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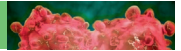
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## MINI REVIEW OPEN ACCESS

# Adaptive Responses of Cyanobacteria to Phosphate Limitation: A Focus on Marine Diazotrophs

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## ABSTRACT

Phosphorus is an essential component of numerous macromolecules and is vital for life. Its availability significantly influences primary production, particularly in oligotrophic environments. Marine diazotrophic cyanobacteria, which play key roles in biogeochemical cycles through nitrogen fixation ( $N_2$  fixation), have adapted to thrive in phosphate ( $P_i$ )-poor areas. However, the molecular mechanisms that facilitate their adaptation to such conditions remain incompletely understood. Bacteria have evolved various strategies to cope with  $P_i$  limitation, including detecting  $P_i$  availability, utilising high-affinity  $P_i$  transporters, and hydrolyzing dissolved organic phosphorus (DOP) with various enzymes. This review synthesises current knowledge regarding how cyanobacteria adapt to  $P_i$  scarcity, with particular emphasis on subtropical marine free-living diazotrophs and their ability to utilise diverse DOP molecules. Omics approaches, such as (meta)genomics and (meta)transcriptomics, reveal the resilience of marine diazotrophs in the face of  $P_i$  scarcity and highlight the need for further research into their molecular adaptive strategies. Adaptation to  $P_i$  limitation is often intertwined with the broader response of cyanobacteria to multiple limitations and stresses. This underscores the importance of understanding  $P_i$  adaptation to assess the ecological resilience of these crucial microorganisms in dynamic environments, particularly in the context of global climate change.

## 1 | Introduction

Phosphorus (P) is a crucial element required for the growth of all organisms, as it plays a central role in the synthesis of central biomolecules (nucleic acids, proteins, and cell membrane lipids). However, P bioavailability limits growth in many terrestrial and oceanic ecosystems. Cyanobacteria, prokaryotes performing oxygenic photosynthesis, are widely distributed across the world and contribute up to 25% of marine primary production (Flombaum et al. 2013). Their biomass is often constrained by the availability and recycling of elements, particularly P; yet they thrive in various environments, from polar regions to the equator (Partensky, Hess, and Vaultot 1999; Garcia-Pichel et al. 2003; Jungblut, Lovejoy, and Vincent 2010; Boyd et al. 2013). Phosphate ( $P_i$ ) is the most readily available

source of P for the growth of autotrophs. The wide ecological distribution of cyanobacteria suggests that they have developed efficient mechanisms to adapt to fluctuations in  $P_i$  levels. Here, we review the cellular mechanisms by which cyanobacteria respond to low  $P_i$  availability. Initially, these P-stress mechanisms prioritise enhancing  $P_i$  transport. However, if the inorganic P source becomes too scarce, cyanobacteria shift towards scavenging organic P sources. Numerous studies have also unveiled additional strategies like a rearrangement of the internal allocation of P to reduce P needs while maintaining the cellular components that most critically need it. We are particularly interested in the response to  $P_i$  limitation in diazotrophic cyanobacteria because they can fix atmospheric nitrogen ( $N_2$ ) and thereby represent a source of new nitrogen (N) for their environment. Their role is particularly

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significant in oligotrophic oceans, where they contribute to sustaining primary production (Karl et al. 2002) and also stimulate microbial activities through the exudation of newly fixed N (Capone, Ferrier, and Carpenter 1994; Mulholland and Bernhardt 2005; Dron et al. 2012). The capacity of diazotrophs to satisfy their N requirements via the reductive assimilation of dissolved  $N_2$  renders them highly competitive in N-depleted regions. This apparent self-sufficiency in N resources means that the growth of diazotrophs is primarily controlled by the bioavailability of  $P_i$  and iron (Fe) in oligotrophic oceans (Mills et al. 2004). Marine diazotrophs have developed adaptive strategies for facing Fe scarcity (González et al. 2018; Jiang et al. 2020). For instance, the genetic regulation of the Fe-mediated stress response increases the Fe scavenging efficiency (Webb, Moffett, and Waterbury 2001). Furthermore, the reduction in the inventory of Fe-metalloenzyme associated with diel variations in the global proteome enables approximately a 40% reduction in the Fe requirements of the diazotrophic cyanobacterium *Crocospaera watsonii* (Saito et al. 2011). Unlike the relatively well-understood influences of nitrate or Fe on diazotrophic growth in marine environments, the regulatory role of  $P_i$  remains significantly less explored. Yet, cumulated evidence from in situ observations demonstrates that diazotrophic cyanobacteria are present in oligotrophic waters known to be particularly  $P_i$ -depleted, like the Southwest Pacific (Moutin et al. 2005), the Northern tropical Atlantic Ocean (Luo et al. 2012), or the Mediterranean Sea (Le Moal and Biegala 2009). The lack of comprehensive understanding of how marine diazotrophs confront and respond to  $P_i$  scarcity may result in unreliable global-scale predictions regarding diazotroph distribution and activity. Leveraging dissolved organic phosphorus (DOP) utilisation could offer a pathway to reconcile these discrepancies (Landolfi et al. 2015).

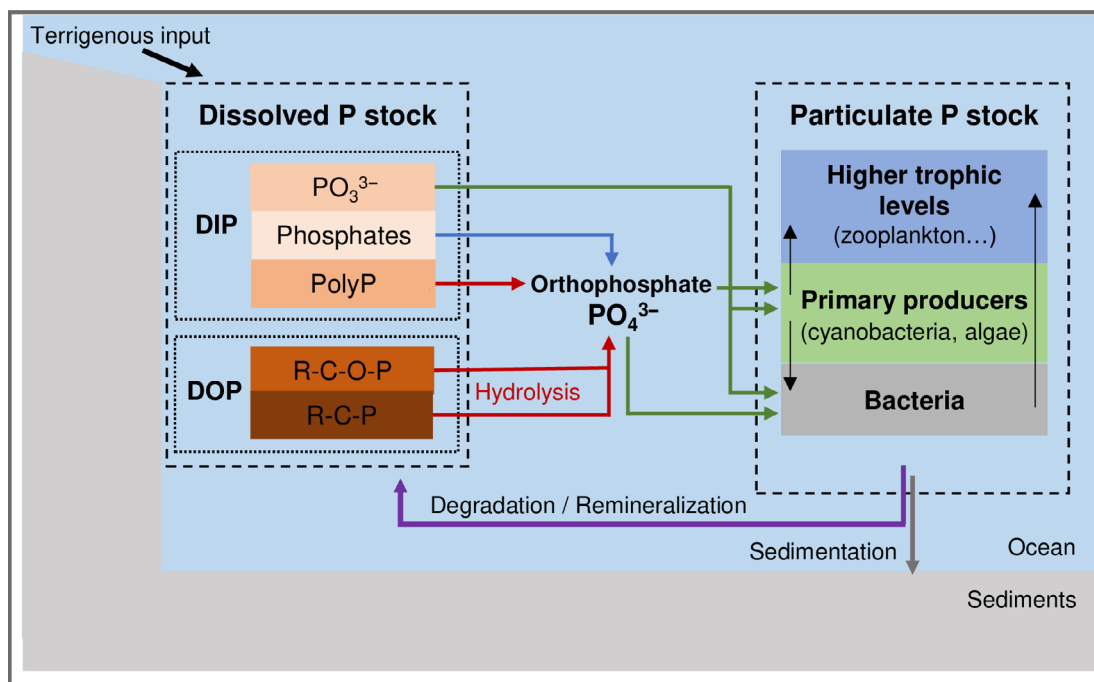
The current understanding of the molecular mechanisms and regulatory processes within cells facing  $P_i$  stress largely stems from studies conducted on model heterotrophic bacteria, in particular, seminal works on *Escherichia coli*. To date, adaptive responses to  $P_i$  deficiency in cyanobacteria have been mostly described in model freshwater strains (Aiba and Mizuno 1994; Scanlan et al. 2009), while our knowledge of the molecular mechanisms involved in marine diazotrophic cyanobacteria remains relatively limited. Therefore, in Section 1, we review the known molecular mechanisms involved in microbial  $P_i$  and DOP utilisation, drawing upon insights gleaned from studies on freshwater and soil bacteria and cyanobacteria. In the second part, we summarise the existing knowledge concerning marine cyanobacteria, with a focus on subtropical, free-living diazotrophic model strains, and we discuss the remaining open questions that merit clarification to understand the dynamics of these phototrophs and their impact on the functionality of marine ecosystems.

