**Phosphate uptake bioassays**

Phosphate uptake bioassays consisted in a set of measurements of P uptake rate at different environmental P concentrations. These P uptake rate-environmental P pairs were subsequently employed to estimate the $V_{max}$ and $K_{eff}$ by fitting the Michaelis-Menten function:

$$V = V_{\max} \frac{P}{P + K_{\text{eff}}} \qquad [S1]$$

The culture aliquot was diluted for the uptake bioassays from cells counted using an inverted microscope and quick-read disposable chambers. We incubated treatments from 0.1 to 10 µM P in the first set of bioassays, but uptake rates at 10 µM P were excluded when fitting the uptake curves because their error was frequently higher and P uptake rate saturated at lower P levels. We incubated two additional vials with the same amount of cells and $H_3{}^{33}PO_4$ but a very high concentration of non-radioactive P (300 µM) to be used as blanks (the uptake of $H_3{}^{33}PO_4$ was negligible). The incubations were conducted in glass vials and finished after 20 min by adding 300 µM of $H_3PO_4$. We employed low incubation times to prevent the occurrence of changes in cell abundance and acclimation responses affecting $V_{max}$ and $K_{eff}$ during incubations. Samples were then gently filtered through 25 mm glass fiber filters of 0.7 µm of pore size. Filters were washed with a solution of oxalic acid to remove the $^{33}P$ attached to the cells (1,2). Filters were subsequently placed in scintillation vials, immersed in scintillation liquid, and kept in the dark for at least 12 h.

After measuring the radioactivity in the filters, the rate of P uptake rate ($V$, fmol cell$^{-1}$ d$^{-1}$) was estimated using the following equation (3):

$$V = \left(R_f - R_b\right) \frac{P}{R_a \cdot T \cdot N}, \qquad [S2]$$

where $R_f$ and $R_b$ represent the radioactivity (corrected by the activity decay between the bioassay and measurement) in non-blank and blank filters, respectively (Table S1); P is the non-radioactive P concentration during the incubation (µM); $R_a$ is the radioactivity added; $T$ is the incubation time (d$^{-1}$); and N is the cell concentration (cell L$^{-1}$).

**Intracellular phosphorus measurement**

We digested the organic phosphorus contained in the filters by placing the filters in glass vials with 2 mL of concentrated (70%) nitric acid. Vials were fitted using a glass stopper as a cold finger cape and placed on a hotplate at a gentle boil for 1 hour. After they cooled, we added 10 mL of MilliQ water and we transferred it to a 100 mL volumetric flask. We rinsed the filters with

three further 10 mL aliquots of MilliQ water. We then neutralized the solution by adding p-nitrophenol indicator (0.1% solution), 5 M NaOH, and 0.5 M sulphuric acid. Once the solution cooled, the flask was made up to volume. Proper blanks (filters washed with salt water and oxalate solution) were also prepared.

The intracellular phosphorus or quota (fmol cell$^{-1}$) was estimated from the inorganic phosphorus concentration measured in the digested solution ($P_{ds}$) according to the following equation:

$$Q = \frac{(P_{ds} - P_{ds\_blank})\, Vol_{flask}}{N \cdot Vol_f}, \qquad [S3]$$

where $Vol_{flask}$ is the volume of the volumetric flask, $N$ is the cell concentration in the culture analyzed, $Vol_f$ is the volume of culture filtered, and $P_{ds\_blank}$ is the phosphorus concentration measured in the digested solution for the blank (Table S1).

**Gene expression: RNA extraction and quantitative Real-Time PCR analysis (qRT-PCR)**

Quantitative RT-PCR was performed using a StepOnePlus Real Time instrument (Thermofisher), Brilliant III UltraFast SYBR QPCR Master Mix (Agilent). Reactions were performed in four technical replicates on two biological replicates. The following cycling conditions were used for quantitative PCR: 2 min at 95°C, 50 cycles at 95°C for 3 s, and 59.5°C for 30 s. Melting curve analysis from 60 - 90°C was performed to monitor the specificity of the amplification. The quantitative real-time PCR procedure used follows the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines (4). The sample specific amplification efficiency was calculated according to the StepOne™ Software v2.2 (Life Technologies) using the slope of the regression line in the standard curve (standard dilution series: serial 4-fold dilutions, number of dilution points: 6). From this standard curve the software interpolated the target quantities of each gene, which was later used to calculate the relative fold differences.

