of much lower RNA:DNA ratios. Lourenço et al. (1998) found a RNA:DNA ratio of ~1 in the small marine cyanobacterium Synechococcus, and Bertilsson et al. (2003) showed that P-limited cultures of the very small marine cyanobacterium Prochlorococcus had over half of the cellular P in DNA, so that the RNA:DNA ratio must be significantly less than 1 to accommodate the very small cell P allocation to phospholipid (van Mooy et al., 2006, 2009) and the unknown allocation to low molecular mass water-soluble phosphate esters and metabolically active inorganic phosphate. This large fraction of the nucleic acid pool which is DNA in these very small cells might relate to the fraction of the cell taken up by the admittedly very small genomes (Raven, 1994; Raven et al., 2013), although such effects would probably only be significant in smaller cells.

Reef et al. (2010) found a RNA:DNA ratio of 0.8–17 in the vascular cambium, and 5.3–14.3 for leaves, of mangroves. Reef et al. (2012) found RNA:DNA ratios of 1.5–8 for macroalgae as they were collected from a coral reef (i.e. an oligotrophic habitat), while the range became 6–8 for these algae when nutrient enriched. Nicklisch and Steinberg (2009) measured RNA:DNA ratios in eukaryotic microalgae, but only give relative units so true RNA:DNA ratios cannot be determined. In all three of these latter data sets, the RNA:DNA ratio increases with growth rate, but there are interspecific differences in the RNA:DNA ratio as a function of growth rate.

**Nucleic acids: RNA**

The possibility here is that there is a smaller allocation of P to rRNA relating to autotrophy if there is a smaller requirement for protein synthesis in some variants of autotrophy, especially with highly expressed proteins such as Rubisco and the family of apoproteins of light-harvesting pigments.

For the apoproteins of light-harvesting pigments, there is a larger mass of protein per mole of chromophore in phycobiliproteins than when the chromophores are chlorophylls and carotenoids (Raven, 1984a, b). This greater requirement for apoprotein on a mole of chromophore basis means that, other things being equal, phycobilin-containing algae (cyanobacteria, glaucocystophytes, rhodophytes, and cryptophytes) would be expected to have a greater allocation of RNA to phycobiliprotein synthesis than is the case for apoproteins of algae with chlorophyll and carotenoid chromophores. There seem to be no data with which this prediction can be tested. For Rubisco, an obvious case is that of oxygenic photosynthetic organisms using a CCM to supply CO₂ to Rubisco at a higher steady-state concentration than would be the case with diffusive supply of CO₂ from an air equilibrium bulk aqueous phase for aquatic organisms, or the bulk atmosphere for terrestrial organisms. This permits, as shown below, a lower content of Rubisco per unit total protein or per unit biomass and, when the additional protein related to the CCM is offset against the decreased protein resulting from a much lower expression of the photorespiratory C oxidation cycle, there is a lower overall requirement for protein related to autotrophic inorganic C assimilation.

The enzyme Rubisco is the core autotrophic carboxylase in all oxygenic photosynthetic organisms, and is involved in >99.5% of the inorganic C assimilated in primary producers (chemolithotrophs as well as photolithotrophs) (Johnston et al., 2009; Raven, 2009) with a global annual flux of at least 10 Pmol CO₂ handled by Rubisco (Field et al., 1998; Raven, 2009, 2013a). This flux requires a large quantity of Rubisco as a result of the enzyme’s low substrate-saturated specific catalytic rate on a protein mass basis, a CO₂ affinity which means that the carboxylation activity is not saturated with CO₂ at the present atmospheric concentration, and expression of oxygenase as well as carboxylase activities (Tcherkez et al., 2006; Savir et al., 2010). There are major constraints on how these properties can vary independently among the range of Rubiscos (Tcherkez et al., 2006; Savir et al., 2010; Young et al., 2012).

A major difference in the quantity of Rubisco in photosynthetic organisms results from a reliance on diffusive entry of CO₂ from the medium to Rubisco rather than the occurrence of a variety of CCMs. In general terms, the former mechanism (‘C₃ physiology’) involves a large quantity of Rubisco with a low substrate-saturated specific reaction rate and a high affinity for CO₂ and a high CO₂:O₂ selectivity, with a total Rubisco CO₂-saturated catalytic activity in the organism several times the rate of photosynthesis in the present atmosphere which is set by the light-saturated catalytic capacity of the thylakoid reactions (Tcherkez et al., 2006; Raven et al., 2012). The latter mechanism (CCM) involves a smaller amount of Rubisco with a higher substrate-saturated specific reaction rate, a lower affinity for CO₂, and a lower CO₂:O₂ selectivity, and a total Rubisco CO₂-saturated catalytic activity in the organism similar to that needed for the light-saturated rate of photosynthesis in the present atmosphere and the light-saturated catalytic capacity of the thylakoid reactions (Tcherkez et al., 2006; Raven et al., 2012). Table 5, which is an extended version of table 1 in Raven (2013a), gives the Rubisco content for a range of oxygenic photosynthetic organisms on the basis of Rubisco protein as a fraction of total protein (for cyanobacteria and algae) and Rubisco N as a fraction of total leaf N (for vascular land plants).

