Schindler's legacy: from eutrophic lakes to the phosphorus utilization strategies of cyanobacteria

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Abstract

David Schindler and his colleagues pioneered studies in the 1970s on the role of phosphorus in stimulating cyanobacterial blooms in North American lakes. Our understanding of the nuances of phosphorus utilization by cyanobacteria has evolved since that time. We review the phosphorus utilization strategies used by cyanobacteria, such as use of organic forms, alternation between passive and active uptake, and luxury storage. While many aspects of physiological responses to phosphorus of cyanobacteria have been measured, our understanding of the critical processes that drive species diversity, adaptation and competition remains limited. We identify persistent critical knowledge gaps, particularly on the adaptation of cyanobacteria to low nutrient concentrations. We propose that traditional discipline-specific studies be adapted and expanded to encompass innovative new methodologies and take advantage of interdisciplinary opportunities among physiologists, molecular biologists, and modellers, to advance our understanding and prediction of toxic cyanobacteria, and ultimately to mitigate the occurrence of blooms.

Keywords: Cyanobacterial blooms, eutrophication, Michaelis-Menten, Monod, nutrient limitation, phytoplankton

1 Introduction

In their book *The Algal Bowl*, Schindler *et al.* (2008) state that 'The greatest threat to water quality worldwide is nutrient pollution. Cultural eutrophication by nutrients in sewage, fertilizers, and detergents is feeding massive algal blooms, choking out aquatic life and outpacing heavy metals, oil spills, and other toxins in the devastation wrought upon the world's fresh waters'. For several decades, nutrient pollution has remained the primary cause of degradation of freshwater (Schindler 1974, Codd and Bell 1985). Despite these early studies highlighting the association between anthropogenic nutrient sources and cyanobacteria (= blue-green algae) blooms (Howard and Berry 1933, Reynolds and Walsby 1975), nutrient pollution in lakes has continued, sometimes unabated, although there are also lakes where restoration through re-oligotrophication has been successful (e.g. Pomati *et al.* 2011). Yet, cyanobacterial blooms continue to develop in countries worldwide (Qin *et al.* 2019). Recent evidence suggests that these blooms are increasing in intensity, frequency, and extent in many parts of the world (Ho *et al.* 2019), fuelled in part by activation of legacy stores of phosphorus (P) in lake sediments that abate only very slowly, even under strong catchment nutrient control

measures (Spears *et al.* 2021). Various changes induced by climate change, in lakes as well as their catchments, also play a role in the promotion of blooms, and Moss *et al.* (2011) spoke of an 'allied attack' between eutrophication and climate change.

Cyanobacteria are found in aquatic systems of all trophic states, from ultra-oligotrophic to hypertrophic (Keith *et al.* 2012, Wood *et al.* 2017, Reinl *et al.* 2021), across all continents from Antarctica to tropical regions (Vincent 2007, Xiao *et al.* 2020), and at thermal extremes associated with frozen water masses or thermal springs, but they most often dominate in eutrophic and hypertrophic lakes (Correll 1998, Smith 2003). Despite growing acknowledgment of the role of nitrogen (N) in driving eutrophication in aquatic systems (Paerl *et al.* 2020), increases in P continue to be a major driver of the global proliferation of cyanobacterial blooms in freshwater systems. An improved understanding of the nuances of P processes which promote cyanobacterial blooms is crucial for developing management strategies to mitigate or avoid the potential of more frequent blooms under future climate scenarios (Burford *et al.* 2019).

Given the key role of P in promoting cyanobacterial blooms, it seems logical that the main eco-physiological traits of cyanobacteria would involve uptake, metabolism, and storage of P. In other

words, when comparing key traits of cyanobacteria with those of their eukaryotic competitors, it might be assumed that P-related eco-physiological traits would be critical. Yet, when scanning the literature on key cyanobacteria attributes, P-related traits are rarely mentioned. For example, Huisman *et al.* (2018) listed N_2 fixation, buoyancy induced by gas-vesicles, a suite of carbon concentrating mechanisms and the production of cyanobacteria toxins under 'traits involved in bloom development'. There are a few studies that refer to cyanobacteria adaptations for survival and growth when P is in limited supply. These studies demonstrate the ability of cyanobacteria to use mechanisms such as phosphatases that hydrolyze P from organic substrates and luxury uptake and storage, that allow continued cell division as P supplies become exhausted for other lake phytoplankton (Carey *et al.* 2012, Burford *et al.* 2016). Indeed, during cyanobacterial blooms, levels of dissolved inorganic P (DIP) are often depleted below the detection limits of standard analytical methods with P being utilized at least as quickly as it is regenerated (Sommer *et al.* 2012). Crucially, key physiological adaptations of cyanobacteria, such as their ability for luxury P storage, cell maintenance at low rates of P supply, and periods of senescence or dormancy, are central to allowing them to form blooms as P supply becomes constrained (Burford *et al.* 2016).

Despite knowledge that eutrophication promotes the growth of cyanobacteria (and other algae), precise relationships are complicated by nutrients other than P that potentially limit growth, including N, carbon, sulphur, and micronutrients. In addition, a suite of other environmental drivers, such as lake water temperature, and consequently, lake mixing regimes, are changing on time scales comparable to those of P loading, so it is difficult to disentangle the role of P from other environmental variables as causal factors in the global expansion of cyanobacterial blooms (Galvanese *et al.* 2019). Complicating matters further, cyanobacteria vary in their morphological and ecophysiological traits among and within species (Mantzouki *et al.* 2016, Xiao *et al.* 2017). For example, substantial intra- and inter-specific variations in P utilization strategies have been identified within the genus *Aphanizomenon* (de Figueiredo *et al.* 2011). Trait variation strongly affects competition among cyanobacteria, extending to strain, population, and community level, especially when P is limited. A clear overview of P utilization mechanisms and the consequences for phytoplankton species dominance would therefore be valuable.

Most lake models have simplified representations of different P forms and the ecophysiological adaptations of cyanobacteria that enable these forms to be exploited. Improved predictions, that include variation in P utilization strategies within and among cyanobacterial species, will help with predictions of the response of cyanobacteria to environmental change, including an understanding of how blooms may proliferate under a combined eutrophication and climate change scenario (Moss *et al.* 2011, Burford *et al.* 2019). Moreover, predictive models that address the complexity of physiological responses in cyanobacteria will provide lake managers with the tools they need to target bloom mitigation measures and safeguard lake ecosystems and the critical ecosystem services they provide (Chorus and Welker 2021, Hamilton *et al.* 2021).

This review synthesises contemporary research on P utilization strategies by freshwater cyanobacteria, with a focus on planktonic taxa in lakes and reservoirs. We consider five main areas allocated to sections in this paper. Section 2 highlights the importance of P for cyanobacteria and evaluates the suitability of different methods to measure P forms. Section 3 examines the different P utilization strategies by cyanobacteria. Section 4 elaborates the inter- and intra-specific relationships among cell growth, species adaption and competition of cyanobacteria. Section 5 summarises the challenges in understanding relationships between P and cyanobacterial dynamics. Finally, Section 6 considers future analytical, interdisciplinary, and modelling approaches, as well as synthesizing the outcomes of this review. The aim of the review is to (i) illuminate the diversity of P forms and understand how to link analytical measurements of P to the bioavailable forms for cyanobacteria, (ii) understand adaptations of cyanobacteria that enable them to be superior competitors for P among freshwater lake phytoplankton, and (iii) demonstrate how to adapt mathematical expressions of these phenomena to enable their routine adoption into predictive models for use in theoretical investigations and hypothesis testing, as well as supporting bloom mitigation assessments for lakes.

2 Sources, metabolism, and measurement of phosphorus for use by cyanobacteria

Phosphorus plays a central structural and functional role in many metabolic processes in cyanobacteria. These include: cellular composition related to P storage, membrane structure and nucleic acids of DNA and RNA; cell metabolism including ATP synthesis; and protein synthesis and transcription of genetic information (Fig. 1). Cyanobacterial cells take up ambient P across the outer membrane and transport it into cells through the plasma membrane, to support metabolism and growth. Orthophosphate ions (Pi) are preferentially taken up and utilized as they are immediately bioavailable. When the Pi pool is depleted, cells may also take up dissolved organic P (DOP) by utilizing metabolically costly enzymatic hydrolysis to produce Pi.

2.1 Measurement of phosphorus concentrations and pools

A key challenge in the study of P utilization and cyanobacteria is that analytical methods for measuring P are only proxies for bioavailable P (Fig. 2). Bioavailable P can be defined as the sum of P taken up directly through the cell membrane and the P transformed into available forms by naturally occurring physical (e.g. desorption), chemical (e.g. dissolution) and biological processes (e.g. enzymatic degradation). Orthophosphate (existing in forms $H_2PO_4^-$, HPO_4^{2-}, or PO_4^{3-}) is the only directly available P source for cyanobacteria but a range of terminologies are often used for bioavailable P. In part this is related to whether reference is being made to an analytical method or to the physiological requirements of cells for Pi uptake. For example, phosphate, soluble reactive P (SRP), filterable reactive P (FRP), and DIP are used, among other terms, to describe orthophosphate, Pi (Fig. 2). There are also different P fractions that are bound to other elements, e.g. Ca-P (calcium-bound P) (Chang and Jackson 1957, Peryer-Fursdon *et al.* 2015) (Fig. 2).

The most commonly used measure of Pi is a colorimetric assay for molybdate-reactive P (American Public Health Association 2012). However, this method is widely recognized to over-estimate P at low concentrations as it includes a portion of acid-labile organic P compounds that are not directly bioavailable (Hudson *et al.* 2000). Measurements also depend on the pre-treatment of water samples. For example, filtration is widely used as a pre-treatment for bioavailable P determination. Membrane filters with pore sizes typically 0.2–0.45 μm allow passage of small bacteria and viruses (Brailsford et al., 2017), resulting in these organisms contributing to the measured Pi. Colloids (size range 0.001 to 0.1 μm), consist-
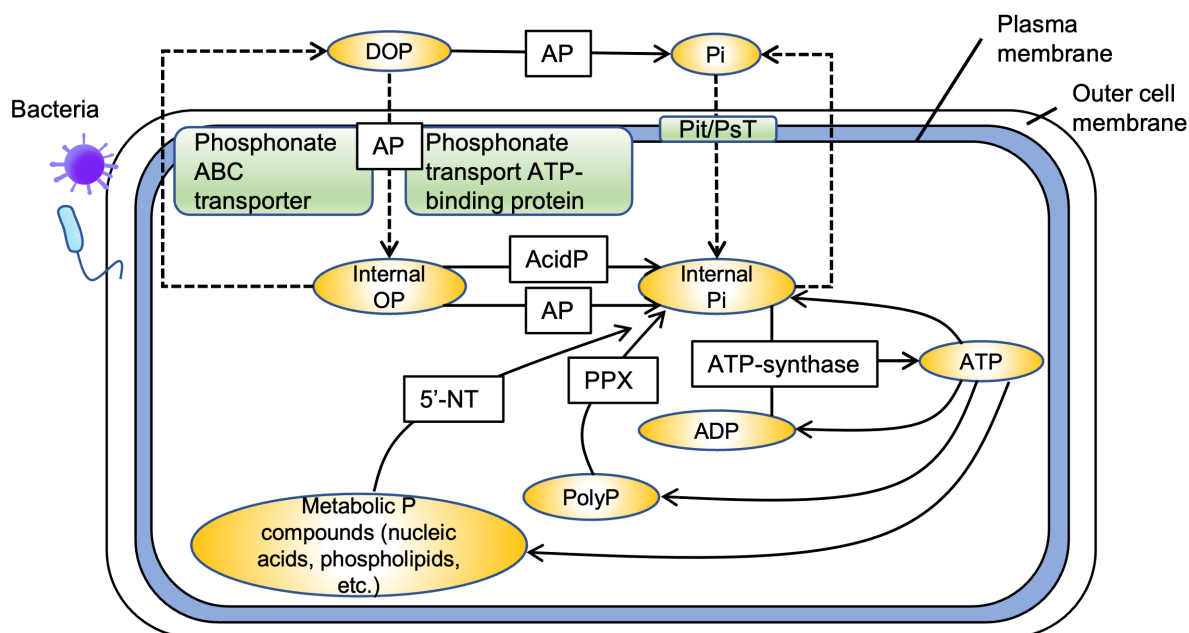
Figure 1. Conceptual diagram illustrating molecular mechanisms in phosphorus (P) uptake, metabolism and storage by a cyanobacterial cell. The bacteria on cyanobacterial cell walls suggests that that they are also involved in utilizing organic P metabolism. Black dashed arrows indicate the transport pathways of P and solid arrows indicate metabolism of P. Ovals—P pools, squares—phosphate-processing enzymes or regulatory proteins, rounded green squares—transporter protein complexes. DOP = dissolved organic P, Pi = orthophosphate, POP = particulate organic P, AP = alkaline phosphatase, NT = 5'-nucleotidase, Acid P = acid phosphatases, ADP = adenosine di-phosphate, ATP = adenosine triphosphate, polyP = polyphosphate, PPX = exophosphatase.
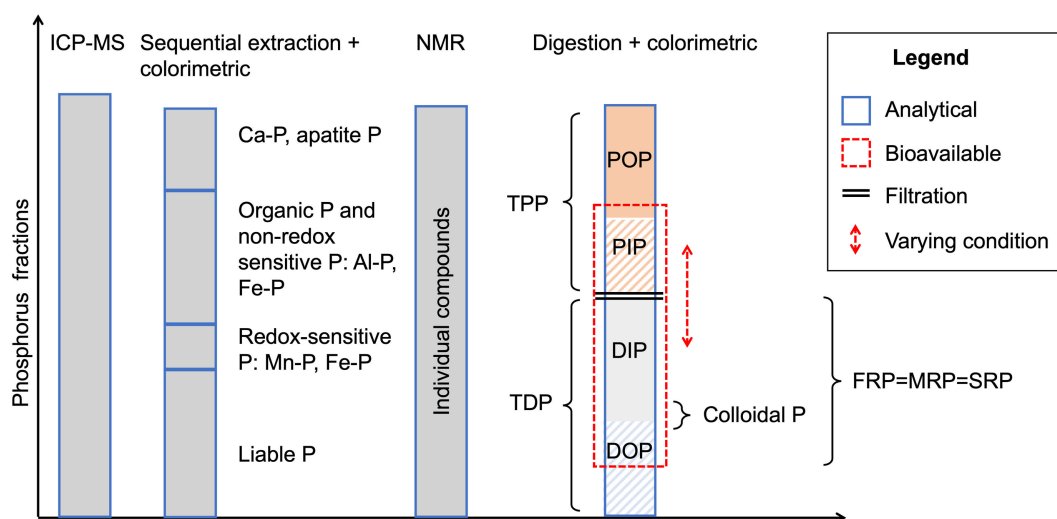