## 2 | Known Phosphorus Intake Mechanisms in Cyanobacteria

To ensure an adequate supply under  $P_i$ -limiting conditions, bacteria induce the production of high-affinity transporters to take up  $P_i$  into the cell, as well as a set of enzymes to cleave the  $P_i$  from organic molecules. They have thus evolved different

pathways by which they may assimilate both inorganic (DIP) and organic (DOP) forms of dissolved P. The usable inorganic forms include phosphate ( $PO_4^{3-}$ ), phosphite ( $PO_3^{3-}$ ), pyrophosphate ( $P_i-P_i$ ), and polyphosphate ( $P_i$  chains, thereafter polyP), the latter two presenting a phosphoanhydride, P—O—P bond. The usable organic forms (DOP) include phosphoesters, with C—O—P bonds, phosphoanhydrides, with P—O—P bonds, and phosphonates, characterised by more stable C—P bonds (Young and Ingall 2010; Karl and Björkman 2015) (Figure 1). Note that the techniques used to quantify DOP bond classes have historically erroneously included polyP due to their phosphoanhydride bonds, which could not be discriminated from phosphoesters bonds (Young and Ingall 2010).

The DOP pool can be categorised into three groups based on molecular weight: low (LMW; <400Da), intermediate (IMW; >400Da and <5kDa), and high (HMW; >5kDa) (Karl and Björkman 2015), although the size ranges vary with the separation technique employed. In aquatic environments, the LMW fraction contains polyP, phospho-aminoacids, but also notable amounts of small phosphoesters like glucose phosphate, fructose phosphate, or dissolved nucleotides, as well as a few phosphonates like methylphosphonate (Young and Ingall 2010; Ged and Boyer 2013). The HMW pool is primarily composed of phosphoesters and phosphonates in the marine environment (Clark, Ingall, and Benner 1998) and is rich in humic-acid-like, aromatic molecules with terrestrial origin in freshwater systems (Ged and Boyer 2013). The IMW fraction shows UV-light absorption properties, indicating it may contain nucleotides; however, it is not well characterised (Karl and Björkman 2015) since modern techniques of ultrafiltration and reverse osmosis can only separate LMW from HMW (Young and Ingall 2010). Still, separation by gel chromatography and characterisation by UV absorbance indicates the presence of nucleotide bases in the IMW fraction of coastal seawater samples (Matsuda and Maruyama 1985). Importantly, the DOP pool composition likely varies over time and is heterogeneous regarding both its composition and bioavailability (Hull and Ruttenberg 2022; Grasset et al. 2023). Monomeric and polymeric phosphoesters derive from well-known primary metabolites (e.g., nucleotides, nucleic acids, phospholipids, phosphoglycans, and phosphoproteins). Phosphonates are a class of compounds with a variety of chemical forms and complexities, and many organisms produce them as secondary metabolites. Some of the simplest forms of phosphonates serve as components of biomolecules, entering, for instance, the composition of lipids and polysaccharides, or can be a  $P_i$  source for microorganisms (Horsman and Zechel 2017). Their use as P supply for growth has long been underestimated, although their utilisation is not as widely observed across taxa as that of phosphoesters (Kononova and Nesmeyanova 2002; Beversdorf et al. 2010; Dyhrman et al. 2006; Ilikchyan et al. 2009; Martinez, Tyson, and DeLong 2010). As techniques to recover LMW fraction improved, phosphoanhydrides, including organic (e.g., ATP) and inorganic compounds (polyP), were found to have a much higher turnover rate than the rest of the DOP pool. Consequently, they are believed to play a substantial role in fuelling the growth of primary producers, in particular in oligotrophic regions (Björkman and Karl 1994, 2005; Moore et al. 2005; Martin et al. 2014; Diaz et al. 2018). The known pathways for the use of DIP and DOP substrates are described below and summarised in Figure 2.



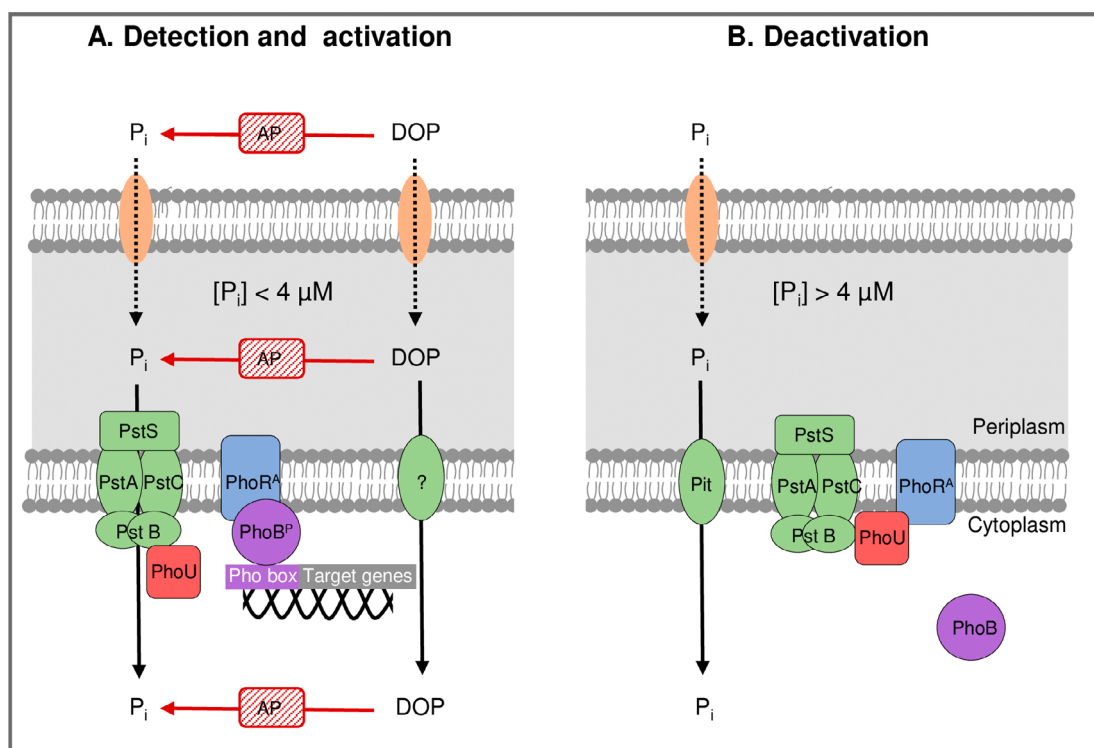
**FIGURE 1** | Simplified schematics of the phosphorus cycle in the open ocean. Arrows represent fluxes of phosphate between the different compartments with a colour coding according to the type of process: Microbial or chemical transformations of DIP into orthophosphate (blue), hydrolysis of polyP or DOP into orthophosphate (red), uptake (green), consumption of particulate P (black), sinking/sedimentation (grey); (i) exudation (by all trophic levels) and (ii) viral lysis, degradation of the particulate matter into DOP, and remineralization into DIP by bacteria (violet). DIP, dissolved inorganic phosphorus; DOP, dissolved organic phosphorus; P, Phosphorus;  $PO_3^{3-}$ , phosphites; polyP, polyphosphates; R-C-O-P, phosphoesters; R-C-P, phosphonates, in which R is a radical (organic group).