How can the leaf Rubisco N as a fraction of the total leaf N values for the vascular plants in Table 4 best be compared with whole-organism (whole-cell) Rubisco protein as a fraction of total protein values for cyanobacteria and algae? Rubisco N on a leaf blade N basis is less than Rubisco N as a fraction of leaf protein N, to an extent indicated in Spinacia oleracea by the finding that, by the approximation that the ‘thylakoid’ plus ‘soluble’ N fractions as a minimum estimate of protein, 0.6–0.8 of leaf N is protein, with the range relating to variation in N and light availability. Working in the opposite direction, protein occurs in the rest of the plant where the fraction of Rubisco protein, contributed by green reproductive structures, aerial stems, and petioles, is much less than in leaf blades. Equating, as a first approximation, total organic N as an (over) estimate of protein, organic N in
the rest of the plant is 0.22–0.40 and 0.34–0.53 of the total in the plant in *Ricinus communis* (Allen and Raven, 1988) and *Phaseolus vulgaris* (Allen et al. 1989); in each case, the range is a function of the N source. The indications from this limited comparison are that leaf blade organic N can be 0.47–0.78 of the total plant organic N, while leaf blade protein is 0.6–0.8 of the total leaf N, so the expression of Rubisco N as a fraction of leaf blade N is likely to overestimate Rubisco N as a fraction of total plant protein. However, a more detailed analysis is needed before it can be concluded that the Rubisco N:leaf N ratios needs downward correction for comparison with the Rubisco:total organism protein ratios in Table 5. A further complication is that there are processes in vascular plants which have no clear analogue in cyanobacteria and microalgae: an obvious example is long-distance transport, which adds to the protein budget of vascular plants.

While Table 4 has no data for plants using the crassulacean acid metabolism (CAM) variant of CCM, there are estimates of Rubisco protein as a fraction of soluble protein (not total protein) in the facultative CAM bromeliad *Guzmania monostachia*, with Rubisco as 0.35 of soluble protein in plants expressing C3 and 0.43 in plants expressing CAM (Maxwell, 2002), although Rubisco protein on a leaf area basis is lower in the CAM than in the C3 physiology phenotype. Niewiasomska et al. (2011) examined the CAM and C3 physiology phenotypes of the facultative CAM dicotyledon *Mesembryanthemum crystallinum* and found that the CAM phenotype had a smaller fraction of soluble protein from leaves contributed by Rubisco than did the C3 phenotype. Further work is clearly needed to provide data on CAM plants which are comparable with the values in Table 4 for other CCM organisms.

The smaller quantity of Rubisco in photolithotrophs using CCMs is often reflected in a greater N-use efficiency (NUE; defined, similarly to PUE defined above, as the rate of biomass gain per unit N in the organism) in C4 plants (Long, 1999; Sage and Zhu, 2011), and predicted (with support from the very limited relevant data available) for cyanobacteria and algae with CCMs (Raven, 1991; Raven et al., 2012). This greater NUE means that the decreased content of Rubisco, and of the machinery used in the photorespiratory C oxidation cycle(s) in organisms with CCMs than in C3 terrestrial plants, is not quantitatively offset by the need for additional N in the catalysts and structures required by CCMs. On the basis of optimal allocation of P (as used above), it would be predicted that less rRNA is needed in the production of

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**Table 5. Rubisco protein as a fraction of total protein and Rubisco N as a fraction of total N for cyanobacteria, algae, and plants with different inorganic C acquisition mechanisms**

