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ORIGINAL ARTICLE

Strict stoichiometric homeostasis of *Cryptomonas pyrenoidifera* (Cryptophyceae) in relation to N:P supply ratios

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ABSTRACT

A common freshwater cryptophyte, Cryptomonas pyrenoidifera*, was cultivated in batch-cultures to analyze intraspecific variation in elemental stoichiometry along a broad gradient of pulsed phosphorus (P) enrichment during the early acclimation period and to determine the immediate homeostatic capacity of the nitrogen-to-phosphorus (N:P) ratio of this alga when nutrients are at saturating levels. Experimental results revealed that nitrogen (N) and P cell quotas significantly increased with increasing P concentration. However, despite the wide range of N:P ratios in the medium,* C. pyrenoidifera *N:P ratios were highly stable at higher P-level treatments, indicating a highly conservative behavior and suggesting strict elemental homeostasis when nutrients are at saturating levels. The strictly homeostatic N:P ratio appears to be attributable to their high potential for a fast luxury consumption of both N and P after a brief and intense episode of increased resource availability and to physiological limits on their nutrient storage capacity. Most importantly, the N:P biomass ratio at nutrient saturating levels converged around 11:1, which was the observed ratio of maximum internal cell quotas for N and P (*i.e., $Q_{max,N}$:$Q_{max,P}$*) under the prevailing experimental conditions. This value is particularly informative for* C. pyrenoidifera *because it represents cell storage quotients and may be a taxon-specific evolutionary optimum, providing a reference point to infer the grade of nutrient-limitation. The experimental data give ranges of variation in* C. pyrenoidifera *elemental composition permitting, among others, proper parameterization of cryptophyte stoichiometry models.*

Key words: Cryptomonas pyrenoidifera*; ecological stoichiometry; elemental homeostasis; optimal N:P ratio; luxury storage capacity.*

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INTRODUCTION

Events of resource pulses are important issues in ecology providing opportunities to investigate how individual consumers confront with change in external conditions. Resource pulses are defined by Yang *et al*. (2008) as 'episodes of increased resource availability in space and time that combine low frequency (rarity), large magnitude (intensity), and short duration (brevity)'. In autotrophic organism, nutrient pulsed perturbations can alter their photosynthetic efficiency and their food quality for consumer organisms. Moreover, interspecific variation in autotroph stoichiometry has implications for the dynamics of food webs, consumer-driven nutrient recycling and detrital breakdown (Sterner and Elser, 2002). In this context, the concept of elemental homeostasis, *i.e.,* the resistance to change of the body composition of consumers in response to variations in the chemical composition of their resources, has been central to the development of the ecological stoichiometry framework (Koojiman, 1995; Elser and Urabe, 1999; Loladze *et al.,* 2000; Sterner and Elser, 2002). The primary focus is on nitrogen (N) and phosphorus (P) as essential nutrients and on the N:P ratio, because autotrophs in aquatic ecosystems are often limited by one and/or the other (Hecky and Kilham, 1988; Downing *et al.,* 1999; Elser *et al.,* 2007). Sterner and Elser (2002) hypothesized that the N:P content of producers would closely match the N:P ratio supplied to ecosystems because of the ability of autotrophs to store nutrients through luxury consumption, which refers to 'increases in organismal nutrients over and above what is immediately required for growth' or the minimal elemental requirements. Generally, luxury uptake of nutrients can be detected in a few hours after onset a nutrient pulsed perturbation (*e.g.*, see Eixler *et al*. 2006). In this short-term, changes in cell elemental composition are involved in the acclimation process (Giordano, 2013).

Renewed attention to the elemental stoichiometry of phytoplankton has revealed that algal species exhibit a wide variety of responses in N:P homeostasis and that nutrient homeostasis is neither a group nor a species-level trait; in fact, the degree of autotroph homeostasis in any given situation appears to be species-specific, depends on the growth rate, on the limits to nutrient storage and on their physiological adaptation (Klausmeier *et al.,* 2004; Hall *et al.,* 2005; Persson *et al.,* 2010; Hillebrand *et al.*, 2013; Nifong *et al*., 2014).