Figure 2. Summary of phosphorus (P) fractions measured in water and sediment samples, including ICP-MS (inductively coupled plasma mass spectrometry) for TP, sequential extraction of P components followed by colorimetry, NMR (nuclear magnetic resonance of individual P components), and digestion followed by colorimetry for TP. The analytical techniques are considered surrogates or proxies for P availability to phytoplankton. Red dashed arrows indicate a varying condition where a fraction of PIP, POP and DOP is available depending on the P forms and phytoplankton species. Other abbreviations are: Ca-P—calcium-bound P (apatite P etc.), Fe-P—iron-bound P, Mn-P—manganese-bound P, Al-P—aluminium-bound P, Fe-P—iron-bound P, TPP—total particulate P, TDP—total dissolved P, POP—particulate organic P, PIP—particulate inorganic P, DIP—dissolved inorganic P, DOP—dissolved organic P. Colloidal P includes a small fraction of dissolved inorganic P and some particulate organic P. FRP = filterable reactive P, and when evaluating these filterable P, MRP (molybdate reactive P) = SRP (soluble reactive P) = FRP. For total reactive P, MRP = SRP + a fraction of particulate P. The P fractions from the sequential extraction are examples using different extraction methods (Chang and Jackson 1957, Peryer-Fursdon *et al.* 2015).

ing of clay and fine silt-sized particles that strongly adsorb P, also readily pass through filters, inflating estimates of Pi (Douglas *et al.* 2016). This problem is further exacerbated by use of glass fibre filters (e.g. GF/C) which allow passage of particulates <∼1.2 µm. As Pi concentrations in surface waters of stratified lakes are often close to detection limits, standard colorimetric methods for Pi are likely to be an inaccurate representation of bioavailable P and could explain our inability to develop quantitative relationships between changes in phytoplankton or cyanobacteria biomass and Pi concentration. Removal of P via precipitation followed by precipitate dissolution and colorimetric analysis is a variation on the standard Pi colorimetric method. This method has a higher accu-

racy at lower concentrations of P, compared with standard colorimetric methods (Karl and Tien 1992), but is labour intensive and is seldom used routinely.

In systems where Pi concentrations are low, cyanobacteria and other algal species may utilize bioavailable P at a similar rate to what it is supplied via biological, chemical or physical processes (Hudson *et al.* 2000, Prentice *et al.* 2015). Even at ambient Pi concentrations ≤ 50 pmol L^{-1} in lakes, rates of phytoplankton Pi uptake can be high, supported by high rates of recycling. These processes indicate a need to focus on ecologically relevant P fluxes (Karl 2000), at least until picomole levels of P can be determined reliably.

Issues with analytical methods are not just limited to measurements of Pi. Several methodological variations exist for total P (TP, i.e. the sum of all P fractions), including digestion of the unfiltered sample using acid-persulphate solution (Wetzel and Likens 2000) or high temperature dry combustion (Solórzano and Sharp 1980). These two methods produce similar results as long as the inorganic content of the sample is low (Suzumura 2008). In both cases sample digestion/combustion is followed by colorimetry for Pi. A single persulfate digestion for total P and total N (Ebina *et al.* 1983) or total P, total N and total carbon (Gibson *et al.* 2015) has been useful in reducing inaccuracies from multiple assays and decreasing analysis times.

Measurement of DOP compounds, some of which can also be utilized by cyanobacteria, is also challenging, particularly when P concentrations are close to detection limits (Worsfold *et al.* 2008). DOP is comprised of two major groups of compounds: phosphoesters that contain a C-O-P ester bond, and phosphonates that contain a C-P bond. DOP is typically measured indirectly, relying on filtration, digestion and colorimetric analysis to first measure total dissolved P (American Public Health Association 2012). The Pi concentration, measured separately, is then subtracted from the total dissolved P to give a calculated value for DOP. Incidentally, measurements of dissolved organic nitrogen (DON), which use the same subtraction approach (nitrate/nitrite and ammonium are subtracted from total dissolved N), are beset by the same problem (Graeber et al., 2012). DON and DOP are calculated as the difference between these two measurements; therefore, small measurement errors are amplified if the difference is close to zero. There are other direct methods to measure DOP, such as ^{31}P nuclear magnetic resonance (NMR) (Valderrama 1981, Özkundakci *et al.* 2013), but they are typically labour intensive and used infrequently (Worsfold *et al.* 2008), and provide only estimates of relative rather than absolute concentrations of P forms. Recent analytical advances in Fourier Transform-Ion Cyclotron Resonance Mass Spectrometer (FT-ICR MS), may allow more accurate determination of DOP dynamics, by directly measuring organic P via solid phase extraction (Dittmar *et al.* 2008), however, the use of this technique has been limited by the ionization efficiency of organic P in complex mixtures relative to other ionizable functional groups (Kurek *et al.* 2020).

2.2 Measurement of phosphorus fluxes

Two approaches are typically used to advance from measurements that quantify P pools to uptake rates. Both are typically carried out for cyanobacterial species or strains in (mono)cultures at different growth stages, usually in chemostats under controlled environments in the laboratory or occasionally using phytoplankton communities *in situ* (Appendix 2 Table S1). These methods involve exposing samples to known Pi concentrations or supply rates for a defined period: (i) by spiking cultures with ^{32}P or ^{33}P-radioisotopic solutions of known activity and concentration, then measuring changes in cell ^{32}P or ^{33}P activity by liquid scintillation, combined with measurement of Pi concentration; or (ii) by measuring the reduction in Pi concentrations in culture media over time, typically using standard colorimetric analysis methods. The first (radioisotope) approach has been used to assess P fluxes of algal and bacterial communities (Thingstad *et al.* 1993, Prentice *et al.* 2015), and was used by Hudson *et al.* (2000) to show that bioavailable P concentrations can be orders of magnitude lower than those determined using the acid-molybdate method. The second (Pi) method makes an assumption of a one-to-one relationship at steady state between changes in Pi concentration and algal cell P quota (Hudson *et al.* 2000). However, bacterial transformations may also change Pi concentrations without incorporation of Pi into algal cells.

Uptake of Pi can also be assessed from measurements of the P content of cells, typically as P in particulate or polyphosphate P (polyP) form (Appendix 2 Table S2). These methods use filtering or centrifugation of cell suspensions to concentrate cells for digestion and subsequent analysis of particulate P, as a proxy of cellular P. The particulate matter collected on filters, especially in the natural environment, is usually contaminated by P in detritus, including dead cells and inorganic sediment. Hence, cell P quotas can be overestimated, although this method has still been used widely as part of a suite of indicators for *in situ* P limitation of lake phytoplankton communities (Hecky and Kilham 1988, Guildford *et al.* 2021). Conversely, high rates of P release from cell lysis in the declining phase of laboratory growth cultures can result in underestimates of actual uptake rates. To ensure that cell P uptake was dominant, Xiao *et al.* (2020) sampled cell cultures before they were physiologically stressed, as indicated by photosynthetic yields (Fv/Fm). In this case, cellular P approximately equated to particulate P of the cultures, as it was assumed that a limited proportion of nutrients was released from the dead cells (Xiao *et al.* 2020). PolyP measurements of cell suspensions involve calibration against a polyP standard, where the polyP across a range of algal taxa is approximately 60 orthophosphate units in length (Martin and Van Mooy 2013, Li and Dittrich 2019). This approach is usually only considered appropriate for measuring relative quantities of cell polyP, as natural polyP has higher fluorescence intensity than synthetic polyP calibration standards (Martin *et al.* 2014).

Phosphohydrolase-mediated conversion of DOP to Pi includes the activity of enzymes such as alkaline phosphatases (AP) and acid phosphatases (AcidP). Alkaline phosphatase activity (APA) has been interpreted as an indirect proxy for DOP scavenging, as the AP enzyme mineralizes the most abundant and labile constituents of the DOP pool. APA has traditionally been measured through colorimetric or fluorometric techniques that measure the phosphomonoester substrates, *p*-nitrophenyl phosphate (*p*NPP) (Guedes *et al.* 2019) or 4-methylumbelliferyl phosphate (4-MUP) (Bar-Yosef *et al.* 2010), respectively (Appendix 2 Table S3). These methods enable the determination of APA within whole-water (bulk) samples (Prentice *et al.* 2019) or size-fractionated samples, to approximate the intracellular and extracellular activity, as well as bacterial and algal activity (Jamet *et al.* 2001). Measured activities are typically determined at saturating (c.f. *in situ*) phosphomonoester substrate concentrations, thus are determinations of maximal activity and not likely representative of activities in natural systems (Prentice *et al.* 2019). These methods have been applied under varying pre- and intra-incubation conditions to enable the isolation and determination of the effects of N and P limitation and other environmental drivers (e.g. temperature, pH and light; see Appendix 2 Table S3) on APA (e.g. Mateo *et al.* 2006, Dong

et al. 2019, Wan *et al.* 2019). These methods, however, (1) are not able to determine the activity of individual taxa within a natural (or composite) sample, and (2) may be confounded by the phosphatase activity of naturally occurring bacteria in field samples (Štrojsová *et al.* 2008) or bacteria in culture media (Harke *et al.* 2012).

Phosphohydrolase-mediated mineralisation of DOP has also been determined by enzyme-labelled fluorescence (ELF). This method involves the use of a fluorogenic substrate—that upon hydrolysis forms a fluorescent precipitate at the site of enzyme activity—and visualisation and measurement under epifluorescence microscopy (Štrojsová *et al.* 2008, Štrojsová and Vrba 2009). ELF, c.f., colorimetric or fluorometric techniques, therefore permits the determination of the activity of individual taxa in a natural (or composite) sample (Nedoma *et al.* 2003, Štrojsová *et al.* 2003), and removes the issue of confounding bacterial activity (Rengefors *et al.* 2001). ELF has also been applied under varying conditions to determine the effects of various environmental drivers on phosphohydrolase activity (Appendix 2 Table S3) (Rengefors *et al.* 2003, Wu *et al.* 2009, Bar-Yosef *et al.* 2010).

2.3 Application of genomics to measure cyanobacteria-phosphorus dynamics

Recent advances in genomic techniques provide new methods to enhance knowledge on the molecular response of cyanobacteria to variations in P concentrations and sources. Both genome sequencing and metagenomics (the sequencing of genomes directly from environmental DNA) allow for the identification of the presence of specific genes, with transcriptomics (single species) and metatranscriptomics (environmental samples) being powerful tools to study changes in the expression of genes. Concurrent assessment of cyanobacterial abundance and differential gene expression via whole-transcriptome sequencing in field studies provides an avenue to identify genetic pathways important in facilitating shifts among dominance genera. Three main methods are used to study the presence and/or regulation of *Pho* and other genes involved in P regulation in cyanobacteria: (i) targeted methods such as quantitative PCR (qPCR) or droplet digital PCR (ddPCR); (ii) sequencing of genomes or metagenomics, and (iii) transcriptomics or metatranscriptomics. Targeted approaches applied to reverse-transcribed complementary DNA (cDNA) have been used to study the expression of a variety of genes including P-binding proteins (*pstS* and *sphX*) and a putative AP (*phoX*) in *Microcystis* (Harke *et al.* 2012) as well as *pit*, *pstS1*, *phoA*, *phoU*, *phnD*, *phnK* gene expression in *Raphidiopsis raciborskii* (previously *Cylindrospermopsis raciborskii*) (Willis *et al.* 2019). Transcriptomics have also been used to show that when grown in low P concentrations, transcript levels of *phoX*, *pstABC*, and the *sphX* gene increased markedly in *Microcystis* (Harke and Gobler 2013). In Lake Erie, researchers have incorporated transcriptomics into field surveys and used manipulative experiments to show that gradients in Pi shape cyanobacterial communities and elicit significant transcriptomic responses (Harke *et al.* 2016). In regions of the lake with low P, *Microcystis* dominates and populations have up-regulated genes associated with P scavenging (e.g. *pstSCAB*, *phoX*); whereas when P availability is high, *Dolichospermum* dominates and gene expression is commonly associated with triggering N$_2$ fixation.

A diverse suite of genes within the *Pho* regulon have been identified that encode for proteins facilitating P assimilation and transport in cyanobacteria (Torriani-Gorini *et al.* 1994, Vershinina and Znamenskaya 2002, Tetu *et al.* 2009). These genes have a range of functions including phosphomonoester metabolism via

AP (e.g. *phoA*, *phoD*, and *phoX*) and AcidP (e.g. a*phA*), glycerol phosphate metabolism via glycerolphosphodiester phosphodiesterase genes (e.g. *ugpQ*), and P-binding proteins (e.g. *pstS*) (Muñoz-Martín *et al.* 2011, Bouma-Gregson *et al.* 2019, Tee *et al.* 2020). Determination of phosphohydrolase-encoding gene expression also permits quantification of the rate at which of phosphohydrolase-encoding genes are expressed over time (Aguilera *et al.* 2019, Willis *et al.* 2019).