## 2.1 | Use of Inorganic Phosphorus

### 2.1.1 | Active Uptake Systems for Phosphate

In Gram-negative bacteria, the entry of ions and small hydrophilic solutes (like most nutrients) from the external environment into the periplasm requires the presence of proteins that form channels, called porins (Nikaïdo and Vaara 1985). In the second step, an active  $P_i$  transport from the periplasm to the cytoplasm is operated by phosphate-binding proteins (PBPs) located in the periplasm, which capture  $P_i$  and carry it to transporters located within the inner membrane. Three different transport systems are known to date (Rosenberg, Gerdes, and Chegwidden 1977; Willsky and Malamy 1980; Lebens et al. 2002): Npt, a low affinity, high-velocity Na-dependent phosphate transport system; Pit, a low affinity, but high-velocity phosphate permease; and Pst, a high affinity, but low-velocity  $P_i$ -specific transport system. Npt is more commonly associated with eukaryotes, notably mammalian cells, although a homologue of the Type II Cotransporters of Eukaryotes has been described in the bacterium *Vibrio cholerae* (Lebens et al. 2002). Pit and Pst, commonly found in microorganisms, have been well described in the model heterotrophic bacterium *E. coli*. The low-affinity  $P_i$  transporter Pit is produced constitutively, while the genes encoding the high-affinity  $P_i$ -specific transporter Pst are induced at low external  $P_i$  concentrations (Rao and Torriani 1990). Genes potentially encoding homologues to PitA and PitB are present in genomes of freshwater cyanobacteria that may encounter high concentrations of  $P_i$  (*Synechococcus* sp. PCC6301, *Synechococcus* sp. PCC7942, *Thermosynechococcus elongatus* BP1, *Nostoc* sp. PCC7120,

and *Anabaena variabilis* ATCC29413), which is consistent with a function as low-affinity permease (Scanlan et al. 2009). Induction of the Pst system in response to  $P_i$  limitation is the most widespread and best-characterised adaptation to low- $P_i$  environments. The Pst system belongs to the ATP-binding cassette (ABC) transporters and is composed of 3 units that are all required for the transporter to function: PstC, PstA, and PstB. These units are encoded by the *pstSCAB* gene cluster, in which *pstS* encodes a periplasmic  $P_i$ -binding protein that carries  $P_i$  to the hydrophobic PstC and PstA, the transmembrane members of the system. PstB is a cytosolic ATPase that uses the energy of ATP hydrolysis to import  $P_i$  into the cytoplasm (Rao and Torriani 1990). The freshwater cyanobacterium *Synechocystis* sp. PCC 6803 harbours two Pst systems, Pst1 and Pst2 (Suzuki et al. 2004), with Pst1 being the principal low-affinity transporter while Pst2 serves as the high-affinity transporter: *pst2* is upregulated to cope with  $P_i$  limitation. Three PBPs proteins are associated with these two Pst transporters: PstS1 and SphX, whose encoding genes belong to the *pst1* cluster, and PstS2 encoded by a gene located in the *pst2* cluster (Pitt et al. 2010; Burut-Archanaï, Eaton-Rye, and Incharoensakdi 2011). The induction of *pstS2* transcription in response to  $P_i$  limitation indicates the involvement of this PBP as part of the low-affinity Pst2 system (Pitt et al. 2010). PstS homologues are found in all sequenced *Prochlorococcus* and *Synechococcus* genomes, some of them in multiple copies (Martiny, Coleman, and Chisholm 2006; Scanlan et al. 2009). Interestingly, genes potentially encoding two Pst systems were found in genomes of other freshwater cyanobacteria such as *Anabaena variabilis*, *Anabaena* sp. PCC7120 and *Gloeobacter violaceus* PCC7421 (Pitt et al. 2010). In the



**FIGURE 2** | Conserved mechanisms for adaptation to inorganic phosphate ( $P_i$ ) limitation in Gram-negative bacteria. (A) Detection of  $P_i$  limitation and activation of the PhoR-PhoB two component system. (B) Deactivation of the PhoR-PhoB system. Porins (in orange) allow inorganic phosphate ( $P_i$ ) and dissolved organic phosphorus (DOP) molecules to enter the periplasm by diffusion (dotted arrows) through the outer membrane. Entry into the cytoplasm is achieved by active transport (solid arrows) via transporters (green). (A) In *E. coli*, the PhoR sensor (blue) is activated when the  $P_i$  concentration in the periplasm is below  $4 \mu\text{M}$ . In response, PhoR phosphorylates the response regulator PhoB (purple), which in turn activates the transcription of target genes, including those encoding the subunits of the Pst high-affinity transporter (green), alkaline phosphatase (dashed red), and PhoU (red). A hypothetical transporter that allows specific DOP molecules to enter into the cytoplasm is represented by an interrogation point. (B) Upon a concentration of  $4 \mu\text{M}$  of  $P_i$ , PhoU dephosphorylates PhoB, leading to the arrest of transcription of its target genes. A, Autophosphorylated; AP, Alkaline phosphatase; P, Phosphorylated.

*Nostoc punctiforme* genome, genes potentially encoding four Pst systems were identified; a functional analysis showed that the PstB1 protein (the ATPase of the Pst1 system) is involved in  $P_i$  internalisation and that functional redundancy with the three other Pst systems may counterbalance the loss of PstB1 in *pstB1*<sup>-</sup> knockout mutants (Hudek et al. 2016). The presence of several copies of uptake systems can therefore provide several selective advantages: adaptation to fluctuating  $P_i$  concentrations and functional redundancy that might compensate for loss of function. Research by Aiba and Mizuno (1994) demonstrated that the SphX protein of *Synechococcus* sp. PCC7942 accumulates when the strain is grown under  $P_i$ -limited conditions. Subsequently, the SphX homologues of cyanobacteria were classified as members of the  $P_i$ -binding proteins family (Mann and Scanlan 1994; Pitt et al. 2010).

### 2.1.2 | A Two-Component System for the Perception and the Response to $P_i$ Limitation

The induction of high-affinity  $P_i$  transporters is the first response to a change in  $P_i$  availability, which cells must first sense. In most bacteria and cyanobacteria, the detection of, and response to  $P_i$  limitation is operated by a two-component system (TCS) which is composed of a histidine kinase sensor and its cognate

response regulator (for a recent review on the mechanisms of TCSs, see the works of Zschiedrich, Keidel, and Szurmant (2016) and Jacob-Dubuisson et al. (2018)). The most conserved TCS proteins that respond to  $P_i$  limitation are homologues of PhoR-PhoB of *E. coli* (Wanner 1996). The PhoR histidine kinase, located in the inner membrane, detects  $P_i$  limitation in the periplasm. The trigger for PhoR autophosphorylation in *E. coli* is a low  $P_i$  level of approximately  $4 \mu\text{mol L}^{-1}$  (Wanner 1996). The subsequent phosphorylation of PhoB activates its DNA-binding activity and hence the transcription of a set of phosphate-starvation-inducible (Psi) genes named phosphate (*pho*) regulon (Wanner and Chang 1987). In addition to genes encoding high-affinity phosphate transporters, the Pho regulon includes genes encoding enzymes releasing  $P_i$  from DOP molecules, and enzymes involved in the storage of nutrients (Wanner and Chang 1987; Santos-Beneit 2015). PhoR-PhoB homologues are widespread amongst bacteria and have in some cases been given different names (for a review, see Santos-Beneit 2015). In addition to the PstSCAB transporter and the PhoR-PhoB regulatory system,  $P_i$ -sensing in *E. coli* involves the metal-binding protein PhoU (Steed and Wanner 1993). Under  $P_i$ -replete conditions, PhoU is somehow required for PhoB dephosphorylation (Steed and Wanner 1993), and for the negative control of the activity and abundance of the Pst transporter to avoid unnecessary  $P_i$ -uptake (Rice et al. 2009).