A matter currently under discussion is the value to

which the N:P ratio converges in algal species when cell elemental stoichiometry is homeostatic. The first mathematical models about phytoplankton stoichiometry assumed that, while nutrients were high, phytoplankton consumed nutrients in an optimal ratio equal to the ratio of minimal quotas for N and P (*i.e*., $Q_{min,N}$:$Q_{min,P}$) (Klausmeier *et al.,* 2004). In Klausmeier *et al.´*s terms (2004), they "eat what they need". According to Klausmeier *et al.* (2004), this is identical to the ratio at which N limitation switches to P limitation assuming a strict Liebig type of single resource limitation. However, a recent mathematical model has suggested that when resource supply rate is large relative to autotroph demand, cell ratios converge on the ratio of maximum internal cell quota for N and P (*i.e.,* $Q_{max,N}$:$Q_{max,P}$) (Nifong *et al*., 2014). Nevertheless, the empirical validity of that prediction needs to be tested. Moreover, it is unclear whether $Q_{min,N}$:$Q_{min,P}$ and $Q_{max,N}$:$Q_{max,P}$ ratios are equal. The latter represents the optimal species-specific cell stoichiometry and it may provide a reference point to infer the grade of nutrient-limitation (Nifong *et al.,* 2014). Nevertheless, there are currently no inventories that report this taxa-specific optimum tissue stoichiometry.

Studies testing stoichiometric hypotheses (Klausmeier *et al.,* 2004, 2007) generally used parameters based on P and N-limited growth of a single species of green algae, the chlorophyte *Scenedesmus* sp. studied by Rhee (1974, 1978). However, *Scenedesmus* is not characteristic of most phytoplankton and is extremely plastic relative to other species (Andersen, 1997). Among algae groups, cryptophytes are a major component of primary production in both freshwater and marine habitats (Klaveness, 1988) and serve as important prey for diverse predators (Pedrós-Alió *et al.,* 1995; Brown *et al.,* 1997). However, the elemental homeostasis of the N:P ratio in Cryptophyta species has been poorly studied and has been limited to conditions of N or P-limitation (Leonardos and Geider, 2005; Bi *et al.,* 2012). Thus, the effects of N and P consumption in excess by cryptophytes on cell stoichiometry need to be explored.

This study investigated C:N:P stoichiometric variations in a common freshwater microalga Cryptophyta, *Cryptomonas pyrenoidifera* Geitler (formerly listed as *Cryptomonas ovata* var. *palustris* E. G. Prinsheim) along a broad gradient of N:P supply during the early acclimation period to examine trends to test the degree of immediate elemental homeostasis of algal cells when nutrients are at saturating levels and, if elemental homeostasis was observed, to determine at which value the N:P ratio converged.

METHODS

Cultivation

A strain of *Cryptomonas pyrenoidifera* (CCAP 979/61) was routinely grown in the laboratory at 17±0.5ºC with sterile-filtered air and continuous PAR of 120 µmol m^{-2} s^{-1} in culture medium-WC (1000 µM N-$NaNO_3$, 50 µM P-KH_2PO_4, N:P molar ratio 20:1; Guillard and Lorenzen, 1972). According to the recommendations of Gervais (1997) to cultivate cryptophytes, no boron was added and the N source was ammonium chloride rather than sodium nitrate. This light intensity does not limit the growth of *Cryptomonas* spp. (Gervais, 1997). Continuous light conditions were chosen to reduce possible diurnal effects on cell P pools. The strain of *C. pyrenoidifera* CCAP 979/61 used in this experiment was grown as free-swimming flagellate cells. During the course of this study *C. pyrenoidifera* never produced palmellae as in Hoef-Emden study (2007).