There are two major Pi import systems in cyanobacteria, Pst and Pit. The Pst system is encoded by a cluster of genes that make up the *Pho* regulon. It is considered highly efficient at transporting Pi (Rao and Torriani 1990). Two copies of Pst systems, *Pst1* and *Pst2*, have been identified in the genomes of freshwater cyanobacteria such as Synechocystis and *Microcystis* (Burut-Archanai *et al.* 2011). Both Pst systems encode ABC transporters and have been shown to be upregulated under Pi deficiency. The Pit system is constitutive, comprised of *PitA* and *PitB*. *PitA* has an affinity for Pi approximately two orders of magnitude greater than the Pst system. *PitB* encodes a functional Pi transporter repressed at low Pi levels by the *Pho* regulon which is assumed to be a secondary transporter system functional in low-Pi environments (Harris *et al.* 2001).

Molecular studies targeting genes involved in the Pi systems are increasing, but still in their infancy. Using a transcriptomics approach, Harke and Gobler (2013) studied *Microcystis* grown under Pi deficiency and showed the *Pho* regulon was induced, leading to increases in transcript levels of the AP phoX gene, the Pst Pi transport system (pstABC), and the sphX gene. Their analysis identified that Microcystis has two Pst gene clusters (pstSCAB), additional copies of the pstA and pstS genes, and a phoA gene that was not expressed, highlighting the complex and diverse P harvesting ability of this genera. In a study on *Nostoc* using a similar approach, *pstABCS* operons were upregulated during P starvation and rapidly decreased following Pi addition (Solovchenko *et al.* 2020). Interestingly, both studies noted shifts in multiple genes not specifically associated with P systems. These genes were often linked with the transport and degradation of organic compounds, highlighting the complex and interdependent nature of these cellular processes. Additionally, expression of *pstB1* increased when Pi-starved *Synechocystis* sp. strain PCC 6803 was exposed to low, normal, or high Pi, but only until the cell quota exceeded that needed for cell maintenance (Hudek *et al.* 2016). These results highlight the need for studies on a wider range of species and a wider range of P compounds to understand the generality of changes in gene expression in cyanobacteria.

In cyanobacteria, polyP kinase (*ppk*) and exo-polyphosphatase (*ppx*) are considered the main encoding enzymes involved in polyP synthesis and utilization, respectively (Gómez-Garcı́a *et al.* 2003, Gomez-Garcia *et al.* 2013, Whitehead *et al.* 2014). The *Pho* regulon, which controls acclimation to P deficiency in most bacteria, also controls polyP utilization in *Synechocystis* (Irby 2021).

The physiology of most cyanobacteria makes them well-suited for nutrient exchange with heterotrophic bacteria, and many species are known to inhabitant their phycospheres (Kim *et al.* 2019, Cook *et al.* 2020). Only a few studies have used axenic cultures, therefore an important caveat when interpreting data from experiment or field studies is that bacterial processes may be confounding the results. Metagenomics and metatranscriptomics provide an avenue to tease apart the contribution and role of bacteria in P cycling and exchange. For example, Tee *et al.* (2020) used metagenomic to analyse a biofilm dominated by *Microcoleus* and showed that the main cyanobacteria species present were equipped with mechanisms that enabling them to scavenge and utilize various forms of organic phosphate, solubilize Pi, uptake

dissolved Pi, and stockpile phosphate. They also showed that although *Microcoleus* genomes lack the ability for pyrroloquinoline quinone (PQQ) synthesis, some biofilm *Proteobacteria* possessed these genes, and could synthesize PQQ as a public good.

2.4 Models of phosphorus loads and lake trophic state

Aside from external P loading, active biological uptake, lakebed sediment inputs and recycling of P create wide variations in P forms and concentrations within and among lakes and reservoirs, and over time (Fig. 3). The abundance of P in the euphotic zone, where photosynthesis occurs, is determined by the net balance between various local supply, removal, and recycling processes. Supply includes inflows, resuspension, and diffusive fluxes from sediments, as well as biological regeneration. Dissolved Pi and DOP are removed (in varying amounts) by phytoplankton and bacterial uptake, and the settling of plankton and biogenic detritus transfers this P to deeper waters or to the bottom sediments. A component of this particulate organic P (POP) is converted to Pi by microbial degradation of the organic matter, either within the water column prior to sedimentation or within the bottom sediments. Pi and DOP are usually depleted by photosynthesis in the euphotic zone, while concentrations generally increase with depth due to dominance of regeneration processes and reduced demand as photosynthesis is curtailed in the aphotic zone (Eager 2017, Prentice 2021). The availability of Pi is also heavily influenced by heterotrophic bacteria, which compete with phytoplankton for resources (Rothhaupt 1992, Rothhaupt and Güde 1992).

Most studies of effects of P on phytoplankton production have focused on northern temperate lakes, where P is considered the key limiting nutrient (Schindler 1977). This research has provided the foundation for many subsequent studies showing that total P is a strong predictor of chlorophyll *a*, the proxy used to represent total phytoplankton biomass (Dillon and Rigler 1974, Jones and Bachmann 1976, Guildford and Hecky 2000, Brett and Benjamin 2008, Smith *et al.* 2016), the proportion of cyanobacteria (Stevenson *et al.* 2008), and the prevalence of nuisance/toxic phytoplankton blooms (Downing *et al.* 2001, Smith and Schindler 2009).

Extensive literature exists, commencing in the 1960s, showing semi-theoretical relationships of P loads to lakes and the resulting in-lake concentrations. Most of these relationships are at steady state and applicable to fully mixed conditions (e.g. at winter or spring overturn), with parameters in the equations derived from measurements scaling from individual lakes up to hundreds of lakes (Jones and Lee 1986). The equations and parameters have been reviewed extensively (Brett and Benjamin 2008, Verburg *et al.* 2018), but should be adopted with caution for individual lake applications, given the many model simplifications, e.g. assumptions of steady state involving equilibration of in-lake total P to external loading, and constant water levels and internal loading. The Vollenweider-OECD approach, for example, models the relationship between average chlorophyll *a* biomass and the P load to the lake, adjusted in-lake according to water depth and hydraulic retention (Vollenweider and Kerekes 1980).

The early empirical models provide a useful starting point for lake trophic state assessment from external loads but lack dynamic representations important for reflecting lake P dynamics, such as internal P loading and cycling from seasonal anoxia-mediated releases. The steady state models have therefore largely been superseded by dynamic lake ecosystem models that resolve multiple physical and biogeochemical processes at high spatial and temporal resolution.

Recent physiological studies of cyanobacterial cultures have identified that the dominance of a particular species in lakes and other lentic systems is not necessarily the result of increased P loading (Carey *et al.* 2008, Willis *et al.* 2015). One example is the filamentous N$_2$-fixing species, *R. raciborskii* (Padisák and Istvánovics 1997). Studies have shown that its growth rate does not increase with increasing Pi concentrations (Willis *et al.* 2015, Xiao *et al.* 2020), and that it has high Pi uptake affinity at low Pi concentrations (Xiao *et al.*, *in prep*), which may help to explain why *R. raciborskii* can outcompete other species at low Pi concentrations. As our need to understand and examine the specificity of blooms has increased, there has been a shift from models that sought to predict broad shifts at an ecosystem scale (e.g. eutrophy and increased chlorophyll *a*) as a result of changes in P loads, to those differentiating forms of P and the resulting species-specific responses.

Additional questions are being asked of these models, including how climate change affects catchment external and internal P loads. In the latter case it is been demonstrated with empirical analyses (e.g. Jankowiak *et al.* 2019) and models (e.g. Me *et al.* 2018) that climate change can be expected to increase surface water temperature and extend the duration of stratification, leading to longer periods of anoxia of bottom waters, greater internal P loading, and increases in cyanobacterial blooms. Hence, a comprehensive review of physiological adaptations of cyanobacteria to P availability provides the basis for how to improve P predictions by differentiating various P forms (Section 2.3), and determining how different cyanobacterial species or groups respond to these P forms and their concentrations (Section 3). Improved predictions require algorithms for species adaptation and competition in relation to the changing P conditions (Section 4), as well as species- and strain-specific traits such as light competition (e.g. chromatic adaptation), carbon acquisition, including the carbon concentrating mechanism (CCM), grazing resistance (e.g. from colonial morphology), N$_2$ fixation, and buoyancy control (see Carey *et al.* 2012).

3 Phosphorus utilization by cyanobacteria

More than 3.5 billion years of evolution have allowed cyanobacteria to develop diverse and highly adaptable eco-physiological attributes (Carey *et al.* 2012). These include strategies to maximize utilization of P, with differences at genus, species, and strain level. Much of the focus has been on the ability of cyanobacteria to: (i) adjust Pi uptake affinity without impacting growth rate; (ii) store P in excess of their immediate cellular requirements; (iii) hydrolyse a variety of organic P compounds into Pi using enzymes; and (iv) reduce the requirement for P by forming colonies and produce lipid membranes with reduced P content. These attributes allow cyanobacteria to maintain cell growth using adaptations to P scarcity or fluctuating P availability. Moreover, variations in responses to P among cyanobacterial species will affect their competitiveness under different P supply regimes, whether those be intermittency from variable catchment loads, internal loads, or low concentrations associated with drought or stratification (Burford and O'Donohue 2006, Deng *et al.* 2014, Wood *et al.* 2017).

3.1 Cell uptake of orthophosphate ions

Uptake of Pi by phytoplankton cells is generally governed by two steps: diffusion of Pi across the outer membrane from the ambient environment into the periplasm, followed by capture with binding proteins and transport across the plasma membrane into the cytoplasm (Fig. 1). These processes are mainly regulated by four se-
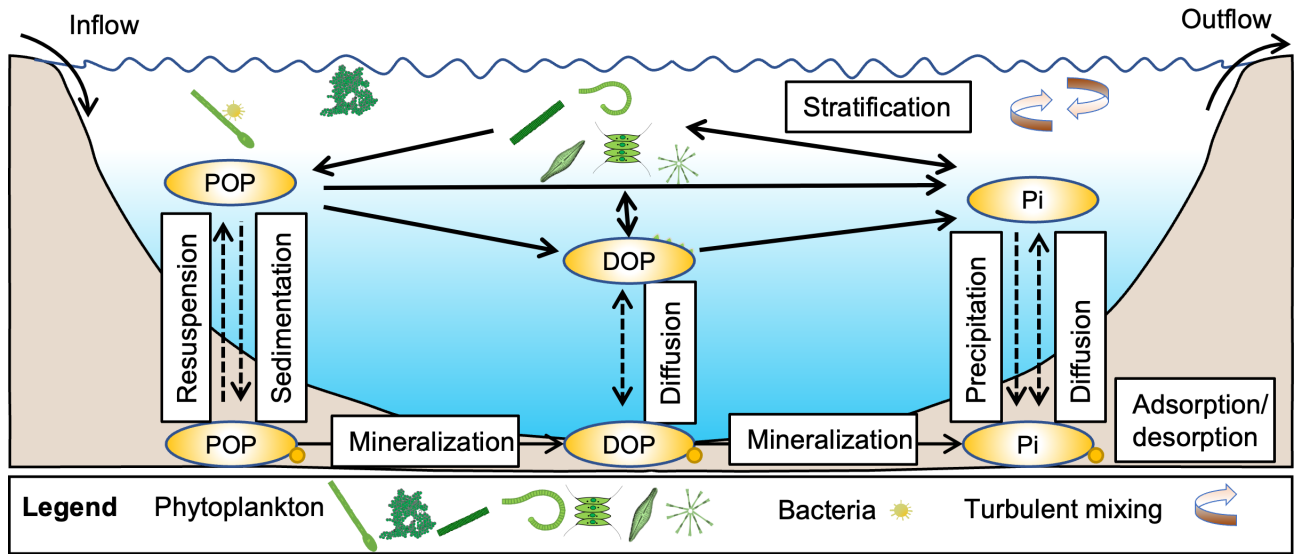
Figure 3. Conceptual diagram illustrating dynamics of P affecting cyanobacterial metabolism and growth in a lake. Background colour (white—low, blue—high) in the water column represents the gradient of ambient P concentration (Pi, DOP) which increases from the surface to the bottom. Black arrows indicate the transport pathways and metabolism of P. DOP = dissolved organic P, Pi = orthophosphate, POP = particulate organic P. The green symbols indicate phytoplankton, and the yellow symbol indicates bacteria that attach phytoplankton cells. Note that this is a simplification and does not include interactions with particulate inorganic P that can adsorb or desorb P according to inorganic sediment properties (sorption isotherms), Pi concentrations and environmental variables (e.g. temperature, dissolved oxygen).

quential factors: (i) extracellular Pi concentration; (ii) cell size and shape, (iii) thickness of the diffusive boundary layer; and (iv) the affinity and turnover rate of Pi transporter proteins embedded in the plasma membranes of cells. Assuming that the transporters are always available, nutrient uptake rate of a spherical cell can be expressed as:

$$v = 4\pi\, D\, r\, (S - S_r) \qquad (3.1)$$

where D (m^2 s^{-1}) is the nutrient molecular diffusion coefficient, r (m) is the radius, S (mg L^{-1}) is the extracellular Pi concentration at a theoretical infinite distance away from the cell, and S_r (mg L^{-1}) is the nutrient concentration at the cell surface (Lindemann *et al.* 2016). This equation uses an idealised nutrient diffusion rate, with no explicit representation of internal cellular constraints such as nutrient 'handling' or limitation by transporter availability.