<table>
<thead>
<tr>
<th>Organisms, carbon assimilation pathway</th>
<th>Rubisco N as fraction of total N</th>
<th>Rubisco protein as fraction of total protein</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>C3 physiology flowering plants</td>
<td>0.095–0.28 (leaves)</td>
<td></td>
<td>Lowest value is for a shade-adapted plant</td>
<td>Evans (1989)</td>
</tr>
<tr>
<td>C3 physiology flowering plant</td>
<td>0.10–0.26 (leaves)</td>
<td></td>
<td>Range is for low to high N supply to plants</td>
<td>Sage et al. (1987)</td>
</tr>
<tr>
<td>Chenopodium album</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Five species of C3 physiology flowering plants</td>
<td>0.158–0.259 (leaves, low CO2)</td>
<td></td>
<td>Low CO2: 300 ppm</td>
<td>Sage et al. (1989)</td>
</tr>
<tr>
<td></td>
<td>0.120–0.217 (leaves, high CO2)</td>
<td></td>
<td>High CO2: 900 ppm</td>
<td></td>
</tr>
<tr>
<td>C3 physiology conifer</td>
<td>0.087–0.309 (leaves)</td>
<td></td>
<td>Lower values for older needles low in the canopy, higher values for younger needles high in the canopy</td>
<td>Warren and Adams (2001)</td>
</tr>
<tr>
<td>Pinus pinaster</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3-NAD-ME dicotyledon</td>
<td>0.040–0.080</td>
<td></td>
<td>First value is for low N supply, second value is for high N supply</td>
<td>Sage et al. (1987)</td>
</tr>
<tr>
<td>Amaranthus retroflexus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seven C3-NAD-ME monocotyledons</td>
<td>0.040–0.129; 0.042–0.084</td>
<td></td>
<td>First range is for low N supply, second range is for high N supply</td>
<td>Ghannoum et al. (2005)</td>
</tr>
<tr>
<td>Seven C3-NADP-ME monocotyledons</td>
<td>0.043–0.088; 0.044–0.074</td>
<td></td>
<td>First range is for low N supply, second range is for high N supply</td>
<td>Ghannoum et al. (2005)</td>
</tr>
<tr>
<td>Cyanobacteria and microalgae, probably all with CCMs</td>
<td>0.024–0.120 (0.16, 0.23)</td>
<td></td>
<td>Range is for 15 values, many of high and low CO2 cultures of the same organism, with two higher values Some values involve an assumed value for chlorophyll a per total pigment</td>
<td>Raven (1991)</td>
</tr>
<tr>
<td>Microalgae: five species of marine diatoms, two species of marine prymnesiophytes, one species of freshwater green, all with CCMs</td>
<td>0.03–0.06; &lt;0.025</td>
<td></td>
<td>Range is for nutrient-replete laboratory cultures; values for field cultures and nutrient-limited laboratory cultures are all below 0.025</td>
<td>Losh et al. (2013)</td>
</tr>
</tbody>
</table>
proteins related to inorganic C acquisition and assimilation in CCM organisms than in those with C3 physiology, so leaving more for the synthesis and other proteins and hence a higher overall PUE. This is considered below under ‘Differences in phosphorus-use efficiency as a function of autotrophic pathway’, with the conclusion that there is no clear evidence for such a higher PUE in C4 plants.

More work is needed, with due allowance made for phylogenetic bias among the organisms examined, since the possibility of a high PUE for photosynthetic organisms with CCMs could have global biogeochemical implications, as will now be outlined.

Consideration of the global quantitative significance of Rubisco requires that the lower fraction of Rubisco in algae and vascular plants with CCMs is considered in relation to the contribution of these organisms to global primary productivity. Essentially all of the net marine planktonic primary productivity of at least 47.5 P g C year\(^{-1}\) (Field et al., 1998; Raven, 2009) involves organisms with CCMs, and CCMs also function in many of the contributors to coastal benthic net primary productivity (~1 P g C year\(^{-1}\); Field et al., 1998). CCMs are as common in polar as in warmer waters (Mitchell and Beardall, 1996; Beardall and Roberts, 1999; Raven et al., 2002a, b; Cassar et al., 2004; Tortell et al., 2006; see also Henley et al., 2012), although the arguments as to the smaller, or absent, competitive advantage of CCMs at low temperatures used for terrestrial C4 plants (Long, 1999; Sage and Zhu, 2011) also apply to marine primary producers (Raven et al., 2002a, b). On land, Field et al. (1998) suggest a net global primary productivity of 56 P g C year\(^{-1}\). Global values for natural abundance stable C isotope fractionation suggest that 21% of terrestrial primary productivity is due to C4 plants (Lloyd and Farquhar, 1994). Ehleringer et al. (1997) based their conclusion that C4 plants contribute 18% to global terrestrial primary productivity on estimates of productivity per unit area of the various habitats, the fraction of the productivity attributable to the C3 and C4 photosynthesis, and the total area of the habitats. The agreement between the two outcomes is extremely satisfactory (if not surprising), granted the different assumptions involved. There are non-C4 terrestrial plants with CCMs, namely CAM vascular plants and terrestrial free-living and lichenized algae and hornworts, which would have made a contribution to the low C isotope discrimination (C4 plants) category. However, in view of the possible variance of the estimates by Lloyd and Farquhar (1994) and by Ehleringer et al. (1997), the 3% difference between the estimates should not be attributed to the terrestrial photosynthetic organisms, other than C4 plants, with CCMs.

The analysis in the preceding paragraph suggest that at least half of global primary productivity involves CCMs, and the organisms with CCMs have less Rubisco per unit biomass, and a smaller fraction of total protein allocated to Rubisco, than in C3 terrestrial plants. The values in Table 4 suggest that the organisms with CCMs generally have less than half of the Rubisco content of terrestrial C3 plants, so that the global quantity of Rubisco is, then, less than three-quarters, and possibly less than five-eighths, of what would been the case if all primary producers using Rubisco had the quantity of Rubisco found in C3 terrestrial plants. Again appealing to optimal allocation of P, this lower global Rubisco content should decrease the global use of P in rRNA. However, the limited data on PUE (as a surrogate for the allocation to RNA, the major single use of metabolically active P in photosynthetic organisms of organisms with and without CCMs) do not, as discussed above, show uniform differences between the two metabolic types.