A preliminary experiment was performed to determine when the algae reached early stationary phase and to obtain their ecological maximum growth rate, i.e. an estimation of the maximal growth rate (μ_{max} = 0.270 d^{-1}) under the prevailing experimental conditions (Supplementary Material). This value is consistent with other estimations of maximum growth rate of *C. ovata* var. *palustris* in laboratory cultures (see Cloern, 1977). Cell density was estimated every two to three days with an improved Neubauer hemacytometer and logarithmically transformed, considering the linearly increasing part of the log plot as the exponential phase. μ_{max} was defined as the slope of the linear regression between logarithmically transformed cell density and time (days). Mean cell volume was 1078 µm^3 (SD 312 µm^3), mean cell length was 18.56 µm (SD 1.65 µm), and mean cell width was 10.41 µm (SD 1.31µm). Cell volume was determined by measuring length (l) and width (w) of 20 cells preserved in lugol´s solution (final concentration 2% v/v) on an inverted microscope and using the formula: volume = (Π/ 6)·l·w^2, which assumes *Cryptomonas* to be a prolate spheroid.

Experimental procedure

To investigate the effect of a P-pulsed perturbation on the immediate N:P homeostatic capacity of algal cells, aliquots (20 mL) of cell suspension with ca. 1x10^6 cells mL^{-1} from a logarithmically grown culture (hereafter, preculture) were equally distributed into each of four 250 mL Erlenmeyer flasks. Then, 170 mL of fresh WC medium with different concentrations of K_2HPO_4 (10, 25, 150, and 300 µM P) were added to the flasks; the N concentration was adjusted to 1000 µM in all formulations, while the PO_4^{-3} was varied to yield four N:P ratios: 3:1, 7:1, 40:1, and 100:1. The residual soluble reactive P (SRP) in the medium in the preculture, just before inoculations of the experimental flasks was 4.01 ± 2.02 (SD) µM P (Tab. 1). Thus, this aliquot of 20 ml of preculture adds 0.42 µM P to the experimental concentrations. As this concentration is less than any of the experimental concentrations, P concentration decreased slightly at all experi-

mental concentrations. The initial SRP concentration in the experimental flasks was of 9.4 µM P in 10 µM treatment, 23 in 25 µM treatment, 135 in 150 µM treatment and 269 in 300 µM treatment. All experimental flasks were maintained during 24 h at the same physical conditions of aeration and light environment as the preculture. Luxury consumption can be generally detected in a few hours after onset the perturbation (Eixler *et al*., 2006). Preculture was not axenic, but it was unialgal and free of protozoan and fungal contaminants; sterile technique was used throughout.

To check that the P-supply was in excess of demand for the *Cryptomonas* strain, samples were taken for measurement of residual soluble reactive P (SRP) in the medium in the preculture, just before inoculations of the experimental flasks, and after 24 h pulsed nutrient inputs in the experimental flasks. For each experimental set, P uptake rate per cell during the 24 h period was calculated as the difference between the initial and final amount of SRP in the medium in relation to the cell abundance in each culture.

Elemental cell quotas

Cells were harvested after 24 h algal inoculation in the experimental flasks to estimate cell abundance (cells mL^{-1}) and analyze carbon (C), N and P contents. Cell density was estimated with an improved Neubauer hemacytometer. The elemental composition of the microalgae was determined by filtering a cell suspension from the preculture (0.3 mL) and each experimental flask (5 mL) on precombusted (460ºC, 4 h) GF/F filters, measuring the particulate C and N with a CNH analyzer (Perkin Elmer) and the particulate P as SRP after potassium persulfate digestion (Murphy and Riley, 1962). The filters were soaked with 5 mL Milli-Q water after cell suspension filtration. Subsamples were also taken after 24 h algal inoculation to determine the SRP content of the filtered extracts. Three measurements per culture were performed in each analysis. Data were used to calculate (on a molar basis) the P:C biomass ratios (P cell quota, Q_P), the N:C biomass ratios (N cell quota, Q_N) and the N:P ratio. Nutrient cell quotas of *C. pyrenoidifera* on cell basis (pg $cell^{-1}$) were also calculated.