**Statistical analyses**

We fitted the following mixed model to analyze the effects of P addition on the temporal dynamics of $V_{max}$, $K_{eff}$, $V_{max}$: $K_{eff}$, $Q$, and cell abundance:

$$y_{ijk} = mean + (P\ pulse)_i + (Time)_j + (P\ pulse\ x\ Time)_{ij} + (Experiment)_k + e_{ijk} \qquad [S4]$$

where $y_{ijk}$ is the value of the response variable at the level $i$, $j$ and $k$ of the factor *P pulse*, *Time* and *Experiment*, respectively. *mean* is the overall mean of the response variable. $P\ pulse_i$, $Time_j$ and $Experiment_k$ represent the effects of the level $i$ of the factor *P pulse*, $j$ of the factor *Time*, and

*k* of the factor *Experiment*, respectively. $(P\ pulse\ x\ Time)_{ij}$ represents the effect of the interaction between the two fixed factors. $e_{ijk}$ is the unexplained error associated with the observation at the level *i*, *j* and *k* of the factor *P pulse*, *Time*, and *Experiment*, respectively. *P pulse* had three levels, which correspond to the different P concentrations supplied to the cultures at day 6 of the experiment: $P_{ref}$, $P_{low}$ and $P_{high}$. *Time* also had three levels, which are associated with the three time points of 3.5 h, 28 h and 100 h after the nutrient pulse when measurements were conducted. *Experiment* had three levels, corresponding to each of the three replicated experiments. By including experiment as a random factor we account for the potential dependency of residuals from observations coming from the same experiment. A total sample size of 27 observations were employed to fit the model.

Multiple pairwise comparisons were restricted to fixed main effects. Specifically, we focused on comparisons respect to the *P pulse* reference level ($P_{ref}$) and associated with the temporal sequence of the experiments (i.e. 3.5 h - 28 h, and 28 h - 100 h). We compared $P_{ref}$ with $P_{low}$ and $P_{high}$ at each level of the factor *Time*, and *28 h* with *3.5 h* and *100 h* at each level of the factor *P pulse* (Table S3).

**Model parameterization and initial conditions**

Because the acclimation responses we focus on are expected to be generally found in phytoplankton, our model can be applied to any species Here, however, we based the parameterization on either the results empirically observed for *P. tricornutum* or the available literature.

The values $\mu_\infty$ = 1.0 $d^{-1}$ and $Q_{min}$ = 1.31 fmol $cell^{-1}$ were obtained from measurements conducted during our experiments; these values are in agreement with those reported for diatoms or phytoplankton species of similar sizes (5,6).

On the other hand, there is a lack of empirical information about the uptake kinetics for specific phytoplankton P transporters. $K_1$ = 0.085 µM was inferred from $K_{eff}$ and $V_{max}$ measured in $P_{ref}$ by the end of the experiments, when cells were mainly expressing transporters similar to $transporter_1$. We based on the equation for the effective half saturation constant (7):

$$K_{\mathrm{eff},i} = K_i + \frac{V_{\max}}{4\pi D r_c}, \qquad [S5]$$

where *D* ($m^2\ s^{-1}$) is the diffusivity constant for phosphorus in water and $r_c$ is the radius of the spherical cell. This expression considers the possible effect of a boundary layer formed around the cell at low environmental P levels due to diffusion limitation and phosphorus uptake (7,8).

We assumed $r_c$= 2.5 µm from microscopic observations and the size information provided by the supplier of the cultures. $K_2$ = 0.45 µM was based on nonpublished $K_{eff}$ and $V_{max}$ data obtained from semicontinuous cultures.