After Pi diffuses across the outer membrane, two mechanisms regulate uptake affinity. The first is constitutive low-affinity uptake via the Pi transport (Pit) system. This mechanism requires minimal energy and is initiated when extracellular Pi concentration is higher than the intracellular Pi concentration. The second is ATP-driven high-affinity uptake via a specific transport (Pst) system, which is initiated when intracellular Pi declines to a threshold, and the extracellular Pi concentration is too low to allow a trans-membrane electronic gradient for Pi to enter cells through low-affinity uptake. High-affinity transporters bind nutrients more tightly than low-affinity transporters, thus reducing release of nutrients from the cells into the external media (Hudson and Morel 1993). Hence, high-affinity uptake systems are likely to be upregulated under low Pi conditions.

Pi uptake kinetics have been characterised using several modelling approaches, however, none incorporates high affinity uptake explicitly. We consider in detail the different modelling approaches. These include the widely used conventional Michaelis-Menten (M-M) kinetics, which relates uptake only to the extracellular Pi concentration (Michaelis and Menten 1913); the integrated Michaelis-Menten kinetics and Droop quota model, which

is linked to extracellular Pi concentration and internal quota (Suominen *et al.* 2017); the chemical reaction (C-R) model based solely on internal quota (Baird *et al.* 2001); and the flow-force (F-F) model, which is specifically designed to capture uptake of nutrients under fluctuating or pulsed supply (Falkner *et al.* 1989) (Fig. 4).

Conventional Michaelis-Menten (M-M) kinetics. The M-M kinetics was originally designed to describe an enzymatic reaction between a substrate and an enzyme (Michaelis and Menten 1913). This kinetics have been adapted more broadly to describe substrate limited uptake:

$$v = v_{max}\, \frac{S}{K_v + S} \qquad (3.2)$$

where S is the extracellular substrate concentration (e.g. Pi concentration) adjacent to the outer of plasma membrane, v_{max} is the maximum uptake rate and K_v is the half saturation constant where v equals 0.5 v_{max}. Based on the nutrient uptake rate of a spherical cell (Equation 3.1), v equates to the number of proteins on the plasma membrane times the rate of nutrient transport and release per transport protein within the cells. Droop (1974) added a minimum extracellular Pi concentration, S_0, where uptake ceases, to the conventional M-M kinetics equation for uptake:

$$v = v_{max}\, \frac{S - S_0}{K_v + S - S_0} \qquad (3.3)$$

Integrated Michaelis-Menten kinetics and Droop quota model. The conventional M-M equation is a simplified representation that does not include cell quota impacts on nutrient uptake. Various attempts have been made to introduce more fundamental or process-based representations of nutrient uptake, typically as variations on Equation 3.2. Suominen *et al.* (2017) related the uptake kinetics to the internal quota Q, where uptake rate decreases with increasing internal quota (Fig. 4A):

$$v = v_{max}\, \frac{S}{K_v + S} \cdot \frac{Q_{max} - Q}{Q_{max} - Q_{min}} \qquad (3.4)$$
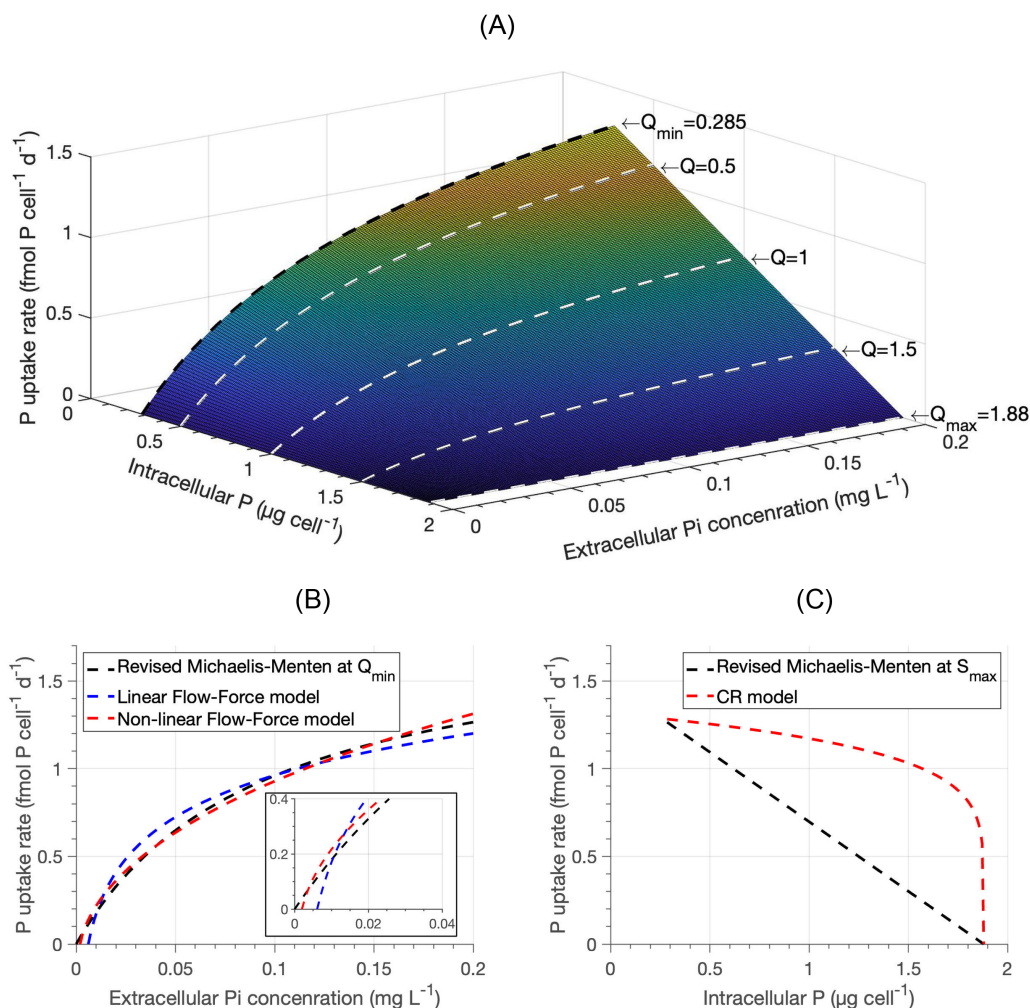
Figure 4. Comparison of cellular uptake of orthophosphate ions (Pi) simulated at an instantaneous value, i.e. for a given Q and Pi at that instance in time, using four equations: (a) comparison of the integrated Michaelis-Menten kinetics and Droop quota model in Equation 3.4 (surface plot) and the conventional Michaelis-Menten kinetics in Equation 3.2 (dashed lines with a given intracellular P quota values denoted); (b) comparison of Equation 3.4 given at the minimum intracellular P quota Q_{min} (black dashed line) with the linear flow-force model in Equation 3.5 (blue dashed line), and the non-linear flow-force in Equation 3.6 (red dashed line); and (c) comparison of Equation 3.4 given at the maximum Pi ($S_{max} = 0.2$ mg L-1) (black dashed line) and the Baird's Chemical kinetics (CR) model in Equation 3.7 (red dashed line). The data were adopted from Pi uptake kinetics of a *Microcystis aeruginosa* strain characterized using Michaelis-Menten kinetics (Suominen *et al.* 2017) with $Q_{max} = 1.88$ fmol P cell-1, $Q_{min} = 0.285$ fmol P cell-1, $K_v =$ 0.093 mg L-1, $v_{max} = 1.853$ fmol P cell-1 d-1, extracellular Pi concentration from 0 to 2 mg L-1, with uptake rates and corresponding extracellular Pi concentrations used to parameterise the linear flow-force model ($L_p = 0.79$ fmol P cell-1 d-1, $S_0 = 0.61$ μg L-1), and non-linear flow-force model ($L_p =$ 0.26 fmol P cell-1 d-1, $S_0 = 0.197$ mg L-1, $L = 0.008$ fmol P cell-1 d-1, $m = 3$). The Baird-Emsley model (Baird and Emsley 1999, Baird *et al.* 2001), referred to as a chemical kinetics (CR) model, was fitted using the same Q_{max} and Q_{min} as in the Michaelis-Menten kinetics (k = 1.3148; see Equation 3.7).

where Q_{max} and Q_{min} are the maximum and minimum nutrient internal quotas, respectively. Algorithms for internal quota provide a nutrient history perspective that reflects how cells moderate their nutrient uptake rates according to their quota. Note that Equations 3.2–3.4 are strictly applicable to steady state conditions (e.g. chemostat case) and may not be suited to variations in nutrient supply that occur under natural conditions, where cells mostly experience pulsed regimes of extracellular Pi supply (Falkner *et al.* 2006).

Physiological measurements to validate Michaelis-Menten kinetics parameters. Cyanobacteria strains and species grown at given Pi concentrations were found to have quite similar K_v val-

ues (Appendix 2 Table S1). For example, Wu *et al.* (2009) noted little difference in K_v measurements between *R. raciborskii*, *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*, and between a toxic and non-toxic strain of *M. aeruginosa* as per Suominen *et al.* (2017). These results contrast with studies which have shown widely different K_v values even at a species level, e.g. K_v of marine *Synechococcus* sp. WH780 was > 20-fold higher in Pi-replete cells compared to Pi-starved cells (Donald *et al.* 1997). A key factor in the differences among studies may be nutrient history, leading to different cell P quotas that are not accounted for in solving K_v according to Equations 3.2 and 3.3.

Many culture studies have examined Pi uptake at a species level using radioisotope, APA and Pi depletion techniques (see Section 2.3). Applying these studies to natural phytoplankton populations or communities *in situ* requires careful consideration of common potential artefacts arising from laboratory methods, including ef-

fects of prior nutrient history for the cultured cyanobacteria, the relevance of high nutrient levels *in vitro* to those *in situ*, and lack of inter-specific competition *in vitro*.

A number of environmental factors, e.g. light, mixing and pH, affect the values of the parameters v_{max} and K_v (Rivkin and Swift 1982, Ritchie *et al.* 1997). Examples include a 5-fold increase in K_v of a cultured *Synechococcus* strain incubated in the dark compared with the light (Donald *et al.* 1997). However, Pi uptake was not responsive to light in a field phytoplankton population unless it was exposed to significantly high Pi concentrations in the dark for several hours (Lean and Pick 1981). Pi uptake may be stimulated by sodium in several cyanobacteria genera, including *Dolichospermum* (formerly *Anabaena* sp. PCC7119) (Valiente and Avendaño 1993), *Synechococcus*, *Gloeothece*, *Phormidium* and *Chlorogloeopsis* (Avendaño and Valiente 1994). Adequate N supply may also be required to attain maximum Pi uptake rates (Aubriot 2019). In *M. aeruginosa,* Pi uptake is inhibited by strong mixing (Zheng *et al.* 2017), but not by increased UV-A or UV-B radiation (Ren *et al.* 2020). Pi forms (i.e. $H_2PO_4^-$, HPO_4^{2-}- or PO_4^{3-}) associated with variations in pH did not affect Pi uptake for a *Synechococcus* strain (Ritchie *et al.* 1997). For *in vitro* experiments on *R. raciborskii* isolated from a natural lake population, there was a strong dependence on extracellular Pi concentration, with a transition from low- to high-affinity uptake at Pi concentrations of ∼4.75 μg L^{-1} (Prentice *et al.* 2015).

Flow-force (F-F) models. The flow-force (F-F) model specifically characterises physiological adaptation of cells to a changing extracellular Pi environment (Fig. 4B). The linear F-F model is given as:

$$v = L_p \left\{ \log(S) - \log(S_0) \right\} \tag{3.5}$$

where S_0 is the threshold of extracellular Pi concertation that reflects Pi uptake affinity and beyond which uptake ceases because of metabolic costs, and L_p is the membrane conductivity coefficient that corresponds to the activity of uptake systems (Thellier 1970, Falkner *et al.* 1989). When uptake systems are in a transient adaptive operational mode, the non-linear F-F model is applied with two additional parameters: the membrane conductivity coefficient L, which is generally two orders of magnitude lower than L_p; and a coefficient m (>1, odd number) shown in Equation 3.6. The second term on the right-hand side of Equation 3.6 is added to reflect transient increases in uptake rate under variable nutrient supply (Falkner *et al.* 2006, Aubriot *et al.* 2011):

$$v = L_p \left\{ \log(S) - \log(S_0) \right\} + L \left\{ \log(S) - \log(S_0) \right\}^m \tag{3.6}$$

Physiological measurements based on the Flow-force (F-F) models. The F-F models have been developed and tested for a range of cyanobacterial cultures (*Anacystis nidulans*, *Anabaena variabilis*, *R. raciborskii*) (Wagner *et al.* 1995, Falkner *et al.* 1998, Isvánovics *et al.* 2000, Plaetzer *et al.* 2005, Falkner *et al.* 2006) and field phytoplankton populations dominated by cyanobacteria (*Gloeotrichia echinulata*, *Planktothrix agardhii*, *Raphidiopsis mediterranea*) (Isvánovics *et al.* 1993, Aubriot *et al.* 2000, Aubriot *et al.* 2011, Aubriot and Bonilla 2012, Aubriot and Bonilla 2018, Aubriot 2019). Using Equation 3.5, a phytoplankton population dominated by *P. agardhii* and *R. mediterranea* was found to have an average threshold Pi concentration S_0 of 0.84–2.74 μg L^{-1} (Aubriot and Bonilla 2018).

Chemical kinetics (CR) model. The Baird-Emsley model developed by (Baird and Emsley 1999), Baird *et al.* (2001), referred to as a chemical reaction (CR) model, was to simulate the interacting effects of nutrient uptake, light intensity, and temperature on growth of *Oscillatoria agardhii* in a phosphate-limited chemostat

culture (Fig. 4C). The uptake rate of Pi was related to the maximum cellular quota of P as:

$$v = k \left\{ \frac{Q_{max} - Q}{Q_{max}} \right\}^{\frac{Q_{min}}{Q_{max}}} \tag{3.7}$$

where v varies with nutrient quotas Q_{max}, Q_{min}, and Q, the instantaneous quota; and k is a coefficient which is closely related to cell growth.