Tab. 1. Cell abundance (cells mL^{-1}) of *C. pyrenoidifera*, residual SRP concentration and P uptake rate in the experimental flasks under initial conditions and after 24 h in response to a pulsed P gradient in the medium (n=1). Values are means (SD).

P-pulse (µM)	Cells mL^{-1}	Residual SRP (µM-P)	P uptake rate (10^{-9} µmol P $cell^{-1}$ h^{-1})
Initial conditions	104,771 (7,098)	4.00 (2.02)	
10	141,875 (10,296)	0.81 (0.06)	2.70
25	115,625 (5,220)	0.70 (0.13)	8.76
150	112,500 (5,944)	95.11 (2.44)	20.33
300	134,375 (12,515)	223.02 (4.45)	23.87

Statistical analysis

All statistical analyses were performed with STATISTICA 7.1 (StatSoft, Inc,. Tulsa, OK, USA). The variability of algal molar ratios (P:C, N:C and N:P) were compared by calculating the propagation of errors from the standard deviations of the particulate C, N, and P (Bevington, 1969). Error propagation (Bevington, 1969) is a method to calculate the uncertainty of a composite calculation from the uncertainty of the component parts. The normality of dependent variables was checked with the Kolmogorov–Smirnov test (Sokal and Rohlf, 1995). The nutrient storage rate S_x (S_P for phosphorus and S_N for nitrogen) was defined as the difference, per unit of time, between nutrient cell quota after nutrient pulsed perturbation was applied (*i.e.* Q_P and Q_N) and nutrient cell quota in the preculture. The relationship between this rate S_x and the different experimental P concentrations was explored by fitting it to a saturation function, based on the Michaelis-Menten model of enzyme substrate interaction, defined by equation 1:

$$S_x = S_{max,x} \cdot \frac{input\ P}{input\ P + K} \quad \text{(eq. 1)}$$

where S_x is the observed nutrient storage rate S_P or S_N (x-mol · C-mol^{-1} · h^{-1}); $S_{max,x}$ is the theoretical maximal storage rate of nutrient (x-mol · C-mol^{-1} · h^{-1}); K is the P concentration at which the storage rate is half of $S_{max,x}$. The realized nutrient-saturated cell quota, $Q_{max,P}$ and $Q_{max,N}$, is attained at the maximum storage rate of nutrient and was calculated as: $Q_{max,x} = (S_{max,x} \cdot 24) + Q_{preculture,x}$. Fitting was computed with a nonlinear least-squares method, and the estimated parameters were obtained using STATISTICA. The relationships between P, N and C cell quotas on a cell basis and pulsed P-input were fitted using a logarithmic equation.

The homeostatic regulation of elemental content was analyzed graphically with a log-log plot of N:P supply ratio *versus* algae N:P stoichiometry. The degree of elemental homeostasis was characterized by the *H* metric of Sterner and Elser (2002), defined by equation 2:

$$H = \frac{\log_{10}(x)}{\log_{10}(y) - \log_{10}(c)} \quad \text{(eq. 2)}$$

where x is the resource nutrient stoichiometry (N:P), y is the organism's nutrient stoichiometry (same units as re-

source), and *c* is a constant. Therefore, 1/*H* is the slope of the regression between log (x) and log (y), with values ranging from zero to one. The regression slope, 1/*H*, was used, following the proposal by Persson *et al.* (2010), because "strictly homeostatic" organisms have an *H* of infinity, which presents some analytical problems. Because the slope was expected to be greater than or equal to 0, one-tailed tests were used with $\alpha = 0.1$. When the regression relationship is not significant (P>0.1), 1/*H* can be set to zero (Makino *et al.,* 2003) and the organism is considered 'strictly homeostatic', as in Persson *et al.* (2010). When the regression analysis is significant, the homeostatic capacity can be classified according to Persson *et al.* (2010) as: $0 < 1/H < 0.25$ = homeostatic, $0.25 < 1/H < 0.5$ = weakly homeostatic, $0.5 < 1/H < 0.75$ = weakly plastic, and $1/H > 0.75$ = plastic.