The effective $V_{max}$ measured in the uptake bioassays can be expressed as the sum of the $V_{max}$ associated with every transporter:

$$V_{max} = \sum_{i=1}^{j} V_{\max,i} = \sum_{i=1}^{j} k_{\mathrm{cat},i}\, n_i\,, \qquad [S6]$$

where $k_{cat,i}$ ($d^{-1}$) is the catalytic rate of transporter *i.* (i.e. the rate of dissociation between the pair transporter$_i$-phosphate ion occurred in the cytoplasm) and it is calculated from

$$k_{\mathrm{cat},i} = K_i\, k_{1,i} \quad [S7]\ ; \text{and } k_{1,i} = 4Dr_{s,i} \quad [S8]$$

with $k_{1,i}$ the encounter rate between P and the transporter *i* in the extracellular environment, and $r_{s,i}$ the radius of transporter *i* (see ref. (7) for further details and references). We used $v_1$ = 2900 $d^{-1}$ and $v_2$ = 32000 $d^{-1}$ based on the values used by Bonachela et al. (7). We set $r_s$ = 2.5 x $10^{-3}$ µm for the two transporters, which is within the range employed in previous studies (7,9). We set $c_H$= 0.51 %, $c_{F,a}$ = 1.6, $c_{F,b}$ = 5, and $g_H$= $10^{-9}$ for the *H* and *F* functions. This $g_H$ term was included in a step function (*H*) with a range between 0 and 1 (Eq. [7]). An alternative and/or additional step function could include negative values to consider the activation-inhibition, in addition to changes in protein expression, of low-affinity transporters as previously observed in yeast (10–12).

Negative density-dependent effects on phytoplankton growth could be mediated, among other processes, by mutual shading and the release of chemical compounds with inhibitory or toxic effects (13–16). Instead of modelling explicitly light and the release of inhibitory compounds, for the sake of simplicity we represented phenomenologically these negative effects by including a generic density-dependent term ($\alpha N^2$) in the equation for the cell growth rate (Eq. [1]), with α = 5.5 x $10^{-21}$ $L^2$ $cell^{-1}$. According to this formulation, the higher the cellular abundance the higher the negative interaction between cells mediated by cell shading or inhibitory compounds. This density-dependent term may lead to unrealistic negative growth rates when *Q* is close to $Q_{min}$ and *N* is very high. This was not the case during our simulations, but it might occur if the model is run in other scenarios, for which other growth rate functions may be more appropriate. Alternatively, the $\alpha N^2$ could be excluded when adapting the model to other systems.

The qualitative patterns predicted by our model for $V_{max}$, $K_{eff}$, *Q* and *N* were robust to changes in the values assigned to parameters and coefficients. Nevertheless, to replicate quantitatively

the magnitude of the responses and the relative performances of the P pulse treatments observed in the experiments some parameters (*e.g.* $c_{F,a}$ , $r_{s,i}$, $c_H$) must be within specific ranges. For example, due to the competition between both types of transporters for the cell surface (Eqs. [5] and [7]), the predicted $V_{max}$ peaks after P pulses were restricted when the threshold $c_H$ related to the surface area occupied by transporters was set to low values. The effects of modifying a specific parameter can be counterbalanced by simultaneously modifying the value of other parameters (*e.g.* in the case of $c_H$, by modifying $r_{s,i}$), so that similar model outputs can be obtained with different parameterizations.

Finally, we set initial conditions aiming to mimic those of the experiments, which resulted in $N$ = 5 x $10^7$ cells $L^{-1}$, P = 3 µM, $Q$ = 1.2 x $Q_{min}$ fmol $cell^{-1}$, $n_1$ = 4500 transporters $cell^{-1}$, and $n_2$ = 50 transporters $cell^{-1}$. These initial $Q$, $n_1$ and $n_2$ values were chosen taking into account that our cultures were intentionally phosphorus starved; nonetheless the model output is robust to modifications of these initial values. Pulses of 0, 3 and 15 µM P were included at t = 6 d to replicate the experimental design.

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