Could there be any alternative autotrophic CO\(_2\) assimilation pathways which function in oxygenic photosynthetic organisms in the present atmosphere with a lower requirement for protein in the CO\(_2\) assimilation pathway, and hence for P in rRNA? Of the five well-characterized alternatives to Rubisco and the Benson–Calvin cycle (Raven, 2009; Bar-Even et al., 2010, 2012; Raven et al., 2012), some are ruled out by their O2 sensitivity or their low affinity for inorganic C (Raven, 2009; Bar-Even et al., 2010, 2012; Raven et al., 2012). All of the alternative pathways have a lower running cost (mol ATP and mol NADPH per mol CO\(_2\) assimilated) than the Rubisco-based system when account is taken of the energetic costs of the photorespiratory C oxidation pathway (PCOC) related to diffusive CO\(_2\) entry, or of a CCM (Raven, 2009; Bar-Even et al., 2010, 2012, Raven et al., 2012). An important aspect of the energetic running costs is that organisms with CCMs do not have complete suppression of the PCOC, for example Lacuesta et al. (1997) for a C4 flowering plant and Roberts et al. (2007) showing significant quantitative differences in the flux through glycolate and glyoxylate in two species of diatom from the genus Thalassiosira with indistinguishable inorganic C concentration dependence of autotrophic inorganic C assimilation. Bar-Even et al. (2012) point out that lower energy inputs mean that the overall reaction sequence is closer to thermodynamic equilibrium, with a greater extent of back-reactions and hence a greater need for enzyme protein than for a reaction sequence further from thermodynamic equilibrium. Bar-Even et al. (2012) also point out that the enzymes with the lowest specific reaction sequence in the pathway are typically carboxylases, so it is not just Rubisco-based autotrophic CO\(_2\) assimilation pathways which have a high protein allocation to carboxylases. The enzyme phosphoenolpyruvate carboxylase (PEPc), the widespread anaplerotic carboxylase, which is also, through gene duplication and different regulatory properties of the resulting protein, the initial carboxylase of C4 photosynthesis, has a high substrate-saturated specific reaction rate; however, the content of PEPc in leaves of the C4 plant Amaranthus retroflexus is half that of Rubisco, although the PEPc value is not corrected for the anaplerotic isoform of the enzyme (Sage et al., 1987).

In terms of the combination of CO\(_2\) affinity, O2 insensitivity, and energetic running costs, the most plausible alternative to the Rubisco-based system is the 3-hydroxypropionate bicycle (Bar-Even et al., 2010, 2012). However, even this pathway would only represent a decrease in protein requirement for a given rate of CO\(_2\) assimilation to about two-thirds of that needed for the Rubisco-based system with the PCOC (Bar-Even et al., 2010, 2012), and a rather smaller decrease in protein requirement relative to a Rubisco-based system with a
CCM (Raven, 1991, 2013a; Long, 1999; Sage and Zhu, 2011). Such decreases in protein requirement may mean, with optimal allocation of P, a lower RNA content needed to support protein synthesis, with a predicted increase in PUE. However, we have seen above that the available data on PUE in organisms with and without CCMs did not support this optimality-based prediction.

A further aspect of possible variations in RNA, and hence P, costs among autotrophs is the rate of protein turnover: faster protein turnover means a greater requirement for P in RNA. Bulk protein turnover rates in oxygenic photolithotrophs were measured, and reviewed, by Quigg and Beardall (2003). Pre-proteomic analyses of turnover of individual proteins focused on the D1 protein (psbA gene product) and associated proteins in the reaction centre of photosystem II (Quigg and Beardall, 2004; Raven, 2011, 2012) and to Rubisco in C₃ and C₄ plants (Ferreira and Davies, 1987a, b; Esquivel et al., 1998; Irving and Robinson, 2006a, b). Proteomics has allowed the simultaneous measurements of the turnover of ≥10 proteins in prasinophyccean (Ostreococcus: Martin et al., 2012) and chlorophyccean (Chlamydomonas: Mastrobuoni et al., 2012) green microalgae. Minimizing the RNA, and hence P, cost of protein turnover requires as constant as possible an environment, for example the absence of high irradiance episodes that could cause photodamage to photosystem II (e.g. the strains of Prochlorococcus that live at the deep chlorophyll maximum of low-latitude low-P regions of the ocean; Raven et al., 2013).

Clearly, more work is needed to test the relationship between growth rate, protein turnover rate, and rRNA content. However, it is important to recall that the relationship of rRNA content and net protein synthesis rate in algae and cyanobacteria does not, in many cases, agree with the growth rate hypothesis and optimal allocation of P (Nicklisch and Steinberg, 2009; Flynn et al., 2010), in contrast to many chemooorganotrophic microorganisms (Karpinets et al., 2006).

Low molecular mass water-soluble phosphate esters

We have seen in the comparison of autotrophs with chemooorganotrophs that the six most common autotrophic CO₂ fixation pathways found in Archaea, Bacteria, and Eukarya (Raven, 2009; Bar-Even et al., 2010, 2012; Raven et al., 2012) have a variable number, sometimes zero, of low molecular mass water-soluble phosphate esters that are unique to that pathway. The discussion above suggests that this variation is likely to have a very small impact on the total low molecular mass water-soluble phosphate ester component of the total P requirement of autotrophic organisms. C₄ and CAM plants have the involvement of the additional C₄ carboxylic acid ester phosphoenolpyruvate in the autotrophic inorganic C assimilation mechanism compared with the photosynthetic carbon reduction cycle with diffusive CO₂ entry to Rubisco or a CCM based on active transport across membranes of an inorganic C species or of protons. However, phosphoenolpyruvate is also produced universally in oxygenic photolithotrophs in glycolysis or by phosphate,pyruvate dikinase, and consumed in the production of pyruvate or in anaplerotic aspects of anabolism, so it is ubiquitous in the cells of oxygenic photolithotrophs.