3.2 Cellular phosphorus storage and nutrient stoichiometry

Cyanobacteria store P intracellularly in the form of polyP granules which could represent up to hundreds of PO_4^- groups linked by phosphoanhydride bonds (Sanz-Luque *et al.* 2020). Many functions have been attributed to polyP, including as an energy reserve, metal chelator, and for DNA synthesis, and it may also be produced under stress (Albi and Serrano 2016, Jiménez *et al.* 2017). PolyP synthesis generally occurs when Pi is abundant compared to other macronutrients such as N and sulphur (S), or when external Pi concentrations increase after a period of P limitation. The continued uptake of Pi, resulting in polyP accumulation beyond immediate cellular maintenance requirements, is referred to as luxury uptake.

Despite luxury uptake during transitions from P-limitation to P-sufficiency, polyP formation is not always linearly related to Pi (Appendix 2 Table S2). Wan *et al.* (2019) reported an inverted u-shape relationship between percentage of cells with polyP bodies and SRP in *Microcystis* and *Dolichospermum*. Similarly, Voronkov and Sinetova (2019) found similar cellular polyP storages in *Synechocystis* cultures receiving different amounts of P over 24 h. The proportion of cells with polyP was also found to be higher in the light than in the dark. This is not surprising, considering that active Pi uptake relies on ATP generated by photophosphorylation. PolyP accumulation is also variable along growth stages (Kulaev and Kulakovskaya 2000). After polyP accumulation in a transition from limitation to sufficiency, polyP can be rapidly depleted in the exponential growth phase, and polyP synthesis is only initiated again when the population approaches the stationary phase (Harold 1966, Li and Dittrich 2019, Solovchenko *et al.* 2020). Conversely, if P limitation follows a stationary phase, polyP is degraded in order to supply P for cell maintenance (Li and Dittrich 2019).

PolyP granules are stored in many areas within vegetative cyanobacterial cells, including near organelles important for carbon fixation and protein synthesis (i.e. carboxysomes and ribosomes). Although polyP can be stored in heterocysts, Braun *et al.* (2018) showed that polyP accumulates primarily in vegetative cells in the marine cyanobacterium *Nodularia spumigena*. PolyP can also be stored in akinetes, i.e. the spore-like cells formed during unfavourable environmental conditions, but not preferentially over vegetative cells in trichomes (Sukenik *et al.* 2012). PolyP that is stored in akinetes contributes to DNA synthesis and serves as P source during cell germination (Sukenik *et al.* 2012).

Cyanobacteria also store intracellular P in organic forms that include esters, nucleotides, vitamins, and phosphonates. These diverse organic P compounds contain macro- and microelements such as C, N, H, O, S, and Co, such that intracellular P is integral to cellular stoichiometry (Karl and Björkman 2015). Cellular P storage capacity and C:N:P stoichiometry vary substantially among species (Appendix 2 Table S2), strains, and even cells from the same trichome. For example, *R. raciborskii* cultures were found to have six-fold higher cellular P quotas under replete P condition than that when there was no P supply (Xiao *et al.* 2020). The P

quota is also linked to physiological functions, with a critical cellular P quota of 0.3–0.45 pg P cell^{-1} required for akinete differentiation in the trichomes of *Dolichospermum circinale* (formerly *Anabaena circinalis*), and the C:N:P ratio differed in trichomes with akinetes compared to those without (van Dok and Hart 1996). Because cyanobacteria use polyP during periods of P deficiency, cellular P storage is lowest during extended periods of P limitation. In turn, P-limited conditions increase cellular C:P and N:P ratios and vice-versa under P-replete conditions (Appendix 2 Table S2).

Mechanisms of P storage can differ within species and strains depending on environmental factors. For example, Thompson *et al.* (1994) found that cells of *Dolichospermum flos-aquae* (formerly *Anabaena flos-aquae*) stored P in different inorganic and organic P fractions depending on the N source. Under N$_2$-fixing conditions P storage was associated with carbohydrates, but when nitrate was supplied acid-soluble P accounted for 15% of cellular P and there was no P associated with carbohydrates. When there is Pi supply, polyP accumulation was independent of the external input for two *Synechococcus* strains and a *Synechocystis* strain (Li and Dittrich 2019), but the proportion of cells containing polyP bodies can be altered by the relative organic or inorganic P supply (Chen *et al.* 2020).

3.3 Dissolved organic phosphorus scavenging

Dissolved organic P (DOP) comprises low molecular-weight esters and organic colloids, a significant proportion of which is unavailable for uptake by primary producers. However, many cyanobacteria, as well as other phytoplankton and bacteria, can access DOP using enzymes known as phosphohydrolases, which cleave phosphate from organic matter using metabolic energy (Healey and Hendzel 1979, Cembella *et al.* 1984, Jansson *et al.* 1988). Phosphohydrolases are highly diverse in their activity, varying in the location of their production within the algal cell, i.e. endogenous or exogenous (Štrojsová *et al.* 2003); their expression, i.e. constitutive or inducible (Kruskopf and Du Plessis 2004); and optimal pH range for activity, i.e. acid or alkaline (Kruskopf and Du Plessis 2004). Critically, they also vary with respect to the DOP constituents they are able to mineralize. For example, the 5'-nucleotidase enzyme mediates mineralization of nucleotides and their derivatives, CP lyase and phosphonatase mediate the mineralization of phosphonates, and AP mediates the mineralization of phosphomonoesters (Harke *et al.* 2012, Bai *et al.* 2014, Willis *et al.* 2019). The most important of the phosphohydrolases is AP because it is produced by phytoplankton, often in response to Pi-depauperate conditions (Rengefors *et al.* 2003). This enzyme mineralizes the most abundant and labile constituents of the DOP pool, i.e. the phosphomonoesters (Heath 1986, Hantke *et al.* 1996, Huang and Hong 1999). In recent years, phosphonatases have been a focus of study because they provide a mechanism to access a substantial portion of the DOP pool. Phosphonate acquisition genes have been identified in *R. raciborskii* (Bai *et al.* 2014, Willis *et al.* 2019), *Nostoc* PCC 7120 and some marine species, including *Trichodesmium erythraeum* (Dyhrman *et al.* 2006).

Phosphohydrolase activity is critical for the supply of P under Pi limiting conditions, affecting the growth and survival of phytoplankton communities (Chróst and Overbeck 1987), especially those dominated by cyanobacteria (Prentice *et al.* 2019). Phosphatases have been identified in 32 unique cyanobacteria taxa, numbering 11, 10, 6, and 5 species from the orders Nostocales, Oscillatoriales, Synechococcales and Chroococcales, respectively

(Appendix 2 Table S3). Phosphonatases have also been identified in Synechococcales, i.e. *Synechococcus* (Kutovaya *et al.* 2013, Willis *et al.* 2019); and Nostocales, i.e. *R. raciborskii* (Willis *et al.* 2019). Cyanobacterial APA is induced by low external Pi concentrations (Guedes *et al.* 2019, Wan *et al.* 2019) or low cellular P stores (Litchman and Nguyen 2008, Prentice *et al.* 2019), and production of AP is generally constitutive (Jansson *et al.* 1988).

Methodological differences in APA measurement make it difficult for a quantitative comparison among studies. Nevertheless, several studies have highlighted substantial variation in phosphatase activity related to changes in ambient Pi conditions. In field conditions, for example, 226-fold and 225-fold increases in phosphatase activity were demonstrated in cyanobacteria-dominated phytoplankton assemblages subject to Pi-depauperate conditions (Nedoma *et al.* 2003, Prentice *et al.* 2019). Moreover, under controlled conditions in the laboratory, a 6.4 to 8.0-fold (Chen *et al.* 2020) and 54-fold (Huber and Hamel 1985) increase in phosphatase activity under Pi-depauperate conditions was observed in *D. flos-aquae* and *N. spumigena*, respectively.

Phosphatase activity is highly variable both within populations (Rengefors *et al.* 2001) and across strains of a given species (Guedes *et al.* 2019, Willis *et al.* 2019). For example, constitutive phosphatase activity varied 2-fold among two strains of *R. raciborskii* (Willis *et al.* 2019), while inducible phosphatase activity differed markedly among *M. aeruginosa* and *R. raciborskii* strains, with two of five *M. aeruginosa* strains and one of five *R. raciborskii* strains exhibiting significant increases in phosphatase activity under P-deplete conditions (Guedes *et al.* 2019). In contrast, inducible phosphatase activity in other species, such as *D. flos-aquae*, does not appear to vary significantly across strains (Chen *et al.* 2020).

Little is known whether phosphohydrolase mediated mineralization of DOP meets P demand in cyanobacteria species under Pi-depauperate conditions, or the extent to which inter-species and inter-strain variability in the production of phosphohydrolases drives successional changes in phytoplankton communities. Uncertainty about the mechanism and impact of DOP on cyanobacterial growth has meant the use of DOP by primary producers is seldom modelled numerically (Franks 2009) or only at an elementary level (Llebot *et al.* 2010).

3.4 Alternative phosphorus utilization strategies

In addition to assimilation of P into cellular storage, cyanobacteria possess strategies to overcome P deficiency by directly adsorbing P onto the cell surface. *Microcystis* colonies have a mucilage sheath composed of polysaccharides that provide binding sites for ions, allowing for a higher rate of P adsorption compared with individual cells. Shen and Song (2007) observed a 25.9% increase in cell wall polysaccharides of colonial *Microcystis* strain XW01, compared with unicellular strain PCC7806, increased P absorption per unit chlorophyll *a* by up to 6.7-fold, especially at low Pi levels (< 50 μM).

Marine phytoplankton species in the open ocean are subject to extremely low levels of Pi and some reduce their cellular P requirements by substituting non-P membrane lipids for phospholipids. The phytoplankton include cyanobacteria *Synechococcus*, *Prochlorococcus*, and *T. erythraeum*, and diatoms *Thalassiosira pseudonana* and *Chaetoceros affinis* (Dyhrman *et al.* 2006, Van Mooy *et al.* 2009). These phytoplankton species often dominate in P-limited multi-species cultures.

4 Cell growth, species adaptations and competition

4.1 Dependence of cell growth rate on phosphorus

Phytoplankton biomass accumulation rate is commonly used in modelling studies to determine responses to P addition. Proxies for biomass include cell concentration, biovolume, wet weight, dry weight, particulate carbon, chlorophyll *a* and, for some cyanobacteria, phycocyanin, and phycoerythrin. The specific growth rate is typically calculated from changes in biomass during the exponential growth phase:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \tag{4.1}$$

where N_1, N_2 are biomass (or its proxy) at times t_1 and t_2, respectively. From Equation 4.1, the doubling time of cells is $\ln(2)/\mu$. The effect on the growth rate of a limiting factor X, other than temperature, can be determined by multiplying the temperature-corrected growth rate, i.e. the maximum growth rate μ_{max} times a temperature limitation function $f(T)$, with $f(X)$ (Montealegre *et al.* 1995, Nicklisch *et al.* 2008, Xiao *et al.* 2020):

$$\mu = \mu_{max} \, f(T) \, f(X) \tag{4.2}$$

where $f(X)$ is growth limitation by factor X that ranges between 0 and 1. This X could refer to light as it plays a critical role in driving cyanobacterial photosynthesis and species dynamics. For the case when light is non-limiting and X is a single limiting nutrient, e.g. P, the limiting effect is expressed by a few equations, i.e. the Monod equation, which is linked to the extracellular nutrient concentration (Monod 1949); the Droop model, which is linked to the minimum intracellular nutrient quotas (Droop 1973); equations that integrate the Droop model with the maximum intracellular nutrient quotas (Rhee and Gotham 1981, Flynn 2008); and a few equations that scale cell size effects (Reynolds 1997, Smith *et al.* 2014).

Monod equation. From chemostat experiments, Monod (1949) related growth rate of heterotrophic bacteria to the extracellular nutrient concentrations, S, as follows:

$$f(X) = \frac{S}{K_S + S} \tag{4.3}$$

where K_S (mg L^{-1}) is the nutrient concentrations where growth rate is one-half of maximal, i.e. $0.5\mu_{max}$. The Monod equation is mathematically identical to the equation describing Michaelis-Menten enzyme kinetics, based on the assumption of direct coupling between uptake of a limiting nutrient and cell growth.

Droop model. Droop (1974) argued that enzymatic reactions relate to intracellular rather than extracellular nutrient concentrations, as cell growth can continue by accessing previously accumulated substrates. The Droop model therefore describes the way in which growth is limited by the intracellular quota Q, and can only occur when the instantaneous nutrient quota Q is greater than Q_{min} of the limiting nutrient:

$$f(X) = \frac{Q - Q_{min}}{Q} \tag{4.4}$$

In the Droop model, nutrient quotas often use P:C ratios rather than absolute P quota per cell (Jauzein *et al.* 2010), to ensure that parameters are comparable across species and/or studies. Droop (1983) subsequently introduced a half-saturation constant K_Q into Equation 4.4, where growth rate took on a Michaelis-Menten dependence for $Q > Q_{min}$:

$$f(X) = \frac{Q - Q_{min}}{K_Q + Q - Q_{min}} \tag{4.5}$$

Droop model integrated with maximum intracellular nutrient quota. Rhee and Gotham (1981) and Flynn (2008) introduced a maximum intracellular quota Q_{max} into the Droop model. When cell quota attains Q_{max}, cell growth rate is maximal. The resulting revisions of Equations 4.4 and 4.5 are, respectively:

$$f(X) = \frac{Q - Q_{min}}{Q} \cdot \frac{Q_{max}}{Q_{max} - Q_{min}} \tag{4.6}$$

$$f(X) = \frac{Q - Q_{min}}{K_Q + Q - Q_{min}} \cdot \frac{Q_{max}}{Q_{max} - Q_{min}} \tag{4.7}$$

Discussion on growth rate models. Growth rates of some cyanobacterial species follow Monod model (Equation 4.4). For example, Amano *et al.* (2010) found a K_S of 0.002 mg P L^{-1} and a maximum growth rate μ_{max} of 0.274 day^{-1} after 10 days of P starvation in *M. aeruginosa* UTEX 2061, while another *M. aeruginosa* strain FACHB 469, had a higher μ_{max} of 0.75 day^{-1} and a higher K_S of 0.718 mg L^{-1} after 30 days of P starvation (Tan *et al.* 2019). Not all cyanobacterial species, however, adhere to the Monod equation. *R. raciborskii*, for example, preferentially stores P internally rather than increasing its growth rate (Willis *et al.* 2017, Xiao *et al.* 2020). Using the intracellular P storage, *R. raciborskii* can grow for ∼7 to 10 days at near maximal rates, when there is no extracellular Pi supply (Willis *et al.* 2017, Xiao *et al.* 2020).