RESULTS

Before perturbation was applied, *Cryptomonas pyrenoidifera* cells logarithmically growing in the preculture had a Q_P of 6.02±0.843 (SD) mmol P mol^{-1} C (Fig. 1a) and a Q_N of 0.106±0.005 (SD) mol N mol^{-1} C (Fig. 1b), which are within previously reported ranges for freshwater phytoplankton of 0.2-20 mmol P mol^{-1} C and 0.014 - 0.180 mol N mol^{-1} C (Sommer, 1988, 1991a, 1991b). These values resulted in a N:P ratio around 18:1, slightly higher than the canonical Redfield N:P ratio.

The residual SRP concentration in the preculture was 4.01±2.02 (SD) µM and in the experimental flasks with higher P-levels ranged between 95.11±2.44 µM (mean ± SD, *n*=3) for the 150 µM treatment and 223.02±4.45 µM (mean ± SD, *n*=3) for the 300 µM treatment (Tab. 1). The residual ammonium concentration in the medium was not determined in this study, but the initial N concentration (1000 µM) was in considerable excess. It can therefore be assumed that nutrient supply rate was in excess of *C. pyrenoidifera* demand in higher P-levels treatments.

After nutrient pulsed perturbation was applied, we observed a clear increase in the Q_P and Q_N values in *C. pyrenoidifera* with increasing P concentration (Fig. 1 a,b). Although the P:C and N:C biomass ratios were similar when P was pulsed with a concentration of 23, 135 and 269 µM-P (Fig. 1 a,b), P cell quotas significantly increased from 4.75 pg-P cell^{-1} in 23 µM-P treatment to 7.33 pg-P cell^{-1} in 135 µM-P treatment and 6.62 pg-P cell^{-1} in 269 µM-P treatment (Fig. 2a). A significant increase was also observed in the N cell quotas in response to short-term changes in N:P supply ratios from 23.25 pg-N cell^{-1} in 23 µM-P treatment to 35.45 pg-N cell^{-1} and 34.84 pg-N cell^{-1} in the 135 µM-P and 269 µM-P treatments respectively (Fig. 2b). C cell quotas showed also a good fit to a logarithmic function with the P input (Fig. 2c). In this short time period, no differences were observed in cell density between treatments after the P-pulse (Tab. 1, Pearson correlation coefficient r^2= 0.0035, P>0.05). Moreover, we observed a clear increase in the P uptake rate per cell in *C. pyrenoidifera* with increasing P concentration