Orthologs of PhoR-PhoB were identified in *Synechococcus* sp. PCC7942 (Aiba, Nagaya, and Mizuno 1993; Nagaya, Aiba, and Mizuno 1994) and later in *Synechocystis* sp. PCC6803 (Hirani et al. 2001; Suzuki et al. 2004), and were named SphS–SphR (for *Synechococcus* phosphate regulon). In *Synechococcus*, a subset of proteins that were induced upon  $P_i$ -starvation in the wild-type strain was absent in a mutant strain lacking the *sphS* and *sphR* genes; the SphS protein was found to both have an autokinase activity in vitro (Aiba, Nagaya, and Mizuno 1993) and to phosphorylate the SphR regulator (Nagaya, Aiba, and Mizuno 1994). DNA microarray assays performed on *Synechocystis* sp. PCC 6803 indicated that SphS–SphR controlled the response to  $P_i$  limitation and induced the transcription of genes in the *pst1* and *pst2* clusters, as well as *phoA* and *nucH*, two genes encoding DOP hydrolytic enzymes (Suzuki et al. 2004). The inactivation of the *sphS* or *sphR* gene abolished the induction of these  $P_i$ -responsive genes, indicating that the SphS–SphR pair is the main regulatory system transducing  $P_i$  limitation response in this cyanobacterium (Aiba, Nagaya, and Mizuno 1993; Hirani et al. 2001; Suzuki et al. 2004). The deletion of the extended N-terminal extremity of the SphS kinase sensor eliminated the activation of the Pho regulon suggesting that this domain is required for sensing the  $P_i$  levels (Burut-Archana, Incharoensakdi, and Eaton-Rye 2009). Like in *E. coli*, the activity of the SphS–SphR system is negatively regulated in  $P_i$ -replete conditions by the homologue of PhoU, the SphU protein (Juntarajumnong et al. 2007). A study that compared the genomes of 19 cyanobacterial species from different environments identified SphR homologues in 16 of them (Su, Olman, and Xu 2007). The DNA-binding consensus of SphR (the Pho-box) was well conserved only in genomes harbouring a *sphR* gene, which suggests a co-evolutionary history between the regulator and its targeted genes (Su, Olman, and Xu 2007). In *Synechococcus* sp. WH8102, PtrA is a transcriptional regulator acting downstream of PhoBR to activate gene transcription in the response to  $P_i$  depletion. In particular, it activates the expression of genes encoding two phosphatases and a photolyase. It also influences additional pathways, including cell-surface modification, metal uptake, and photosynthesis, suggesting a broader regulatory function in cellular adaptation to environmental stress (Ostrowski et al. 2010).

### 2.1.3 | Polyphosphates, a Less Explored Source of Inorganic P

Recent analytical techniques improved the characterisation of polyP in natural samples, revealing their abundance within microbial communities and high turnover rates even in  $P_i$ -limited regions such as the Sargasso Sea, which suggests that they likely support primary production (Martin et al. 2014). PolyP serves important physiological functions across all kingdoms of life, with cellular synthesis via the polyphosphate kinase PpK and degradation with the polyphosphatase PpX (Kornberg, Rao, and Ault-Riché 1999). According to Young and Ingall (2010), extracellular polyP chains of tens of  $P_i$  are likely too large to cross the membrane and so cannot enter cells as a whole. Their use would first require an extracellular hydrolytic activity prior to uptake. Polyphosphatases, in particular, might fulfil this role. These can be exo- or endoenzymes (Akiyama, Crooke, and Kornberg 1993; Wurst and Kornberg 1994; Kornberg, Rao, and Ault-Riché 1999), but their activity has so far been related to

metabolic processes and functions as well as to stress responses (Brown and Kornberg 2004, 2008) while their role in the uptake of extracellular polyP remains unexplored. PolyP is composed of phosphoanhydride bonds, also present in some phosphoesters like ATP. Various enzymes are tasked with hydrolyzing these bonds, including inorganic pyrophosphatases and nucleoside triphosphatases. Incubations of natural and culture samples of the diatom *Thalassiosira* spp. with a selection of P-compounds showed that polyP was not only substantially hydrolyzed but also exhibited higher maximum hydrolysis rates than phosphomonoesters, suggesting that extracellular polyP are actively degraded and sustain primary productivity (Diaz et al. 2018).

## 2.2 | Use of Organic Phosphorus

In the event of a severe  $P_i$  scarcity in the environment, even an active  $P_i$  transport into the cytoplasm through the high-affinity  $P_i$ -specific transporter Pst will not alleviate the phosphorus limitation and allow cells to meet their P demand. The use of DOP (phosphoesters and phosphonates) may represent an additional survival strategy.

### 2.2.1 | Phosphoesters Utilisation

Alkaline phosphatases (APs), also called phosphoesterases, play a vital role in microbial P metabolism, as they cleave the  $P_i$  group from organic compounds (mainly phosphoesters). APs are critical for responding to  $P_i$  depletion, as they increase their synthesis and activity under low  $P_i$  conditions. However, their function extends beyond addressing  $P_i$  scarcity. AP activities have also been reported in  $P_i$ -rich seawaters, such as coastal regions (Hoppe 1983; Labry et al. 2016) and the deep ocean (Koike and Nagata 1997; Tamburini et al. 2002). In  $P_i$ -replete regions, AP can facilitate carbon uptake from dissolved organic matter to mitigate carbon limitation (Hoppe and Ullrich 1999; Baltar et al. 2009, 2016). For example, higher APA can be associated with fast-sinking particles (Koike and Nagata 1997; Davis and Mahaffey 2017).

The archetype of this enzyme family is PhoA of *E. coli*, and six classes of AP have been described to date: PhoA, PhoD, PhoX, an atypical PhoA, PafA and Psp1. The first three (PhoA, PhoD, and PhoX) have been widely described in prokaryotic organisms. They display different substrate specificity and may require different metal ions for their activity (Coleman 1992; Yamane and Maruo 1978; Wu et al. 2007). First identified as periplasmic enzymes (Coleman 1992), APs have also been described as extracellular or cell surface-located (Poole and Hancock 1983; Von Tigerstrom 1984). Furthermore, a recent in silico analysis of the Global Ocean Sampling metagenomic database (Rusch et al. 2007) revealed a high prevalence of cytoplasmic AP potentially encoded by marine bacterial genomes (Luo et al. 2009). The ability of cyanobacteria to produce APs and utilise organic P-sources contributes to their ecological success in various ecological habitats. The presence and function of AP in various cyanobacterial strains have been investigated using a range of molecular and enzymatic approaches. A PhoD phosphatase was described in the halotolerant cyanobacterium *Aphanothece halophytica* (Kageyama et al. 2011) and PhoX was characterised in

*Microcystis aeruginosa* PCC7806 (Hong et al. 2021). *Anabaena* sp. PCC7120 harbours the typical PhoA (Luo et al. 2010) while the atypical PhoA was reported for *Synechococcus* (Ray et al. 1991) and *Synechocystis* (Hirani et al. 2001). A family of putative AP, PhoV, was found in *Synechococcus* sp. PCC7942, but as its activity is not inhibited by  $P_i$ , the physiological role of this enzyme in  $P_i$  acquisition remains to be demonstrated (Wagner, Masepohl, and Pistorius 1995). PafA, a 5th class of APs, was described in Bacteroidota and presents the peculiarity of being phosphate-insensitive (Berlutti et al. 2001; Lidbury et al. 2022). PafA appears to be the only one not reported in cyanobacteria so far. Last, the most recently described class of phosphatases, Psp1, is found in picocyanobacteria (Torcello-Requena et al. 2024).

### 2.2.2 | Phosphonate Utilisation

Phosphonates, which are characterised by stable C—P bonds, represent another class of organophosphorus molecules that can be used as a source of P under  $P_i$ -limited conditions (Kononova and Nesmeyanova 2002). In *E. coli*, the *phn* operon encodes a phosphonate transporter (PhnC-PhnD-PhnE), a C—P lyase multisubunit complex (PhnG-M), potential transcriptional regulators (PhnF and PhnO), and accessory factors (PhnN and PhnP) (Metcalf and Wanner 1993; Martinez, Tyson, and DeLong 2010). This operon also includes the genes *phnY* (possibly encoding a 2-oxoglutarate dioxygenase) and *phnZ* (encoding a protein of the HD phosphohydrolase family) that are required in the utilisation of 2-aminoethylphosphonate (Martinez, Tyson, and DeLong 2010).

Numerous laboratory investigations have documented bacterial utilisation of phosphonates when provided as the sole P source. Although the *phn* operon is part of the Pho regulon and typically induced under  $P_i$ -starvation, certain strains have demonstrated the ability to utilise phosphonates for cell growth even when  $P_i$  is available (Schowanek and Verstraete 1990). *Prochlorococcus* strain MIT9301 has been shown to grow using phosphonates as its sole phosphorus source through the phosphonate oxidation pathway, which involves the PhnY and PhnZ proteins. Homologues of the *phnY* and *phnZ* genes have been identified in several *Prochlorococcus* genomes (Sosa, Casey, and Karl 2019). The marine bacterium *Pelagibacteriales* sp. strain HTCC7211, is believed to facilitate methane formation through the cleavage of the C—P bond in methylphosphonate under P-starvation (Metcalf et al. 2012; Carini et al. 2014). Similar observations have been made with freshwater bacterial isolates (Yao, Henny, and Maresca 2016) and cyanobacteria (Gomez-Garcia et al. 2011; Teikari et al. 2018), shedding light on the methane paradox (Karl et al. 2008). These studies have shown that  $P_i$  availability can mitigate the excessive production of methane by microorganisms.