It is therefore unlikely that the difference among CCMs, and between CCMs and CO₂ diffusion, will be reflected in a significant influence on the pool size of low molecular mass water-soluble phosphate esters.

Differences in phosphorus-use efficiency as a function of autotrophic pathway

There are very few data sets obtained with the objective of investigating PUE of the metabolism or growth of autotrophs as a function of the autotrophic pathway used, and they all involve the comparison of C₃ and C₄ flowering plants. Halsted and Lynch (1996) examined 12 species, four C₄ monocots (all grasses, of which only three species were used in comparisons because the other showed extreme P limitation responses), two C₃ monocots (both grasses), a C₃-C₄ intermediate monocotyledon (a grass), two C₄ dicotyledons, two C₃ dicotyledons, and a C₃-C₄ intermediate dicotyledon. The C₃ species had a larger dry weight than the C₄ species when grown for 7.5 weeks under P-replete conditions, but there was no significant difference in DM accumulation under P-limiting conditions. No whole-plant P concentrations are given, so PUE as the rate of DM gain per unit plant P could not be calculated. However, the growth rate of C₄ plants was less limited by P deficiency than was the case for C₃ plants. Photosynthetic PUE (PPUE), with the units of μmol CO₂ fixed s⁻¹ g⁻¹ (or mol⁻¹) leaf P, showed no significant difference between C₃ and C₄ plants. The main conclusion from this work was that photosynthesis of the grasses is more P efficient than that of the dicotyledons.

Other data on C₃ and C₄ grass responses to P limitation are provided by Ghannoum and Conroy (2007) and Ghannoun et al. (2008). Ghannoum and Conroy (2007) examined a C₃ grass and two C₄ grasses. While P deficiency decreased the plant dry mass accumulation rate to a similar extent in all three species, PPUE was higher in the two C₄ grasses than in the C₃ grass; the reverse was true of the rate of DM accumulation per unit plant P. Ghannoun et al. (2008) examined four tropical grasses, one of which was C₃ and the others were C₄, with one each from the NAD-ΜE (NAD-malate enzyme), NADP-ΜE (NADP-malic enzyme), and PEPC (phosphoenolpyruvate carboxykinase) subtypes. The C₃ grasses had higher PPUE, expressed on the basis of leaf P, as was found by Ghannoum and Conroy (2007), but contrasting with the findings of Halsted and Lynch (1996).

Mantiana et al. (2008) measured PPUEs for C₄ plants from an African savannah/wetland mosaic; values were significantly higher than the mean for C₃ plants (Wright et al., 2004), but the authors caution that a larger C₄ data set is needed to examine further the effects of P on C₄ relative to C₃ photosynthesis. While the work of Yu et al. (2012) only deals with one C₃ and one C₄ perennial grass species (and an annual C₃ Chenopod), the PUE for the C₄ species is less than that of the C₃ species for growth under both P-limited and N-limited conditions.
Attempts were made to estimate the PUE of growth of natural populations of three freshwater isoetid life form vascular plants, Isoetes lacuusistris and Litorella uniflora, which take up almost all of their CO₂ through their roots and use CAM, and Lobelia dortmanna, which also takes up almost all of its CO₂ through the roots but uses C₃ photosynthesis (Richardson et al., 1964; Christensen and Sand-Jensen, 1998; Christensen et al., 1998). However, the wide variations in P concentration and problems with estimating growth rates under natural conditions meant that no significant differences could be found within the high variance data sets. Comparing the [¹⁴C]inorganic C assimilation rates per unit plant P in the dark plus light for the natural populations of the C₃ L. dortmanna and the CAM I. lacustris does not yield significant differences, again within the rather large variances (Richardson et al., 1984).

There is clearly a need for more work, using both total plant P and leaf P, as well as measurements of the four main P pools, for a more detailed analysis of the determinants of variations of PPUE and the PUE of growth in terms of the relationship of photosynthetic and growth rates to nucleic acid P, phospholipid P, low molecular mass water-soluble phosphate esters and metabolically active inorganic phosphate, and stored (metabolically inactive) phosphate. The interpretations could be related to the possible differences in uses of the various P pools in C₃ and C₄ plants, as discussed above. In particular, we note the absence of clear evidence of an increased P paralleling the higher NUE in many photosililithotrophs with CCMs, which would be predicted from the decreased requirement for rRNA if less protein has to be made.

**Water-use efficiency of vascular plants with different photosynthetic pathways under P limitation**

Water and P can co-limit plant growth, so an important aspect of P deficiency in terrestrial plants is its relationship to water loss per unit DM gain. The typical response of nutrient deficiency in terrestrial vascular plants is a decrease in water-use efficiency (WUE), measured as g DM gain g⁻¹ water lost in transpiration (Raven et al., 2004). While almost all of the data relate to N deficiency, there are some data indicating a decrease in WUE in P-limited C₃ plants (Raven et al., 2004).