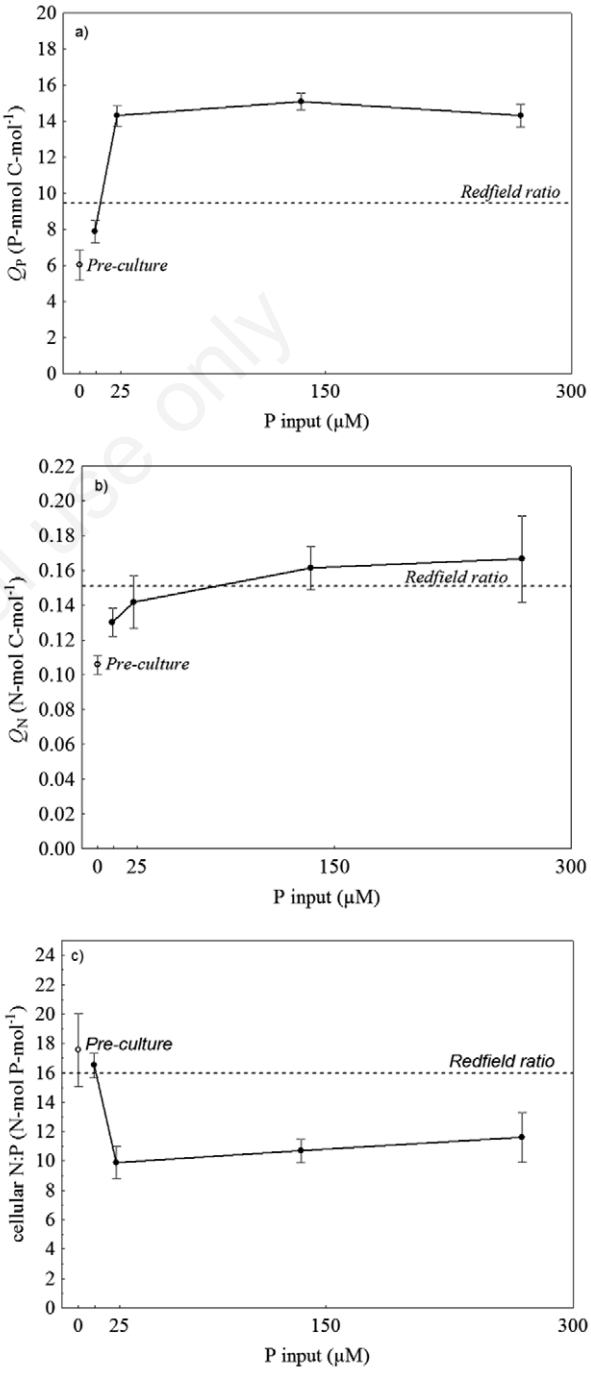

Fig. 1. Behavior of Q_P (a), Q_N (b) and N:P ratio (c) of *Cryptomonas pyrenoidifera* after 24 h in response to a P gradient in the medium. Value for preculture (open circle) is also shown. Vertical bars represent the propagation of errors of the molar ratios of algae calculated from the standard deviations of the particulate C, N, and P (Bevington, 1969). The Redfield ratio is shown as a broken line for visual reference.

(Tab. 1). According to these results, the response to the N and P input was an obvious immediate luxury consumption of both nutrients and an increase in *Cryptomonas* biomass.

Both storage rates of P and N (S_p and S_N) showed a good fit to eq. 1 (Fig. 3 a,b), with a maximum P storage rate $S_{max,P}$ of 0.4005 P-mmol C-mol^{-1} h^{-1}C and a maximum N storage rate $S_{max,N}$ of 0.0026 N-mol C-mol^{-1} h^{-1}. Saturating Q_P ($Q_{max,P}$) was of 15.65 mmol P mol^{-1} C and saturating Q_N ($Q_{max,N}$) of 0.17 mol N mol^{-1} C. These values resulted in a $Q_{max,N}$:$Q_{max,P}$ ratio around 11:1, slightly different to the N:P ratio of algal cells in the preculture and in the lower P-level treatment (Fig. 1c).

The N:P ratio with the lowest P-pulse was similar to N:P ratio in the preculture (Fig. 1c). Most importantly, the observed N:P ratio decreased nonlinearly and was highly stable around $Q_{max,N}$:$Q_{max,P}$ ratio at higher P-level treatments (Fig. 1c), indicating a highly conservative behavior despite the wide range of P concentrations in the medium. This response pattern suggests that the early acclimation to P-enrichment is a homeostatic process. Moreover, the N:P ratio of *Cryptomonas* cells deviated strongly from a 1:1 relationship with the N:P supply ratio (Fig. 4), and it was clustered around the observed $Q_{max,N}$:$Q_{max,P}$ ratio. The regression relationship of the N:P supply ratio with the algae N:P stoichiometry was not significant ($F_{1,2}$ = 0.76, P>0.1). Hence, 1/*H* was considered zero (see Methods), classifying *C. pyrenoidifera* as "strictly homeostatic".