## 3 | Phosphorus Scavenging in Marine Cyanobacteria

In the upper layer of the oceans, DIP, and especially  $P_i$ , are usually depleted, making DOP the dominant source of dissolved P (Benitez-Nelson 2000; Kolowitz, Ingall, and Benner 2001; Young

and Ingall 2010; Karl and Björkman 2015). The composition of DOP measured by solid-state P nuclear magnetic resonance ( $^{31}\text{P}$  NMR) spectroscopy following a tangential-flow ultrafiltration is estimated at around 75% phosphoesters and 25% phosphonates, and considered to be rather well-conserved between oceanic regions (Kolowitz, Ingall, and Benner 2001). A more recent technique combining electro dialysis and reverse osmosis coupled to  $^{31}\text{P}$  NMR yielded estimations of 80%–85% phosphoesters, 5%–10% phosphonates and 8%–13% polyphosphates, with the latter probably present in the LMW fraction (Young and Ingall 2010).

At a functional and mechanistic level, knowledge of the mechanisms operating in marine cyanobacteria, particularly diazotrophs, is comparatively less advanced (see below). However, recent advancements in comparative genomics, coupled with the extensive analysis of metagenomic data collected during large-scale campaigns, have culminated in a comprehensive understanding of the gene repertoire that cyanobacteria have acquired throughout evolution to flourish in ecological niches characterised by  $P_i$  depletion. Moreover, when coupled with global transcriptomics approaches or metatranscriptomic data surveys, these analyses are even more powerful because they reveal the specific genes that are expressed under varying environmental conditions. To sum up a few, the survey of the distribution of APs encoding genes in the marine metagenomic Global Ocean Survey database (Rusch et al. 2007) showed that the *phoX* and *phoA* genes were widespread in genomes of marine bacteria, including cyanobacteria (Sebastian and Ammerman 2009). In light of an improved knowledge of PhoA sequence diversity in the World ocean, recent analyses have shown that *phoA*, and *pafA* as well in some regions, are more abundant than *phoX* across marine bacteria (Lidbury et al. 2022). More specifically, in Cyanobacteria, the atypical *phoA* (and also *psp1* in certain regions), is more prevalent than *phoX* (Torcello-Requena et al. 2024). Analysis of the metagenomes dataset of TARA ocean focusing on picocyanobacteria revealed the presence of the two-component system *phoB-phoR* and the atypical *phoA* gene in nearly all populations of *Prochlorococcus* thriving in  $P_i$ -depleted regions, whereas genomes of *Synechococcus* mainly harboured *phoX* and the  $P_i$ -binding protein-encoding gene *sphX* (Doré et al. 2023). The protein Psp1, induced in response to  $P_i$  deficiency, was very recently characterised as a high-affinity, alkaline phosphatase expressed in some picocyanobacteria found in particularly  $P_i$ -impoverished oceanic regions; structural modelling predicted that it is close to PhoX, to which it also shows convergent evolution (Torcello-Requena et al. 2024). Two loci of genes potentially encoding phosphonate transporters (*phnCDE*) were identified in *Prochlorococcus* genomes isolated from  $P_i$ -limited oceanic regions, and the *phnYZ* operon, involved in the C—P bond cleavage, was found in *Synechococcus* genomes (Doré et al. 2023). The *phnD* gene has been proposed as a proxy of phosphonate assimilation in cyanobacteria (Ilikchyan et al. 2009). In the Sargasso Sea, *phnD* was found to be constitutive in *Prochlorococcus* and to show an expression pattern in *Synechococcus* that correlated negatively with  $P_i$  availability (Ilikchyan et al. 2010). Altogether, these observations suggest that phosphonate is an alternative source of P for picocyanobacteria in oligotrophic environments. A recent survey of 2568 cyanobacterial genomes for the presence of genes potentially involved in phosphonate metabolism revealed a widespread occurrence of *phn* genes in cyanobacterial genomes with a high

prevalence in filamentous strains (Zhao, Lin, Chen et al. 2022). These results suggest that the use of phosphonates may not be limited to marine cyanobacteria. Moreover, phosphonate impact on cyanobacterial growth in aquatic environments is likely to be underestimated not only due to poor knowledge of the role of putative phosphonate-lyase of cyanobacterial strains in these niches but also due to a limited understanding of the relationship cyanobacteria might establish with other microorganisms of their natural consortia. In this context, a functional study of a microbial consortium during a *Microcystis* bloom period in a freshwater system revealed that this cyanobacterium, which does not possess the ability to hydrolyze phosphonate, benefits from the phosphonate catabolised by heterotrophic bacteria (Zhao et al. 2023).

In addition to the global approaches discussed above, the ability of cultivable strains representative of major groups of marine diazotrophic cyanobacteria to cope with  $P_i$  limitation has been assessed by wet lab approaches, as summarised in the following sections. Readers with a specific interest in picocyanobacteria can refer to the following publications (Tetu et al. 2009; Ostrowski et al. 2010; Doré et al. 2020; Shah et al. 2023).

### 3.1 | P Scavenging in Diazotrophic Marine Cyanobacteria

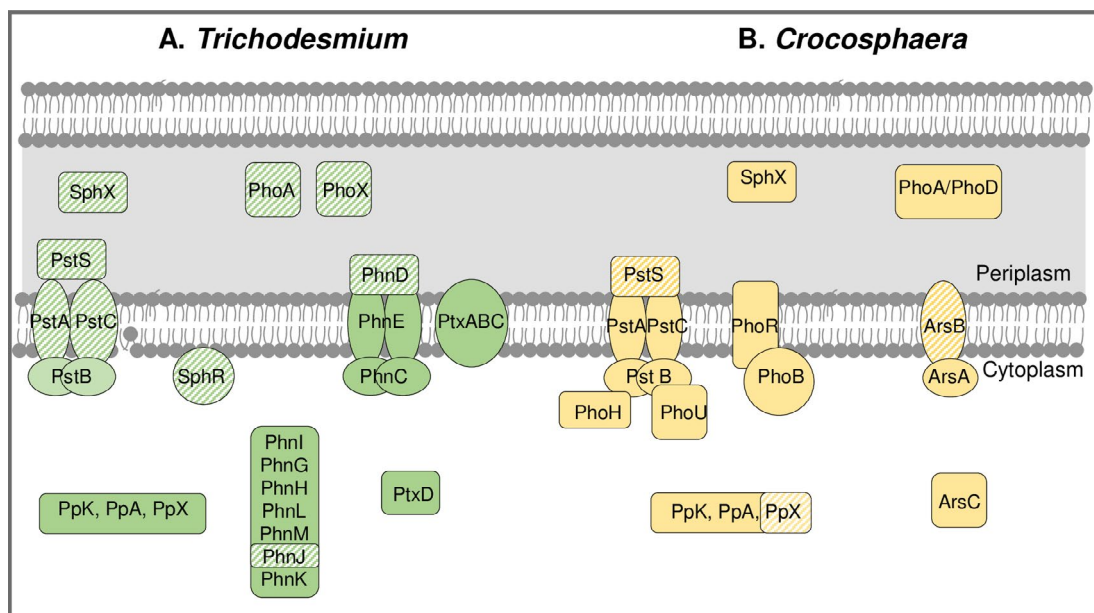
The ecological success of  $N_2$ -fixing cyanobacteria, and hence the biogeography of  $N_2$  fixation, relies on their ability to compete for nutrients other than  $N_2$ . For a review on marine  $N_2$ -fixation see Zehr and Capone (2020), and for a review on the impact of nutrient limitation on  $N_2$ -fixation rate in the ocean, see Sohm, Webb, and Capone (2011).  $N_2$ -fixation rates measured in the open ocean were originally attributed to the filamentous cyanobacteria *Trichodesmium* sp. and *Richelia* sp. (Mague, Weare, and Holm-Hansen 1974; Carpenter and Romans 1991; Carpenter, Capone, and Rueter 1992; Capone et al. 1997; Karl et al. 1997). The advent of molecular techniques (Zehr et al. 1996; Zehr, Mellon, and Zani 1998) revealed the unsuspected diversity, occurrence, and activity of unicellular cyanobacteria and bacterial strains with diazotrophic capability, not only in the oligotrophic surface open ocean (Zehr et al. 2001, 2008; Montoya et al. 2004; Bombar et al. 2013; Foster and Zehr 2019) but also in a variety of habitats where  $N_2$ -fixation was rather unexpected (Foster et al. 2007; Hewson et al. 2007; Moisaner et al. 2010, 2017; Harding et al. 2018; Turk-Kubo et al. 2023). In the open ocean, three groups of diazotrophic, cyanobacterial strains have been identified to date (see Zehr 2011): the filamentous, non-heterocystous and free-living *Trichodesmium*, the heterocystous-forming *Richelia* sp. and *Calothrix* sp., which live in symbiosis with diatoms, and an ensemble of unicellular, diazotrophic cyanobacteria (UCYN). Heterocystous, free-living strains such as *Anabaena* and *Nodularia* are rare in the open ocean and their exclusion from (sub)tropical areas was associated with temperature (Staal, Meysman, and Stal 2003). UCYN are further divided into three groups named UCYN-A, B, and C. UCYN-A is peculiar as it is heterotrophic, due to the loss of photosystem II; it lives in symbiosis with a prymnesiophyte (Thompson et al. 2012) and is now described as an early evolutionary stage  $N_2$ -fixing organelle (Coale et al. 2024). UCYN-B is distributed in open-ocean, (sub)tropical areas; a cultivated