There are some additional data on P deficiency effects on WUE. Conroy et al. (1988) examined the effect of P limitation of growth rate on WUE efficiency of the C₃ tree Pinus radiata. Limiting P supply decreased WUE at all three levels of water supply and at both CO₂ levels (330 and 660 mm³ DM⁻¹). Brück et al. (2000) examined the C₄ Pennisetum glaucum (pearl millet), and found a decreased WUE with P limitation of growth for well-watered, but not for droughted, plants. Singh et al. (2008) found that P limitation decreased the WUE of the C₃ herb Trifolium repens. Cernusak et al. (2011) examined 19 C₃ woody tropical tree seedlings and a liana, and found a negative correlation between shoot P:C ratio and WUE; that is, the reverse of the ‘typical’ response of a decreased WUE with decreasing plant P concentration. Cernusak et al. (2011) attributed their findings to a P movement to the root surface driven by transpirational water flow. A tentative conclusion is that the conflicting results arise from situations in which processes internal to the plant dominate and WUE decreases with plant P concentration, and situations where mass flow of P with the transpiration stream external to the plant dominate, when WUE increases with decreasing plant P concentration.

**Phosphorus effects on the expression of CCMs**

There is some information on the effects of P availability on the expression of CAM in facultative CAM plants, which can express either C₃ or CAM photosynthesis, as well as on algae in relation to the expression of CCMs. C₄ photosynthesis in land plants is constitutive, so there are no parallel data sets on the effects of P availability on the extent of C₄ expression.

Starting with algae, Kozlowska-Szrenos et al. (2004) examined the effects of P deficiency on the rate of photosynthetic oxygen production under a range of light and CO₂ levels in the green (trebouxiosisphycean) freshwater microalga Chlorella vulgaris. The cells were grown in originally air-equilibrated media at pH 6.8 in vessels, which were subsequently sealed with bacteriological cotton wool, and with no subsequent attempts to re-equilibrate the cultures with the atmosphere, so the culture medium was at or below air equilibrium CO₂ during cell growth. While P deficiency decreased the initial slope (α) of the photosynthetic rate on a chlorophyll basis versus an irradiance relationship, the light-saturated rate of photosynthesis was higher in the low-P cells. The affinity of whole-cell photosynthesis for inorganic carbon was higher in the low-P than in the control cells, with K₀₅ values significantly decreased by ~30% in the low-P cells (Table 6).

Beardall et al. (2005) grew the green (trebouxiosisphycean) freshwater microalga Chlorella emersonii at a range of P concentrations in air-equilibrated culture media. In contrast to the results of Kozlowska-Szrenos et al. (2004) with C. vulgaris, the K₀₅ increased (affinity decreased) with increasing P limitation, although this was only significant at the lowest P level. Beardall et al. (2005) also measured the accumulation ratio (internal/external) for CO₂ achieved by the CCM, and found that this was more than halved at the lowest relative to the highest P treatment. A less effective CCM in the low-P cells is also indicated by the increased inhibition of photosynthesis by oxygen (air equilibrium versus a quarter of air equilibrium) for low-P than for higher P cells, and also the significantly more negative δ¹³C values of cell organic matter for the cells grown under lower relative to higher P supply. On all four of the grounds tested, the CCM was less effective in low-P cultures (Table 6).

Xu and Gao (2009) examined the red (florideiosisphycean) marine macroalga Gracilaria lemaneiformis. The thalli were grown in air-equilibrated seawater at two P levels. The
Table 6. Effects of phosphorus limitation on the expression of CCMs in a cyanobacterium, an algae, and facultative CAM flowering plants

<table>
<thead>
<tr>
<th>Organism</th>
<th>CCF</th>
<th>$K_{0.5} \text{CO}_2$</th>
<th>$O_2$ inhibition</th>
<th>$\delta^{13}\text{C}$</th>
<th>CAM $\Delta\text{pH}$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cylindrospermopsis raciborski</td>
<td>NM</td>
<td>Decrease</td>
<td>NM</td>
<td>NM</td>
<td>NA</td>
<td>Wu et al. (2012)</td>
</tr>
<tr>
<td>(Cyanobacterium)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>NM</td>
<td>Decrease</td>
<td>NM</td>
<td>NM</td>
<td>NA</td>
<td>Kozłowska-Szerenos et al. (2004)</td>
</tr>
<tr>
<td>(Trebutiophyceae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorella emersonii</td>
<td>Decrease</td>
<td>Increase</td>
<td>Increase</td>
<td>Decrease (more –ve)</td>
<td>NA</td>
<td>Beardall et al. (2005)</td>
</tr>
<tr>
<td>(Trebutiophyceae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydomonas acidophila</td>
<td>Decrease</td>
<td>Increase</td>
<td>NM</td>
<td>NM</td>
<td>NA</td>
<td>Spijkerman (2011); Spijkerman et al. (2011)</td>
</tr>
<tr>
<td>(Chlorophyceae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gracilaria lemaneiformis</td>
<td>NM</td>
<td>Increase</td>
<td>NM</td>
<td>NM</td>
<td>NA</td>
<td>Xu and Gao (2009); Xu et al. (2010)</td>
</tr>
<tr>
<td>(Floridiophyceae)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesembryanthemum crystallinum</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>Increase</td>
<td>NA</td>
<td>Paul and Cockburn (1990)</td>
</tr>
<tr>
<td>(Magnoliophytopsida)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clusia minor (Magnoliophytopsida)</td>
<td>NM</td>
<td>NM</td>
<td>NM</td>
<td>Increase</td>
<td>NA</td>
<td>Maiquetia et al. (2009)</td>
</tr>
</tbody>
</table>