The higher growth rates were also found under a pulsed Pi supply instead of a single Pi addition, including for cultures of *R. raciborskii* strains CCMP1973 and MVCC19 (Amaral *et al.* 2014), and field populations dominated by filamentous *P. agardhii* and *R. mediterranea* (Aubriot and Bonilla 2012), and *R. raciborskii* (Posselt *et al.* 2009).

One challenge in simulating growth rate is the inference implied by Monod kinetics that growth is directly linked to uptake rates of the limiting nutrient. Comparing the Monod nutrient uptake kinetics models of Equations 3.5 and 3.7 with the Droop formulations of Equations 4.4 to 4.7, it is evident that the addition of the intracellular quota uncouples the growth rate from the uptake rate. When $Q > Q_{min}$, the uptake rates is reduced while the growth rate increases. Smith *et al.* (2016) characterised growth rate of phytoplankton at steady state based on the assumption that nutrient uptake rate (v) is linearly related to growth rate (μ) through the coefficient of the intracellular quota Q:

$$v = \mu \, Q \tag{4.8}$$

The growth equations above are all predicated based on Liebig's law of the minimum (after Justus von Liebig, 1803–1873), that at any one time only one substrate is limiting growth. It is known, however, that the limiting substrate can change through time or that there is interaction among potentially limiting substrates. Montealegre *et al.* (1995) attempted to address this issue by modelling growth based on interactions between N and P, using a multiplicative function as:

$$\mu = \mu_{max} \, f(T) \, f(N) \, f(P) \tag{4.9}$$

However, Droop (1983) demonstrated that the multiplicative formulation based on intracellular nutrient storage underestimated growth rates in some continuous cultures by as much as 30%. Despite some interaction among growth-limiting nutrients, Liebig's law of the minimum still appears to provide the closest theoretical and empirical basis for nutrient-limited growth, see

also Guven and Howard (2006) and Saito *et al.* (2008):

$$\mu = \mu_{max} \, f(T) \cdot min \left\{ f(N), f(P) \right\} \qquad (4.10)$$

However, O'Neill *et al.* (1989) used an additive model to describe limitation by two nutrients after comparing eight different models, including those using multiplicative (Equation 4.9) and minimum (Equation 4.10) functions. The additive model included the extracellular concentrations of each nutrient N_1, N_2, and a half saturation constant for growth for N_1 and N_2 as in (Equation 4.11). Note that, Equations 4.9–4.11 are valid when light is non-limiting.

$$\mu = \mu_{max} \, f(T) \cdot \frac{N_1 \, N_2}{K_2 \, N_1 + N_1 \, N_2 + K_1 \, N_2} \qquad (4.11)$$

Growth responses to dissolved organic P versus inorganic P. Cyanobacterial growth rates also differ within and between species and strains depending on P forms. *M. aeruginosa* preferentially utilizes Pi over polyP and organic P sources such as β-glycerol phosphate (Peng *et al.* 2018). By contrast, there was no difference in growth rate of a *Nostoc* strain with either dipotassium phosphate (K_2HPO_4) or β-glycerol phosphate as the P source, but the growth rate was significantly slower when the P source was (2-aminoethyl)-phosphonic acid (Dong *et al.* 2019). *D. flos-aquae* could also grow with two different organic P forms: nucleotide adenosine monophosphate (AMP) and lecithin, as the sole P source for cell growth (Lin *et al.* 2018). These researchers also incorporated a qPCR-based method that identified the relative expression of *phoD* and *phoX* genes, to demonstrate that AP regulates the release of P from DOP. *R. raciborskii* showed no significant difference in growth rate when the P sources were: K_2HPO_4, β-glycerol phosphate, D-glucose-6-phosphate or (2-aminoethyl)-phosphonic acid, but it could not grow with glyphosate addition (Bai *et al.* 2014). Glyphosate is a form of phosphonate, which is not bioavailable for freshwater cyanobacteria but is used by some marine cyanobacteria when Pi is scarce (Moore *et al.* 2005, Dyhrman *et al.* 2006, Dyhrman *et al.* 2009).

Genomic studies of the unicellular marine diazotroph *Crocosphaera watsonii* indicate a capacity for high-affinity Pi transport using homologs of the *pstSCAB* transport genes in low-P, oligotrophic systems and a capacity for phosphomonoester hydrolysis, e.g. a putative AP (Dyhrman and Haley 2006). Consistent with these genomic observations, growth of *C. watsonii* was possible using phosphomonoesters as the sole source of P but not on phosphonates. Genomic analysis of *T. erythraeum* has also identified proteins predicted to be associated with the high-affinity transport and hydrolysis of phosphonate compounds by a C-P lyase pathway (Dyhrman *et al.* 2006).

4.2 Species adaptation to phosphorus supply and forms

In lakes, P concentrations typically vary due to a combination of multiple dynamically interacting sources and sinks (Rigler 1956, Dodds 1993, Hudson *et al.* 2000). Phytoplankton species, including cyanobacteria, develop physiological adaptations that are activated to align with those on which transient environmental fluctuations of P occurs (Falkner *et al.* 2006, Posselt *et al.* 2009, Aubriot and Bonilla 2012, Amaral *et al.* 2014). However, most physiological studies of phytoplankton responses to Pi, including uptake kinetics, gene expression, and growth rates, are often designed around discrete steady state scenarios, e.g. P-starved versus P-replete. This hinders our understanding of the dynamics of Pi responses of phytoplankton, including cyanobacteria, especially in the natural environment (Li and Dittrich 2019, Prentice *et al.* 2019). In addition, the use of fixed and species-specific Pi uptake constants, as outlined in Section 3.1, is inconsistent with current knowledge of microbial metabolism and genomics, including the notion of a continually changing ambient Pi.

Cyanobacteria acclimate to environmental Pi fluctuations by physiological adjustments of Pi uptake systems (Falkner *et al.* 1995, Wagner *et al.* 2000, Aubriot *et al.* 2011, Aubriot and Bonilla 2012) and changing the expression of genes involved in Pi acquisition (Juntarajumnong *et al.* 2007, Pitt *et al.* 2010, Cáceres *et al.* 2019, Willis *et al.* 2019) (see Section 3). P-deficient populations exposed to short-term P fluctuations may have short reaction time responses within generation times (minutes to hours) (Aubriot and Bonilla 2012). For this case, Pi uptake regulation is performed by an existing array of uptake systems, in order to optimize growth and P acquisition, and to sustain long-term growth (Plaetzer *et al.* 2005, Falkner *et al.* 2006, Aubriot *et al.* 2011, Aubriot and Bonilla 2012). Over time scales of several hours to days (spanning generation times) up- or down-regulation of genes integral to Pi uptake, such as high affinity P-binding proteins (*pstS*) and alkaline phosphatases (*phoX*, *phoD*), provides an adaptive response to varying Pi supply (Solovchenko *et al.* 2020). Expression of *pstS* ocurrs within hours (e.g. 4 to 6 h) of tranferring cell suspensions in exponential growth phase from P-replete to P-free media, while AP enzymes take a longer time to activate (Wagner *et al.* 1994, Scanlan *et al.* 1997, Orchard *et al.* 2009, Bar-Yosef *et al.* 2010, Pitt *et al.* 2010, Bench *et al.* 2013, Pereira *et al.* 2019). For example, a study of N-deficient natural cyanobacterial populations showed that Pi uptake is activated 20 h after addition, but full activation occurred only after 39 h (Aubriot 2019).

Increases in ambient Pi concentrations in the natural environment can be sudden, e.g. a rainfall-runoff event contributing high P to a lentic system, but the decrease in P may take much longer, depending on the physical and biogeochemical fluxes (Steinman *et al.* 2009, Moura *et al.* 2020). The time scales on which these fluxes alter Pi are not easily accommodated in laboratory studies. Many P-limitation assays in the laboratory involve centrifuging, washing, and resuspending cells in P-free media, which results in immediate removal of external Pi and upregulation of several genes involved in Pi scavenging, even if these cells still have sufficient polyP reserves to maintain growth for several generations (Bar-Yosef *et al.* 2010, Li and Dittrich 2019, Pereira *et al.* 2019). This scenario is unlikely to occur *in situ* where Pi depletion is likely to occur on longer time scales. Laboratory studies have usefully established that cyanobacteria react to Pi removal by inducing the expression of *Pho* regulon genes (Suzuki *et al.* 2004, Schwarz and Forchhammer 2005, Juntarajumnong *et al.* 2007) but do not immediately activate internal P stores because polyP granules are osmotically inactive (Aubriot *et al.* 2011). The laboratory studies therefore provide clues that cyanobacteria are extremely well adapted to optimise growth at short time scales (minutes to hours) preferentially over storing P (Falkner and Falkner 2011, Bernhardt *et al.* 2020).

When P-deficient organisms with highly active uptake systems experience a sudden rise in ambient Pi concentrations from pico to micromolar levels, the transporters can transfer large amounts of P into the cytoplasm before downregulation takes effect (Voronkov and Sinetova 2019, Solovchenko *et al.* 2020). Luxury Pi uptake or 'overplus' follows due to slow disengagement of the previous acclimation response to P deficiency, before downregulation of the expression of genes encoding Pi transporter/binding proteins (Solovchenko *et al.* 2020). This rapid and excessive P accumulation, which may be mostly an artifact of rapid P transitions in laboratory experiments, appears to contradict an expected optimization of the balance of P utilization and storage (Falkner *et al.*

1989). At the same time, adequate acumulation of P is needed to fulfill cell maintenance requirements, but excessive accumulation of polyP needs to be avoided so as not to disturb cell function. Falkner *et al.* (1989) has proposed that sensory features for Pi uptake by cells are an adaptation to optimize the balance of growth, maintenance and storage under different environmental P levels.

Evidence of adaptation of Pi uptake to short-term P fluctuations is found in experiments with cyanobacterial cultures (*A. nidulans* (*Synechococcus*), *A. variabilis*, *R. raciborskii*) (Falkner *et al.* 1989, Falkner *et al.* 1995, Wagner *et al.* 1995, Falkner and Falkner 2003, Falkner *et al.* 2006, Amaral *et al.* 2014) and natural blooms (*P. agardhii, R. mediterranea*) (Aubriot *et al.* 2000, Aubriot *et al.* 2011, Aubriot and Bonilla 2012, Aubriot and Bonilla 2018). In these studies, Pi uptake kinetics by P-deficient cyanobacteria are altered within minutes of a P pulse (<10 min in cultures and < 20 min in natural samples). The adaptation consists of a slowdown of the uptake rate and an increase in the P threshold value; a regulatory response that occurs well before the P storage capacity of cells is maximal. These kinetic alterations can be observed after a sequence of Pi pulses (Falkner *et al.* 2006, Aubriot *et al.* 2011, Aubriot and Bonilla 2012) or by diluting a cell suspension (Falkner *et al.* 1995, Wagner *et al.* 1995, Wagner *et al.* 2000), such that the exposure time of cells to ambient Pi is extended via increased P residence time. By slowing down uptake rates and increasing the Pi threshold values, cells attain a stationary state of least energy dissipation and significant P storage can be avoided. Sequences of Pi pulses in experiments with *R. raciborskii* cultures can result in high growth rates (Amaral *et al.* 2014). Therefore, the energetic optimization of Pi uptake with minimal energy dissipation seems to be more relevant to cell growth than for storage. Further studies are needed to integrate these findings of flexible Pi uptake behavior displayed by cyanobacteria into current concepts of co-existence and competition when P becomes the main limiting resource.

4.3 Species competition and co-existence in relation to phosphorus

When multiple species share the same limiting resource, competition is expected. Accordingly, in the case of P limiting conditions, the superior competitor is considered to be the species that generally has the lower P requirements (Tilman 1977). In this context, species coexistence can be considered a consequence of non-equilibrium conditions (sensu Hutchinson 1953), because environmental conditions change before reaching competitive exclusion. However, Li and Chesson (2016) provided a different perspective about nonequilibrium dynamics on phytoplankton coexistence. At the inter-specific level, functional groups have been proposed to aggregate species according to their spatio-temporal patterns *in situ* (Reynolds 2006). In this sense, the dominant group possesses advantageous traits for the specific environmental conditions. At the intra-specific level, trade-offs between physiological traits amongst strains (see Section 3) also provide resilience of populations and species to continuously low or variable P supplies. For example, Xiao *et al.* (2020) suggested that variations in P allocation to growth and storage in six *R. raciborskii* strains isolated from a single *in situ* population provided resilience to fluctuating Pi supply.