representative is *Crocospaera watsonii*. UCYN-C is most similar to the strain ATCC51142 (Foster et al. 2007) initially described as a *Cyanothece* by Reddy et al. (1993) but recently reclassified as belonging to the *Crocospaera* genus (Mareš et al. 2019). UCYN-C strains seem to be more present in coastal areas than in open oceans (Church et al. 2008; Halm et al. 2012; Cheung et al. 2017; Shiozaki et al. 2018). To date, *Trichodesmium* spp. and *Crocospaera watsonii* remain the main biological models for experimental studies.

#### 3.1.1 | Increased $P_i$ Uptake Abilities Induced by $P_i$ Limitation in *Trichodesmium*

The mechanisms known so far for *Trichodesmium* adaptation to  $P_i$  limitation are summarised in Figure 3A. The expression of the *sphX*, *sphR*, *phoA*, *phoX*, *phnD* genes (with *phoA* being homologous to the atypical AP of *Synechococcus* PCC 7942) is induced in response to  $P_i$  scarcity in *Trichodesmium erythraeum* IMS101 (hereafter *T. erythraeum*; Orchard, Webb, and Dyhrman 2009; Frischkorn, Haley, and Dyhrman 2019). Proteomics assays further indicated that the corresponding protein levels followed the same regulated pattern (Frischkorn, Haley, and Dyhrman 2019). Moreover, homologues of these four genes were found in the genomes of three other cultured *Trichodesmium* strains (*T. tenue*, *T. thiebautii*, and *T. spiralis*), which suggests that the internalisation of  $P_i$  and use of phosphoesters might be conserved across the *Trichodesmium* genus (Orchard, Webb, and Dyhrman 2009). Further, the genome of *T. erythraeum* also harbours gene homologues to those encoding a phosphonate transporter (*phnCDE*), and a phosphonate lyase (*phnG* to *phnM*). The expression of this gene cluster (studied through the analysis transcription of the *phnD* and *phnJ* genes) was found to be induced in axenic  $P_i$ -starved culture of *T. erythraeum* and also in field populations from an oligotrophic region of the western North Atlantic (Dyhrman et al. 2006). The ability of *Trichodesmium* to metabolise phosphonate may have been acquired through horizontal gene transfer (Dyhrman et al. 2006). Besides, *T. erythraeum* contains intracellular phosphonate, suggesting that it might be able to produce it (Dyhrman et al. 2009). Natural *Trichodesmium* sp. colonies were indeed shown to intracellularly reduce up to 16% of the  $P_i$  (which has a +5 oxidation state) taken up into LMW phosphonate molecules with a +3 oxidation state (Van Mooy et al. 2015). Most of this  $P_i$  reduced into phosphonates does not accumulate in cells but instead is released in the environment, where it may benefit the associated microorganisms (Van Mooy et al. 2015). In metatranscriptomic analyses of *Trichodesmium* colonies collected from the North Atlantic Subtropical Gyre (NASG), transcripts for *ptsA* and *ptsC*, which encode the membrane subunits of a high-affinity transporter, were detected. Their abundance was negatively correlated with concentrations of dissolved inorganic phosphate (DIP). In the same study, transcripts of the *phoX* gene were also identified, but *phoA* transcripts were not detected (Cerdan-Garcia et al. 2022).

In its natural environment, *Trichodesmium* forms colonies with heterotrophic marine bacteria, forming complex consortia. And in turn, the chemical signals produced by these heterotrophic bacteria, used in quorum sensing regulation, can also benefit *Trichodesmium*. It was for instance shown that bacteria stimulated *Trichodesmium* AP activity (Van Mooy et al. 2012). This



**FIGURE 3** | Potential mechanisms for adaptation to  $P_i$ -limitation in diazotrophic marine cyanobacteria: Proteins encoded by genes found in *Trichodesmium erythraeum* IMS101 or in metatranscriptomic analysis of *Trichodesmium* (panel A) are in green, and those found in *Crocosphaera watsonii* WH8501 (panel B) are in yellow. Proteins encoded by genes whose transcription is induced by  $P_i$ -limitation are highlighted in hatched boxes. Note that we opted not to include details on phosphite utilisation (discussed in Section 3.1.3) in this figure; therefore, the PtxABC proteins are not represented here.

important observation, together with the previous mention of *Microcystis* benefiting from the activity of their associated bacteria (see Section 2.2.2) represents just a couple of examples that underscore the role of metabolic and chemical signals within microbial consortia. A careful consideration of biotic interactions will provide a more thorough understanding of how marine cyanobacteria adapt to their oligotrophic environment, an approach that is now emerging in the concept of holobiont, which considers the ecological unit composed of an organism and the species associated around it (Simon et al. 2019; Aldana and Robeva 2021).

### 3.1.2 | Increased P Uptake Abilities Induced by $P_i$ Limitation in *Crocosphaera*

Comparative genomic analysis revealed the presence of an entire gene cluster for  $P_i$  transport (*pstCAB* genes) in the *Crocosphaera watsonii* WH8501 genome, with an homologue of the ABC-type phosphate transport system substrate-binding protein SphX/PstS (Shi et al. 2010). The *pstS* gene in this cluster had been identified as potentially encoding a  $P_i$ -binding protein (Dyrhman and Haley 2006) and several copies of *pstS* orphan genes were thereafter identified (Bench et al. 2013). The transcription of the *pstS* gene belonging to the *pstCAB* cluster is induced under low- $P_i$  conditions and is inhibited in the presence of  $P_i$  (Pereira, Shilova, and Zehr 2019). In line with *pstS* gene belonging to the  $P_i$ -binding protein, the expression of the *pstS* gene was reported to be more responsive to  $P_i$  addition than selected DOP molecules (Pereira, Shilova, and Zehr 2016). Altogether, the data suggest that *C. watsonii* likely produces a high-affinity Pst transport system for the uptake of  $P_i$  (Figure 3B). In addition to the presumed high-affinity  $P_i$  transporter, a gene homologous to those encoding the low-affinity transporter PitA was also identified

(Dyrhman and Haley 2006). Its transcription was unresponsive to  $P_i$  limitation, which is characteristic of this family of transporters as discussed above. Comparative genome analysis of six unicellular *C. watsonii* strains, representing two morphological phenotypes (small and large cells), revealed variations in the copy number of genes involved in phosphate ( $P_i$ ) transport and utilisation. Notably, the number of  $P_i$  stress-related genes was higher in large-cell strains (30–32) than in small-cell strains (19–25), with *pstS* showing the greatest variation (5–7 copies in large-cell strains versus 3–4 copies in small-cell strains). Another difference lies in the type of alkaline phosphatase encoding gene present in the genomes: *phoD* was found exclusively in large-cell strains, while *phoA* was unique to small-cell strains. These findings suggest that the acquisition of  $P_i$ -related genes by the large cells or their loss by the small cells may have played a role in shaping the evolutionary trajectory of this bacterial group (Bench et al. 2013).