NM, not measured; NA, not applicable.

growth rate, and a significantly higher rate for high C and low P, and even (significantly) higher rates for the two high-P treatments. In this case, the $K_{0.5}$ for inorganic C only varies with P supply, with a significantly higher $K_{0.5}$ at the higher P concentrations, with no effect of inorganic C supply within a P treatment. These results resemble those of Kozłowska-Szerenos et al. (2004), namely a high affinity for inorganic C at the lower P concentration, but not the other data sets (Table 6).

The conclusion is that, in the two cases where it was measured, the CCF decreased with P limitation, and in the majority of cases examined the affinity for external inorganic carbon decreased with P limitation (Table 6). A rather different approach was taken by Garcia et al. (2013) who measured cellular growth rates at high light and low light combined with high (2100 AD?) CO$_2$ and low (last glacial maximum) CO$_2$ as a function of P supply. The maximum growth rate of P-replete increases in the order low light–low CO$_2$, low light–high CO$_2$, high light–low CO$_2$, high light–high CO$_2$ (Garcia et al., 2013). These authors also measured the CO$_2$ and N$_2$ fixation rates per unit cell P under the four sets of light and CO$_2$ conditions, and found that both rates were highest for P-replete cells in high light–high CO$_2$, with all other treatments giving lower values. Garcia et al. (2013) suggest that high light–high CO$_2$ permits a greater allocation of energy to processes other than the CCM.

The other potential role of P in acclimation of inorganic C acquisition is in facultative CAM plants, which can switch from C$_3$ to CAM. Paul and Cockburn (1990) examined the interaction of phosphate and nitrate with salinity in the expression of CAM in Mesembryanthemum crystallinum. P deficiency led to some CAM expression in the absence of salinity treatment, and also increased CAM expression after induction by salinity treatment (Paul and Cockburn, 1990) (Table 5). Maiquetia et al. (2009) showed that P deficiency promoted the induction of CAM by drought in seedlings of Clusia minor; addition of mycorrhizal fungi, or of phosphate, did not promote the induction of CAM by drought (Table 5).
A final point concerns the effects of P deficiency on the constitutively expressed $C_4$ CCM in terrestrial vascular plants (Jacob and Lawlor, 1991, 1992). P deficiency decreases the maximum (light- and CO$_2$-saturated) rate of photosynthesis, and the carboxylation efficiency (increment of photosynthetic rate per unit increase in external CO$_2$ concentration) resulting from effects on metabolism in photosynthetic cells rather than effects on diffusion through stomata (Jacob and Lawlor, 1991, 1992, 1993a, b). The decreased photosynthetic capacity is not a function of limitation by electron transport, but rather of ATP availability in regenerating ribulose-1,5-bisphosphate (Jacob and Lawlor, 1993b). This decreased affinity for inorganic C under P deficiency resembles many of the effects noted for cyanobacteria and algae. However, similar effects are found for $C_3$ terrestrial vascular plants, albeit based on a lower P-replete CO$_2$ affinity than is the case for $C_4$ plants (Jacob and Lawlor, 1991, 1992).

The discussion above shows that P deficiency decreases the CCF in the two algae with CCMs that have been tested, and that most data sets show decreased affinity for external inorganic C in photosynthesis in cyanobacteria and algae. P deficiency increases the expression of CAM in facultative CAM plants, either alone or in conjunction with decreased water availability. The CO$_2$ affinity of both $C_3$ and $C_4$ terrestrial flowering plants is decreased by P deficiency.

**Conclusions on differences on phosphorus allocation among photolithotrophs**

Most evidence shows no greater PUE in $C_4$ than in $C_3$ plants. The effect of P availability on the expression of CCMs is variable; CAM expression is increased by P deficiency, while in most cases algal CCMs show lower expression under P deficiency.

**Interactions of phosphorus and photosynthetically active radiation in organisms with CCMs**

Hessen et al. (2002) examined the growth of cultures of the freshwater green alga *Selenastrum capricornutum* under a range of P concentrations and of PAR levels. The cell C:P ratio decreased with increasing external phosphate and/or decreasing PAR, so that the PUE is lower for growth at low PAR. Hill et al. (2009) examined growth of diatom-dominated communities of stream algae in a range of PAR and P treatments. The effects of external P were decreased at low PAR, and PAR effects were decreased at low external P, but there was only weak evidence of PAR–P interactions. As with the work on *Selenastrum*, PUE is lower at low growth PAR, although there is the complication of changes in species composition of the communities. A decreased PUE with decreasing growth rate as a function of availability of a non-P resource is to be expected, since there is a baseline content of P-containing components (e.g. membrane lipids) for survival. The absence of clear evidence for kinetic interaction of P and PAR recalls the absence of such interactions between P and CO$_2$ in Chlamydomonas acidophila (Spijkerman et al., 2011). P-deficient cells have a lower PAR use efficiency, as indicated in the work discussed in this paragraph, in Kozłowska-Szerenos et al. (2004), and in the work of Jacob and Lawlor (1991) on flowering plants with and without CCMs.