Chesson (2000) proposed a theory of species coexistence, where two main mechanisms are assumed to modulate the inter and intra-specific competition in a community at equilibrium: equalizing and stabilizing forces. The equalizing force corresponds to reduced fitness among species or strains. Here fitness refers to the number of descendants. The stabilizing force

is an abundance-dependent outcome. By increasing intra- over inter-specific competition, stabilizing mechanisms favour the less abundant species. In the classic view of competition, the exclusion of some phytoplankton species with slow Pi uptake and growth rates is expected during transition from Pi deficiency to sufficiency (see Section 4.2). However, the adaptability of uptake systems (see Section 5.2, Issues 1) affects the extent of competition intensity and co-existence. The incomplete P depletion (high-threshold value) and the decrease in uptake rates, when cyanobacteria are exposed to Pi fluctuations, are interpreted as a state of energetic optimization of uptake systems (Falkner *et al.* 1989, Plaetzer *et al.* 2005) at which the intensity of inter- and intra-specific competition is expected to decrease (Welden and Slauson 1986). Thus, the competitive exclusion would likely be prevented if a common P threshold value is attained by the whole community (Wagner *et al.* 2000, Aubriot *et al.* 2011). Evidence of a common threshold can be identified when, after Pi fluctuations, the resulting high Pi threshold value is stable (exceeding generation times) and independent of the P storage capacity of organisms, indicative of the regulation features of the uptake systems in cultures (e.g. Wagner *et al.* 1995, Isvánovics *et al.* 2000, Wagner *et al.* 2000) as well as in P-deficient phytoplankton dominated by cyanobacteria (e.g. Aubriot *et al.* 2000, Aubriot *et al.* 2011, Aubriot and Bonilla 2012). Interestingly, the resulting Pi uptake kinetic by phytoplankton could be fitted to the flow-force model (see Section 5.2, Issues 1), meaning that they are operating like a single population. Therefore, P-deficient populations with flexible uptake systems may be able to synchronize their uptake kinetics when responding to the same ambient Pi fluctuation pattern (Aubriot and Bonilla 2012). When no further Pi fluctuation takes place, populations with the highest affinity for Pi and/or the capacity to hydrolyze DOP may have better chances to persist using recycled P (Prentice *et al.* 2015, Prentice *et al.* 2019). The hydrolysis of DOP into Pi forms by cyanobacteria and heterotrophic bacteria (see Section 3.3) could favour the persistence and coexistence of many species under limited Pi conditions. For instance, Schoffelen *et al.* (2018) reported distinct preference for P among the cyanobacteria species *Nodularia*, *Dolichospermum* and *Aphanizomenon* during summer in the Baltic Sea, while the enhanced capability of *Aphanizomenon* to utilize organic P favoured the persistence of the three species by partitioning the P source.

Deterministic models typically use parameters obtained from monoculture experiments under fixed P levels for predictions of competition in mixed species or strain assemblages (Marinho *et al.* 2013). The predicted outcomes of competition in mixed communities may disagree (Suominen *et al.* 2017) or agree (De Nobel *et al.* 1997) with the results obtained from monoculture experiments. Laboratory culture experiments indicate that *R. raciborskii* is highly competitive for limiting resources, e.g. P (Ryan *et al.* 2017). This species has high invasion potential, as evidenced from its rapid spread to many areas of the world where it was previously absent (Padisák 1997, Sinha *et al.* 2012). However, low invasive capability of *R. raciborskii* was also observed in natural samples even under favourable environmental conditions (Bolius *et al.* 2019), possibly due to the coexisting competing species (Govaert *et al.* 2021). Beyond N and P, some micronutrients also limit cell growth and species invasion, which need to be included in process-based modelling predicting species competition (Hofmann *et al.* 2021).

The mismatch between model predictions and measured population level responses in the field might be partially attributed to the variable environmental conditions and the resulting variability of physiological responses in the field (see Section 3). Some models are based on Lotka-Volterra equations, therefore, still

quite descriptive (sensu Tilman 1977), mostly due to oversimpli-fication of the processes being considered (Spaak and De Laender 2020). The temporal and spatial scale of changes in environmental conditions leads to dynamic and complex species traits (Giordano 2013), which are likely expressed through competitive interactions and changes in algal community composition (Sommer 1985, Suttle *et al.* 1987). Thus, predictions based on laboratory experiments fail to incorporate all the complex processes and interactions present in natural environments.

There is also mismatch between theoretical consideration and experimental studies in evaluating species competition. Most theory of phytoplankton dynamics is based on equilibrium or steady-state conditions to evaluate the competition outcomes in terms of the minimum resource requirements (Tilman 1982) and the invasion criterion, i.e. the capability of a species to recover when in low abundance with other species in equilibrium (Grainger *et al.* 2019). Equilibrium and steady-state can be artificially achieved in chemostat systems, however, batch cultures are more similar to the discontinuous epilimnetic conditions found in natural environments (Wagner and Falkner 2001). Additionally, batch culture experiments are often stopped after reaching stationary phase, where equilibrium could occur. The estimation of cell death or loss rate is critical in evaluating species interaction is rather simplified in laboratory experiments. The loss rate is usually adjusted by applying different dilution rates in chemostats, while in reality, is variable depending on interactions among many environmental conditions (Kozik *et al.* 2019).

It is worth mentioning that in spite of differences in Pi uptake rates (Popendorf and Duhamel 2015), interactions between bacteria and cyanobacteria could result in a range of possible outcomes from positive to negative (Berg *et al.* 2009, Becker *et al.* 2019). This complex scenario is rarely considered in theoretical prediction, and negative interactions (e.g. competitive exclusion) receive a disproportionate attention in comparison to that of positive interactions (e.g. facilitation, Simha *et al.* 2022). An improved understanding of the roles of flexible physiologies under Pi fluctuation, and the partitioning of resource acquisition under low P (e.g. high affinity uptake systems and APA) upon competition intensity, could lead to advances in integrating physiological features in coexistence-competition models.

5 Summary of challenges for understanding phosphorus and cyanobacteria dynamics

5.1 Issues in physiological research linking with models

Issue 1. Problems with definitions and measures of phosphorus

Laboratory-scale experiments have identified that cyanobacteria have a wide range of strategies for utilizing P when it is limiting. Here we used the word 'limit' but point out a range of terminologies used to define the processes in which phytoplankton physiological responses are linked to availability of P. We collated published studies using cultured marine and freshwater cyanobacteria which linked terms to P concentrations (see results in Fig. 5, and methods in SI Appendix 1). The use of multiple terms and definitions has led to confusion and misinterpretation about the physiological state of cells in relation to P. For example, a given Pi concentration can be denoted as 'sufficient' or 'deficient', depending on the study and the studied species. In other studies, cultures grown without external P supply are defined interchangeably as
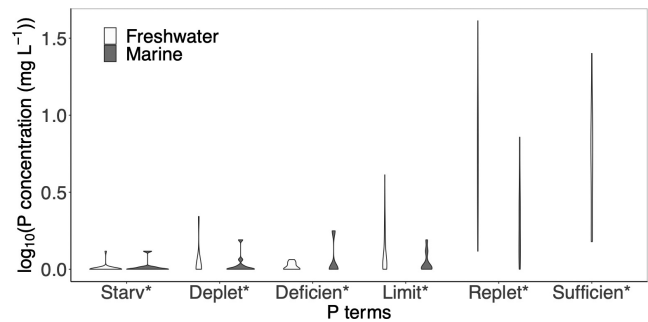

Figure 5. Violin plot of phosphorus terms and concentrations from published culture studies of freshwater and marine cyanobacteria. As there were lots of zero concentrations in P 'starv*', 'deplet*', 'limit*' and 'deficien*' treatments, P concentrations were transformed by applying log10(x + 1), where x is the individual P concentration from each study. The shape of the violin shows the probability density of the data. Note that no culture studies from marine cyanobacteria used 'sufficien*'. For 'starv*', laboratory cultures were mostly in media with a given P concentration (i.e. no supply). The terms of 'deplet*', 'limit*', and 'deficien*' were sometimes used interchangeably within a study.

depleted (or in depletion) or starved (or in starvation). A good example to define these terms is provided in Xiao *et al.* (2020), where starvation is defined from cell physiological responses linked to cellular P storage. Most laboratory studies have not measured nutrient quotas for individual species or strains. As a result, it is unclear in these studies whether cells are in fact P limited. This has resulted in inappropriate use of starvation to describe the physiological state of cells. It is evident that external Pi concentration provides a poor proxy for growth limitation and that quantitative indicators and proxies should be developed to be linked to cell internal P quota. However, for cases where external Pi concentration can be measured accurately at high frequency, changes in the concentration can be linked to cell uptakes rates and, by extrapolation, growth rates. For example, the autonomous sensors (optical or chemical) are providing an opportunity to greatly advance our understanding of Pi uptake rates, from collecting high spatial and temporal resolution of phosphate measurements (Mahmud *et al.* 2020).

Box 1. Phosphorus terms and concentrations from published culture studies of freshwater and marine cyanobacteria.

P limit* (limiting, limitation); P deficien* (deficient, deficiency), P deplet* (deplete, depletion)—controls whether phytoplankton, including cyanobacteria, grow. Reynolds (2006) provides a practical definition that limitation is based on the magnitude of a phytoplankton response to P augmentation.

P starv* (starvation, starved)—at the extreme end of limitation is P starvation where cellular P reaches a minimum quota, beyond which cells senesce.

P reple* (replete, repletion); P sufficien* (sufficient, sufficiency)—phytoplankton species have sufficient internal P available either within cells or in the media/water column to sustain growth at optimal or near-optimal levels for some period of time (e.g. one or more generations).

Other P terms including depauperate, P-stressed and P-free.

It is also clear that analytical methods for determination of P are only a proxy, and they can either overestimate or underestimate the bioavailability of P. Many species appear to be able to survive and even grow *in situ* at concentrations below routine an-

alytical detection limits. Additionally, the accuracy and sensitivity of methods to measure organic P and cellular P storage is still limited, which hampers the accurate quantification of physiological responses of cells to P supply.

Issue 2. Difficulties in comparing inter and intra-population variations to phosphorus availability

Cyanobacteria populations in freshwater systems are inherently variable in terms of morphological characteristics, physiological function and genetics (collectively referred to as strains, or phenotypes, ecotypes, chemotypes and genotypes) (Lakeman *et al.* 2009). For the purposes of this review, strains are referred to as isolates of individual cells, colonies or trichomes. Researchers are only now starting to understand intra-species variability, but the initial findings have revealed interesting results that still require some interpretation. For example, intra-specific variation in growth rates of *R. raciborskii* and *M. aeruginosa* was greater than inter-specific variation (Xiao *et al.* 2017). Collating physiological information at a species level based on laboratory experiments across studies has yielded variable results, it now seems likely that the main reason for this is strain variation within species (Xiao *et al.* 2020).

Comparing inter- and intra-population variations within and among species and strains is complicated by physiological acclimation, morphological plasticity, and genomic evolution under different environmental conditions. In a laboratory study, *R. raciborskii* acclimated to P-free media had longer trichomes and narrower cells, but also developed higher rates of Pi uptake and APA compared with cultures in higher P concentrations (Xiao et al., *in prep*). Another *R. raciborskii* strain was found to undergo substantial morphological and physiological changes during 23 years of culturing, resulting in isolates no longer being representative of their characteristics at the time of isolation (Willis *et al.* 2020). Such adaptations are expressed through horizontal gene transfer and gene loss, and can even lead to loss of ecological functions, such as N_2-fixation (Willis *et al.* 2020).

The inter- and intra-population variations in responses to P are reflected in variability of cellular P storage, based on antecedent environmental conditions. Although it is well documented that periods of P deficiency result in short-term regulation of the cellular uptake system, most laboratory studies fail to mimic these fluctuating conditions and have limited ability to represent acclimation and evolution of species. Conducting laboratory experiments with concentrations and pulsing regimes of Pi relevant to the field is one mechanism to overcome this (Aubriot *et al.* 2011, Aubriot and Bonilla 2018). However, explicitly measuring internal P stores of cyanobacteria population is still difficult for field populations, due to detection limits in analytical methods and the lack of methods to remove all microbes attached to the cells.

Inconsistencies in measurement of cyanobacteria biomass across studies has made it difficult to assess results among studies (Table 1). Physiologists have tended to focus on quantifying growth rates, APA and Pi uptake rates that are normalized by cell number (Kelly *et al.* 2019), dry weight (Li *et al.* 2013), chlorophyll *a* (Dong *et al.* 2019), volume (Wan *et al.* 2019), carbon (Xiao *et al.* 2020), among other measures (Table 1). The number of trichomes (for filamentous cyanobacteria), protein content, and cell surface area are sometimes used, but not often. Although unit conversion, e.g. between carbon and chlorophyll (Jakobsen and Markager 2016), or normalization, e.g. transforming values by comparing to the maximum values (Xiao *et al.* 2017), can be used to standardise units for direct comparison, uncertainties caused by species and strain variations are likely to lead to inaccuracies. However, as carbon and chlorophyll *a* are considered to co-vary with cellular P (Lyck

2004), they could be considered inferior to cell concentration or biovolume. Many ecological models, however, have phytoplankton state variables expressed in units of carbon and chlorophyll *a* (Shimoda and Arhonditsis 2016). Studies that incorporate quantitative molecular analysis of P genes are limited but increasing. These studies also lack consistency in quantitation, with attempts to normalise gene expression sometimes using reference genes such as *rpoC* or 16S rRNA (Yang *et al.* 2018, Willis *et al.* 2019).