The presence of *phoR*, *phoB*, and *phoU* gene homologues in the genome of *C. watsonii* (Bench et al. 2013) suggests that the perception and the transduction of the  $P_i$ -starvation signal could be mediated, as in other Gram-negative bacteria, by the two-component system PhoR-PhoB, with the activity potentially regulated by PhoU under  $P_i$ -replete conditions (Figure 3B). An open question about *C. watsonii* adaptation to its oligotrophic environment is which enzyme(s) enable(s) it to hydrolyze DOPs. Indeed, while AP-type activity has been measured by biochemical approaches (Rabouille et al. 2022), none of the three genes annotated as potentially belonging to the AP family was induced in response to  $P_i$  deficiency (Dyrhman and Haley 2006; Pereira, Shilova, and Zehr 2016). Yet, laboratory experiments showed that *C. watsonii* WH8501 can grow on  $P_i$  as well as on the phosphomonoesters glycerophosphate and adenosine monophosphate (Rabouille et al. 2022) as the sole

source of *P. C. watsonii* is also able to use the phosphate diester bis-p-nitrophenyl phosphate (Yamaguchi et al. 2020). However, the gene exhibiting the highest homology to an *E. coli* phosphatase-like gene demonstrated no expression, even under low  $P_i$  conditions (Dyhrman and Haley 2006). The homologues to *phoA* or *phoD* identified in the genomes of small and large *Crocospaera* strains, respectively (Bench et al. 2013) may thus be responsible for the measured hydrolytic activities.

Unlike what is reported for *Trichodesmium*, no homologues to genes known to be involved in phosphonate utilisation were identified in the *C. watsonii* genome (Dyhrman and Haley 2006). This absence is consistent with the inability of this strain to grow when phosphomonofomic acid serves as the sole source of P, indicating that *C. watsonii* likely does not use phosphonate (Dyhrman and Haley 2006). A putative phosphonate-binding protein (PhnD) was identified in the UCYN-C *Crocospaera* strain ATCC51142 (Feingersch et al. 2012), which was thereafter found in all *C. watsonii* strains (Bench et al. 2013). However, PhnC and PhnE, essential components for a functional PhnCDE ABC transporter, are absent from the published draft genome, which further supports the thought that *Crocospaera* is unable to use phosphonate. Although not all the mechanisms involved in the response to  $P_i$  limitation have been elucidated in *Crocospaera* spp., recent researches are clarifying the types of P-compounds they can use (Yamaguchi et al. 2020; Rabouille et al. 2022; Filella et al. 2022) and the impact of micronutrients on their P metabolism (Liu et al. 2020; Yang et al. 2022).

### 3.1.3 | Other Adaptive Strategies to $P_i$ Limitation in Diazotrophic Cyanobacteria

The wide distribution of *ptxABCD* genes in genomes of marine microorganisms thriving in oceanic regions with low  $P_i$  concentrations, including *Trichodesmium* and *Crocospaera* ATCC 51142 (Martinez et al. 2012), suggests that phosphite ( $PO_3^{3-}$ , an inorganic form of P) may be an underestimated contributor to the P cycle in marine environments. *T. erythraeum* can uptake phosphite (Van Mooy et al. 2015), likely through the PtxABC transporter (Polyviou et al. 2015). Phosphite oxidation to  $P_i$  is then catalysed by the phosphite dehydrogenase, PtxD (Martinez et al. 2012). Besides phosphite, *Crocospaera* was also shown to utilise phosphoanhydrides in the form of inorganic triphosphate (3polyP), even when  $P_i$  is not severely limiting in the environment, supporting the hypothesis that phosphoanhydrides may be used as a source of P and energy (Filella et al. 2022). Given that *Trichodesmium* is genetically equipped to use polyP, the absence of a positive response to the addition of 3polyP in the same study was associated with an absence of  $P_i$  limitation (Filella et al. 2022). As previously mentioned, an unresolved question persists regarding the potential utilisation of longer extracellular polyP chains, given their inability to cross the extracellular membrane. In addition to these inorganic P sources, some organic phosphoanhydrides are also bioavailable to diazotrophic cyanobacteria, as evidenced by the utilisation of ATP in *Trichodesmium* (Orchard, Ammerman et al. 2010).

Another widely used strategy to face  $P_i$  scarcity is the mobilisation of phosphorus reserves. PolyP can be stored as granules and

used when the available  $P_i$  is low. Therefore, polyP storage represents a strategy better adapted to environments experiencing pulsed events (Scanlan et al. 2009). Genes encoding cytoplasmic enzymes involved in the synthesis (*ppk*) and degradation (*ppX*, whose protein is described as an exo-polyphosphatase (Akiyama, Crooke, and Kornberg 1993), and an inorganic pyrophosphatase *ppA*) of polyP have been identified in several cyanobacterial genomes (Sanz-Luque, Bhaya, and Grossman 2020), including *C. watsonii* WH8501 and *Trichodesmium* (Dyhrman and Haley 2006; Orchard, Benitez-Nelson et al. 2010). The transcription of *ppX* was reported to be induced in  $P_i$ -limited cultures of *C. watsonii*, suggesting capacities for internal polyP hydrolysis (Deng et al. 2022). For more details on the metabolism of internal polyP, the reader can refer to the review of Sanz-Luque, Bhaya, and Grossman (2020).

When facing a sustained  $P_i$  limitation, the cellular P content of microorganisms tends to decrease (Kilham et al. 1997; Vrede et al. 2002) as a consequence of reduced uptake rate. Microorganisms may compensate for this reduction by remobilizing the P content of some biomolecules towards critical metabolic activities. For instance, in the Sargasso Sea, the proportion of phytoplankton P uptake allocated to phospholipids synthesis was positively correlated with environmental  $P_i$  availability (Van Mooy et al. 2009). Notably, a decrease in phospholipids coincided with an increase in sulphur- and nitrogen-rich lipids. This strategy appears widely used in photosynthetic microorganisms (Bolik et al. 2022). Laboratory experiments demonstrated that marine cyanobacteria, including *Synechococcus*, *Prochlorococcus*, *Crocospaera*, and *Trichodesmium*, are all able to substitute phospholipids with the non-P membrane lipid sulphoquinovosyldiacylglycerol when grown under  $P_i$ -limiting conditions (Van Mooy et al. 2009). In *Synechococcus* PCC7942, inactivation of the *sqdB* gene led to an inability to replace phosphorus with sulphur in lipids; as a consequence, cells exposed to  $P_i$ -limiting conditions could not remobilise P contained in their lipids to support metabolic processes and decayed sooner than the wild type (Güler et al. 1996), demonstrating the active nature of this substitution and its role in the  $P_i$  stress response. This remobilisation does not affect photosynthetic efficiency (Güler et al. 1996) and so represents an efficient mitigation measure for  $P_i$  stress in phototrophic organisms. This is due to the similar biophysical properties of sulfoquinovosyldiacylglycerol and phosphatidylglycerol bilayers, which makes them suitable mutual substitutes in photosynthetic membranes (Bolik et al. 2022).