**Conclusions**

The interactions in evolution between autotrophy and P availability are complex, but some conclusions can be drawn.

The PUE of autotrophs (specifically photolithotrophs) is lower than that for chemoorganotrophs in comparisons of the fastest-growing Bacteria and Eukarya of the two trophic modes that are otherwise as closely alike as possible. However, there is a very wide range of PUEs in each trophic mode and so a large range of overlap. More experiments are needed to measure specific growth rates and C:P ratios in parallel for a range of organisms of each trophic mode, each under a range of P supplies, to test further the hypothesis of a higher PUE in chemoorganotrophs than in autotrophs. Among rapidly growing organisms, the higher PUE in chemoorganotrophs is unlikely to be a result of a smaller P content in DNA because of the small fraction of P in DNA, and the finding that the smaller genome size of autotrophic than of chemoorganotrophic bacteria is not necessarily the case in eukaryotes. Chemoorganotrophs generally have closer to optimal allocation of P to rRNA than do photolithotrophs, especially under P-limited conditions, but there is a greater occurrence of non-phospholipid polar lipids in membranes in oxygenic photolithotrophs than in many chemoorganotrophs.

Among photolithotrophs, there seem to be no data available for comparison of PUE of growth between anoxic andoxic bacterial photolithotrophs, although the resource-saturated maximum specific growth rate of the anoxygenic photolithotroph *Chlorobium* sp. exceeds that of oxygenic photolithotrophic cyanobacteria. There is little possibility that the different numbers of low molecular mass water-soluble phosphate esters specific to the six well-described pathways of autotrophic inorganic carbon assimilation alter the PUE of autotrophic inorganic carbon assimilation or of growth. The data on PUE of growth and of photosynthesis for $C_3$ and $C_4$ flowering plants give very limited support for a greater PUE of $C_4$ than $C_3$ plants, though the major difference noted was the greater PUE of grasses than dicotyledons independent of fixation pathway, correlated with the site of P storage. The WUE of growth of terrestrial photolithotrophs is generally decreased by P limitation. For photolithotrophs with facultative expression of CCMs, P limitation increases CAM expression in combination with other predisposing factors such as salinity or drought. For algae and cyanobacteria, the most complete data sets show a decrease in the CCF and the affinity for extracellular inorganic C when P is limiting, but some data on other organisms show a higher inorganic C affinity under P limitation. PUE is decreased in organisms with decreased supply of (non-P) resources, such as CO$_2$ and PAR.
An organism using exergonic inorganic chemical reactions, or photons, as the energy source for growth, and inorganic chemicals taken up on a molecule by molecule basis across the plasma membrane to supply nutrient elements. 

A photolithrophic organism which uses H₂O as electron donor and generates O₂. This paper shows that photolithotrophs have lower phosphorus-use efficiencies than do aerobic osmochemoorganotrophs.

A chemoorganotrophic organism taking up organic and inorganic nutrients on a molecule by molecule basis across the plasma membrane to supply nutrient elements. Only found in Eukarya. This paper shows that phagochemooorganotrophs have lower phosphorus-use efficiencies than do aerobic osmochemoorganotrophs.

A chemoorganotrophic organism combining autotrophy and chemolithotrophy; usually applied to organisms which combines phototrophy with chemoorganotrophy.

A chemoorganotrophic organism taking up organic and inorganic nutrients on a molecule by molecule basis across the plasma membrane to supply nutrient elements. Only found in Archaea and Bacteria. This paper shows that anoxygenic chemolithotrophs have lower phosphorus-use efficiencies than do aerobic osmochemoorganotrophs.

A photolithotrophic which uses S or SO₂ as electron donor and generates, respectively, no oxidant, S or SO₄²⁻, and Fe³⁺. Only obligate in non-cyanobacterial Bacteria. This paper shows that anoxygenic photolithotrophs have lower phosphorus-use efficiencies than do aerobic osmochemoorganotrophs.

No oxygenic photolithotroph. A photolithotrophic which uses an electron donor other than H₂O (e.g. H₂, H₂S, Fe²⁺) as electron donor and generates, respectively, no oxidant, S or SO₄²⁻, and Fe³⁺. Only obligate in non-cyanobacterial Bacteria. This paper shows that anoxygenic photolithotrophs have lower phosphorus-use efficiencies than do aerobic osmochemoorganotrophs.

An organism using the catalysis of organic compounds as their energy source for growth, and organic compounds as the source of carbon and, in many cases, the source of nitrogen. Heterotroph is a synonym of chemoorganotroph.

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