5.2 Issues in models linking with physiological research

Issues 1. Michaelis-Menten kinetics are widely used but not always correctly

The classic Michaelis-Menten (M-M) enzyme-catalyzed reaction has been widely applied to characterise not only nutrient uptake, phytoplankton growth and carbon fixation, but also prey and predator relationships (See SI Appendix 2). Its application, however, has several inconsistencies identified as early as the 1970s. Firstly, the model predicts that uptake ceases only when all the substrate is converted to product (zero substrate concentration), and for this reason the hyperbolic relationship of uptake rate versus substrate (e.g. Pi) passes through the origin. Since the uptake of Pi ceases at a measurable limiting value, Droop (1974) argued to include a threshold of substrate concentration (S_0) in the classic M-M kinetics. The existence of a threshold was supported by other researchers based on cellular energetics (Falkner *et al.* 1989, Falkner *et al.* 1995, Button 1998, Bonachela *et al.* 2011). The practical solution was to replace the substrate concentration S by $S-S_0$, as noticed by Droop (1974). Secondly, the M-M model assumes fixed parameter values, e.g. the maximum rate for nutrient uptake and the half saturation constant, but fails to consider the uptake physiology of phytoplankton communities, as well as species that have more than one uptake system and can adapt to fluctuating nutrient conditions (Brown and Harris 1978, Falkner *et al.* 1989, Wagner *et al.* 1995, Aubriot *et al.* 2011). Thirdly, cyanobacteria species are able to modify the functional organization of the high- and low-affinity Pi uptake membrane proteins, via the *Pho* regulon expression, in order to acclimate to limiting and sufficient P levels (Orchard *et al.* 2009, Pitt *et al.* 2010, Wang *et al.* 2018). To solve some of these inconsistencies, some models have adapted the M-M model and incorporated the P acclimation processes by combining changes in low and high affinity Pi uptake transporters that operate over a time scale of hours to days (Cáceres *et al.* 2019). However, this approach is not yet widely used. It is clear that for improved predictions, the M-M model needs to incorporate physiological parameters measured over time and concentration scales corresponding to those of short-term fluctuations in P concentration. Moreover, almost all Pi uptake modelling does not even specify whether it is low- or high-affinity that has been modelled. We have assumed in our evaluation of the algorithms associated with Pi uptake that the fitted parameters are representative of high-affinity uptake, but we recommend that such considerations of recourse limitation explicitly state the nature of uptake, i.e. low- or high-affinity.

At present, the flow-force (F-F) relationship is the only physiological model that describes the adaptation properties of the uptake system itself, since it represents the relationship between nutrient fluxes and the driving force for polyP formation from external phosphate (Thellier 1970, Falkner *et al.* 1989). As the F-F model is driven by external phosphate concentrations, a possible effect of internal P storage on nutrient uptake is not quite clear. This is because the effect of internal polyP reserves is not expected

Table 1. Phytoplankton biomass indices, analysis, and applications.

Phytoplankton biomass indices	Analysis and applications
Wet weight	Commonly obtained from cell volumes measured microscopically at species and taxon levels; units g L^{-1}.
Dry weight	Obtained from air-dried wet samples, including organic and inorganic fractions; units g L^{-1}. Generally found to have a linear regression with cell volume (Reynolds 2006).
Ash-free dry mass	Oxidation of organic fractions of the dry weight, which is approximately the inorganic constituents; units g L^{-1}.
Biovolume	Commonly used in quantifying biomass of laboratory and field samples; units include μm^3 L^{-1}.
Cell concentration	Commonly used in quantifying number of cells of laboratory and field samples; units include cells L^{-1}.
Carbon	Makes up approximately one half of the ash-free dry weight. Commonly used for all phytoplankton; units g L^{-1}.
Chlorophyll *a*	A biomass proxy for photoautotrophic algae and cyanobacteria using solvent extraction and measurements of fluorescence or absorbance. Commonly converted from carbon using a ratio of 50:1 by weight, and average about 1% of ash-free dry mass.
Phycocyanin	Commonly used for cyanobacteria, including C-Phycocyanin (single absorption peak at ∼621 nm, and emission peak at ∼ 642 nm) and R-Phycocyanin (absorption peaks at 533 and 544 nm, and emission peak at 646 nm).
Phycoerythrin	Used for cyanobacteria and red algae, with absorption peaks ∼495 and ∼545/566 nm and emission peaks ∼575 nm. Some planktonic picocyanobacteria and benthic species often have much more phycoerythrin than phycocyanin.

since the granules are osmotically inert structures, as far as we know. Although this model has been tested in cyanobacterial cultures (e.g. Falkner *et al.* 1998, Isvánovics *et al.* 2000, Falkner *et al.* 2006), as well as field populations dominated by cyanobacteria (e.g. Isvánovics *et al.* 1993, Aubriot *et al.* 2000, Aubriot 2019), it is still not widely used. This is probably because experimental design and interpretation of results are complex to perform in comparison to traditional measures involving M-M kinetics. In addition, considering that new properties of polyP are being revealed (Sanz-Luque *et al.* 2020), the possible effect of internal P storage on the regulation of Pi uptake needs to be clarified in future experiments, which may allow for the validation or otherwise of the current suite of widely used kinetic models.

Issue 2. Oversimplified processes and parameters

Species and strains vary in physiological attributes, allowing populations to grow and develop under changing environments; hence, it is anticipated that not all physiological responses are equally important for individual species or strains. Including key physiological attributes is needed to avoid bias in model predictions. However, one deficiency in current models predicting cyanobacterial blooms and species interactions is that some key physiological processes are missing, for example, utilization of DOP. Ghyoot *et al.* (2015) developed a comprehensive model that represented the (de)repressive regulation of APA for a colony-forming prymnesiophyte, *Phaeocystis globose*, in utilizing DOP when external Pi is limited, adapted from the AQUAPHY model. However, the effect of internal P storage was not directly linked to APA. The other key issue for this model is that it requires a significant number of parameters, resulting in difficulties in parameterization and validation when applying it to other species, notably when it would be useful to simulation inter-specific competition.

Interactions of a range of physiological responses with P utilization tend to be oversimplified in models or often not represented at all. For example, diazotrophic cyanobacteria require more P to fix atmospheric N$_2$ under limitation of dissolved inorganic nitrogen (Moisander *et al.* 2003, Moisander *et al.* 2007). However, correctly simulating N$_2$ fixation rates, and how N affects P metabolism remains unsolved (Salk *et al.* 2021). Debates remain also on using either multiplicative, Liebig or additive models to simulate interactions among multiple potential limiting factors on cell growth. We attempted to collate the available datasets to test different models in simulating nutrient uptake rate using the key words 'Michaelis-Menten' and 'plankton∗' on Scopus (SI Appendix 2), but these datasets are mostly incomplete, often missing the effects of temperature, light, extracellular N (ammonium, nitrite, or nitrate) or Pi concentrations, or intracellular N or P quotas. We narrowed the selection from 850 to 154 studies by specifically focusing on references that included studies on phytoplankton, Pi uptake and/or APA. Of this selection, 75.3% (116 studies) studied nutrients other than P, 19.5% (30 studies) were related to Pi uptake or APA, 3.2% (5 studies) included impacts of other environmental factors (e.g. light, silica) on Pi uptake, and only 1.9% (3 studies) included both extracellular Pi concentrations and intracellular P quotas in experimental or modelling work.

Scaling from laboratory culture studies, at the level of cellular responses, to cyanobacterial communities in freshwater systems is challenging. The wide range of parameters measured in physiological studies needs to be carefully assessed in model parameterizations and simulations. For example, Franks (2009) found that although individual species take up nutrients following Michaelis-Menten kinetics, the whole algal community takes up nutrients following a linear relationship with P concentrations. This is because many of the parameters including growth and nutrient uptake, were obtained from monoculture experiments, not observations from multi-species communities where there are interactions among species and strains. Moreover, Xiao *et al.* (2017) have argued that applying single values for each parameter is fundamentally flawed, as the uncertainty and variability within and between species is not adequately represented.

6 Future approaches

Ideally, physiological studies on P strategies of cyanobacteria should focus across scales from molecular to ecosystems, ensuring that measured fluxes of different P fractions and forms most closely matches *in situ* conditions, and captures intra- and inter-specific variability, adaptation, and competition. This information will greatly assist in improving models that can help develop hypotheses and support theoretical understanding of how P affects cyanobacterial and phytoplankton communities. Instead of running small-scale laboratory experiments in monocultures that test one or more environmental factors, future research approaches could move towards *in situ* experiments to reflect real-world dynamics and complexity. Molecular techniques, such as metagenomics, metatranscriptomics and metabolomics, when paired with environmental data, will provide new insight into

how specific species or strains respond to differing quantities and forms of P. These techniques allow the responses of individual species to be studied *in situ* as opposed to in the laboratory, where conditions rarely mimic those in the environment.

Despite the issues discussed in Section 5.2, Michalis-Menten kinetics remain the mostly widely used model for nutrient uptake. However, including the threshold of internal cellular P quota and adaptive responses is urgently required. Here, as an example, we propose a new approach to simulate the DOP utilization (APA) and uptake of Pi based on the threshold of cellular P quota Q_{thres} and the effects of external Pi and DOP concentrations:

$$v = \begin{cases} v_{max,PO_X} \frac{[PO_X]-[PO_{X\_0}]}{K_v+[PO_X]-[PO_{X\_0}]} \frac{Q_{max}-Q}{Q_{max}-Q_{min}}, Q > Q_{thres} \\[2ex] v_{max,PO_X} \cdot \left\{ \frac{[PO_X]-[PO_{X\_0}]}{K_v+[PO_X]-[PO_{X\_0}]} + f \cdot \frac{[DOP]-[DOP_0]}{K_v+[DOP]-[DOP_0]} \right\} \cdot \frac{Q_{max}-Q}{Q_{max}-Q_{min}}, \ Q \le Q_{thres} \end{cases} \quad (6.1)$$

where Q_{thres} is the threshold of intracellular P quota that ranges between the maximum quota Q_{max} and the minimum quota Q_{min}. Here, an intracellular quota above Q_{thres} indicates that cells have sufficient external Pi for luxury uptake, while below Q_{thres} indicates that utilization of DOP is also switched on. v_{max,PO_X} is the maximum uptake rate of phosphate; $[PO_X]$ is the concentration of external Pi concentration; $[PO_{X\_0}]$ is the minimum Pi concentration below which uptake of Pi is energetically not viable; $[DOP]$ is the concentration of ambient DOP; $[DOP_0]$ is the minimum external DOP concentration below which cells stop utilizing DOP; f is a non-dimensional coefficient that apportions preference for DOP uptake relative to Pi uptake. This model (Equation 6.1) would address many of the issues that we have identified in this paper, including the importance of cell quota for uptake, the minimum Pi concentration for uptake and the threshold Pi concentration for DOP utilization, and the utilization of P in different forms.

New analytical methods are also urgently needed to accurately detect P fractions at levels and frequencies beyond current detection limits and sampling methods, to more rapidly characterise the forms of DOP available for uptake by cyanobacterial cells, especially in natural freshwater systems; and to precisely detect cellular P content without interference from dead cells or nutrients released from cell lysis.

Improved modelling approaches are needed that include parameters identified in physiological studies. Individual-based models (= agent-based models) provide an example which could be combined with 'lumped' models to represent variability within and between species and strains (Hellweger and Kianirad 2007, Ranjbar *et al.* 2021, Hellweger *et al.* 2022). These approaches are needed to determine how the variability at strain and species levels affects population dynamics. For short-term acclimation and long-term adaptation of species, the effects of antecedent conditions on cells will be reflected in the 'memory' attribute of individual-based models (Hellweger *et al.* 2008). These models will support specific mitigation assessments that are required to develop targeted mitigation of blooms that are specific to species or strains.

Most importantly, transitioning from siloed research to multidisciplinary collaboration is critical to better understand P dynamics in aquatic ecosystems. Instead of running experiments or models separately, we advocate that physiologists, ecologists, molecular scientists, chemists, modellers work interactively. Projects can be designed collaboratively from the outset, allowing for a shared understanding of the limitations and opportunities of techniques available.

7 Conclusions

Our comprehensive review is timely given the recent passing of Dr. David Schindler, who pioneered the early studies linking P concentrations and cyanobacterial proliferations. Schindler's large body of work on P limitation in freshwater lakes has laid the foundation for many subsequent studies showing the close relationship of P, in various dissolved and particulate inorganic and organic forms, to chlorophyll *a*, the proxy used for total phytoplankton biomass. However, we also feel that it is time to build on this paradigm as we learn more about the complicated relationships between P and phytoplankton, and seek ways to capitalize on methodological advances, including 'omics', autonomous sensors, and new modelling techniques.

Cyanobacteria can directly take up and utilize a range of bioavailable P sources. The main source is orthophosphate (Pi), but dissolved organic P (DOP) compounds can also be scavenged enzymatically. Cells store excess P during P replete conditions, termed luxury uptake, and for some species, luxury uptake is prioritized over allocation of P to produce an immediate increase in growth rate. These P utilization strategies also vary within and between strains and species, and hence, populations, resulting in adaptation of communities to changes in the P environment.

Despite insights into P dynamics from many studies, substantial challenges remain in accurately measuring bioavailable P and indicating the P availability in relation to cell physiology, as well as standardising methodologies across studies in quantifying cell physiology in responses to varying or pulsing P conditions. There is also a need to continue to understand population- and community-level responses to P *in situ*. Metagenomic techniques, run in parallel with conventional approaches, could provide a step-change in our understanding of cellular-level responses of specific cyanobacteria to shifts in P sources and concentrations.

A range of process-based models has been applied to simulate Pi uptake, including the conventional Michaelis-Menten (M-M), integrated M-M, Droop quota, chemical reaction (CR) and flow-force (F-F) models. These models relate growth directly to the extracellular substrate concentration, however, not the intracellular nutrient quota, and they often lack threshold effects (e.g. for changes in Pi uptake affinity or upregulation of Pi uptake genes with reductions in P supply). Additionally, other environmental factors interact with P, but this interaction term is often over or under-represented depending on the type of model used (e.g. multiplicative, minimum, additive, etc). The last, but most challenging issue is the ability of models to represent inter- and intraspecific variability that is critical to capture competition, dominance, and bloom formation in cyanobacteria. The models should ideally include short-term acclimation and long-term adaptation using 'memory' attributes that reflect the antecedent conditions.

Supplementary data

Supplementary data is available at *FEMSRE* online.

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