### 3.1.4 | Cross-Talk With Other Stresses

Arsenate is a toxic element that, because of its analogy to  $P_i$ , can be internalised by  $P_i$  transporters.  $P_i$  stress can therefore be exacerbated by the presence of arsenate, as demonstrated in *Thalassiosira oceanica* (Snow, Holdship, and Rickaby 2020). This could potentially result in cellular arsenate toxicity, particularly in oligotrophic areas, where its relative concentration can be high relative to  $P_i$  (Hashihama et al. 2019). Evolving mechanisms to cope with arsenate-toxicity is part of the adaptation to  $P_i$ -scarcity; in culture, *C. watsonii* WH8501 can sustain growth in the presence of up to 30nM of arsenate, albeit at reduced rates (Dyhrman and Haley 2011), and natural *Crocospaera* populations may even show a stronger resistance (Hashihama

et al. 2019). The underlying tolerance mechanism was attributed to the reduction of arsenate to arsenite by the arsenate reductase (ArsC), and by the efflux of arsenite outside the cell by the arsenite efflux pump (ArsA-ArsB). In axenic cultures of *C. watsonii* WH8501, the expression of *arsB* gene was induced both in the presence of arsenate and in response to  $P_i$  limitation; *arsB* transcripts were also detected in field samples from a low  $P_i$  region (Dyrhman and Haley 2011). Consequently, arsenate detoxification genes can be used as a molecular marker of  $P_i$  limitation. For instance, a combination of *pstS* and *arsB* gene expression analysis in natural populations of *C. watsonii* was used to discriminate the early and late stages of  $P_i$ -limitation (Pereira, Shilova, and Zehr 2016).

$P_i$  deficiency is often coupled with Fe limitation in oligotrophic marine environments. An unexpected and puzzling result was the observation that Fe and  $P_i$  co-deficiency is more favourable to *Crocospaera* WH0003 and *Trichodesmium* growth than  $P_i$  deficiency alone. In *Crocospaera*, this was related to smaller cell size, lower cellular P quota, and higher affinity for  $P_i$  under co-limiting conditions (Garcia et al. 2015). Transcriptomic analyses performed on natural populations of *Trichodesmium* under Fe- $P_i$  co-limitation indicated that metalloenzyme switching, such as increased transcription of the Fe-insensitive PhoA compared to the Fe-requiring PhoX, could alleviate Fe-limitation in DOP metabolism (Rouco et al. 2018). Moderate Fe- $P_i$  co-limitations can also induce a reduction in the transcription of Fe-demanding proteins in the photosynthetic apparatus to increase the turnover rate of Photosystem I, thereby boosting ATP production and ultimately enhancing  $N_2$ -fixation rates (Cerdan-Garcia et al. 2022). Yang et al. (2022) examined the molecular regulations induced in *Crocospaera* WH0005 under conditions of either Fe or  $P_i$  limitation individually or under co-limitations. They observed that the pathways involved in the substitution and conservation of metalloenzymes induced in response to Fe limitation led to a reduction in the nutrient requirements of cells. This resulted in a smaller sized, fast-growing and resource-efficient phenotype, which exhibited enhanced growth performance compared to the  $P_i$ -limiting condition. Revealing the molecular mechanisms governing the interplay between Fe and  $P_i$  availability and cell growth rates in other marine cyanobacteria representatives poses a significant challenge in this field.

In addition to the nutrient (co)limitations typically observed in oligotrophic areas, the manifestations of climate change, such as ocean acidification or temperature increase, impose additional stresses, and the combined impact of those stresses remains to be assessed for many species. For instance,  $P_i$ -limited cultures of *T. erythraeum* responded to medium acidification with a stronger increase in the transcription levels of genes encoding phosphonate and/or phosphite utilisation, suggesting heightened P requirements (Zhang et al. 2022). The authors suggested that these requirements were related to a buildup of intracellular polyP to maintain pH homeostasis under an acidified culture medium (Zhang et al. 2022). As for ocean warming, it can be hypothesised that warmer temperatures below their optimum will generally enhance enzymatic activities, which may result in more efficient phosphatase activities. A positive correlation was for instance reported between temperature and APA in the Northern Adriatic Sea (Ivančić et al. 2021) and between temperature and DOP degradation rates in incubation experiments

using water collected in the North Subtropical Pacific Gyre (White et al. 2012). However, colimitation may result in effects that are not the sum of individual stressors; the combined effect of  $P_i$  limitation and global warming on regulating  $P_i$ -stress responses is still an open field of research. Temperature will also affect the overall metabolism; co-limitation experiments showed for instance that temperature alleviates  $P_i$  limitation in *C. watsonii* through an effect on several metabolic functions, including  $P_i$  uptake,  $CO_2$  and  $N_2$  fixation (Deng et al. 2022).

### 3.1.5 | Cyanophage-Mediated Modulation of Phosphate Metabolism in Marine Cyanobacteria

The impact of bacteriophages on the dynamics and metabolism of marine cyanobacteria has been an active field of research. The discovery that some cyanophages carry auxiliary metabolic genes that positively impact the metabolism of their host bacteria has opened up an exciting area of research (Gao, Huang, and Ning 2016). For instance, marine cyanophages infecting marine picocyanobacteria encode the  $P_i$ -binding protein PstS and the AP PhoA (Sullivan et al. 2005; Zhao, Lin, Cai et al. 2022), and phage infection increases the  $P_i$ -uptake of the host bacteria (Zhao, Lin, Cai et al. 2022). Furthermore, the expression of the *pstS* and *phoA* genes in cyanophage S-SM1 is induced during infection under  $P_i$ -limited conditions and regulated by the host PhoR-PhoB TCS (Zeng and Chisholm 2012). The interaction between the cyanophage and its host results in the release of P from cellular debris and viral particles, which enriches the marine DOP pool. This, in turn, promotes the growth of phytoplankton (Zeng and Chisholm 2012). Undoubtedly, there remains much to uncover regarding the precise mechanisms through which phages interact with their host cyanobacteria, especially the mechanisms involved in adapting to nutrient-poor environments.

## 4 | Conclusion

Cyanobacteria combine multiple strategies to survive  $P_i$  deficiency. While examining genes associated with  $P_i$  metabolism from other organisms offers valuable insights into potential responses, a comprehensive understanding of the intricate mechanisms has been achieved for only a limited number of diazotrophic marine cyanobacteria strains to date. Particular attention should be directed towards analysing the molecular mechanisms governing the use of DOP by the identified enzymes, as well as understanding the regulation of their synthesis as a function of DOP composition and concentration. Besides, in their natural niches, cyanobacteria exist in microbial consortia where the interaction between microorganisms shapes metabolic activities and ecological success. Exploring how these interactions influence the availability and utilisation of  $P_i$  for cyanobacteria could provide valuable insights. Specifically, studying the consortia composition and dynamics in niches where cyanobacteria face  $P_i$  fluctuation or limitation, will shed light on potential symbiotic or competitive relationships and their impact on  $P_i$  recycling. Also, this review focused on subtropical, free-living diazotrophic cyanobacteria. A similar analysis is yet to be done on other diazotrophs, notably symbiotic associations with eukaryotes (Foster, Carpenter, and Bergman 2006; Foster and Zehr 2019), the more recently identified UCYN-A (Thompson

et al. 2012; Coale et al. 2024) and the spheroid body associated with the marine diatom *Epithemia* sp. (Schvarcz et al. 2022).

A breakthrough in microbiology and ecophysiology is underway thanks to metatranscriptomic approaches, enabling the identification of both the spatial and temporal expression patterns of genes involved in  $P_i$ -stress response. To go further, investigating the role of environmental factors, (such as temperature, pH, and light intensity) in modulating cyanobacteria response to  $P_i$  variations would provide a more comprehensive understanding of their adaptive mechanisms. This is particularly important in the case of marine diazotrophic cyanobacteria, where the coordination of all these factors impacts their metabolic capacity, and consequently the nitrogen balance in the ocean. In the context of a changing climate, global warming threatens to increase upper ocean stratification and affect oceanic currents and thus the provision of nutrients to surface waters. A better understanding and representation in global models, of DIP stress responses and DOP use capabilities in the phytoplankton, including diazotrophs, could provide a more accurate description of primary production, as well as carbon and nitrogen budgets in oligotrophic oceans under present and future conditions.

#### Author Contributions

**Chloé Caille:** investigation, visualization. **Solange Duhamel:** writing – review and editing, investigation. **Amel Latifi:** conceptualization, investigation, writing – original draft, writing – review and editing, methodology, validation, supervision, resources. **Sophie Rabouille:** conceptualization, investigation, funding acquisition, writing – original draft, writing – review and editing, methodology, validation, project administration, supervision, resources.

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#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Data Availability Statement

The authors have nothing to report.

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