DISCUSSION

Experimental results revealed that, despite the enormous variation in N:P ratios in the medium, during early acclimation period *Cryptomonas pyrenoidifera* exhibited N:P ratios highly stable (Fig. 4). According to the homeostatic regulation coefficient (*H*) (Sterner and Elser, 2002), the N:P ratio of *C. pyrenoidifera* was strictly homeostatic under conditions of nutrient saturation. Cells, therefore, were able to adjust their metabolism in a relatively short time to these changes in N:P supply ratios. The degree to which autotrophs actively regulate their internal nutrient composition remains unclear. The stoichiometric homeostasis of *C. pyrenoidifera* in relation to N:P supply ratios appears to be attributable to their high potential for a fast luxury consumption of both N and P after a brief and intense episode of increased resource availability and to physiological constraints on their nutrient storage capacity. It is generally accepted that, under conditions of N-limited growth, autotrophs can show low N:P ratios due to P storage (Sterner and Elser, 2002). Similarly, they may also consume N in excess under conditions of P-limited growth (Greenwood, 1976; Miyashita and Miyazaki, 1992; Bi *et al.,* 2012). However, N cell quotas of *C. pyrenoidifera* also increased under conditions of P-enrichment (Figs. 1b and 2b), probably due to N concen-

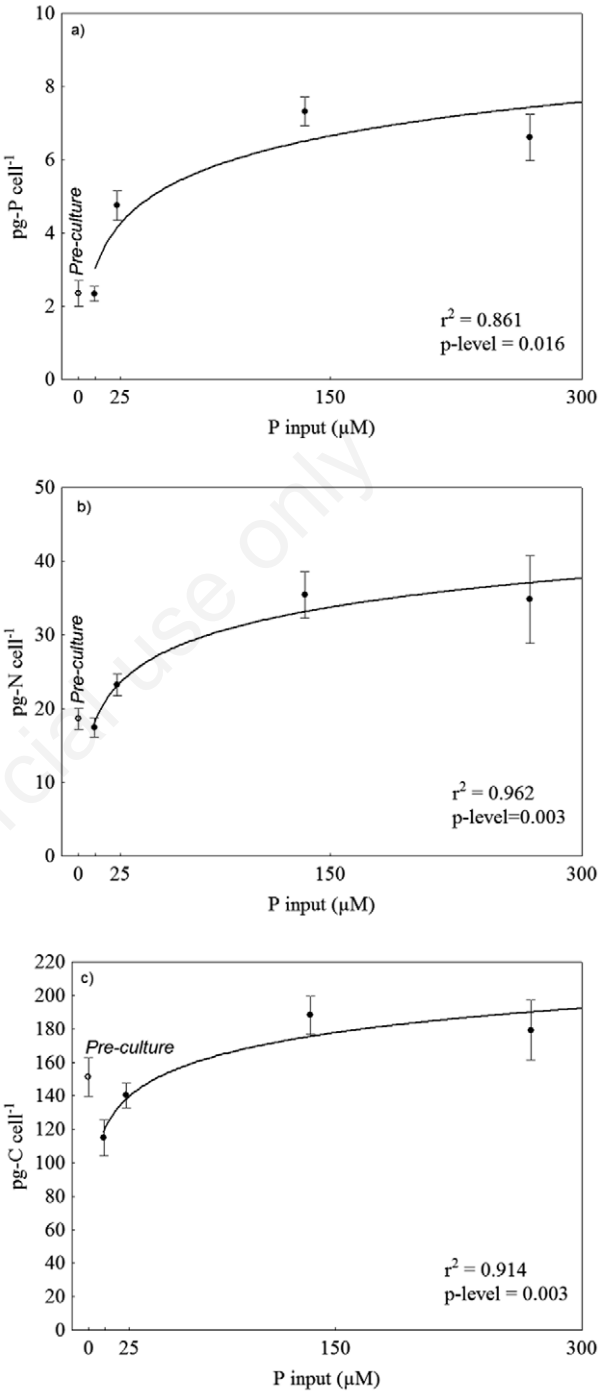

Fig. 2. Behavior of (a) P, (b) N and (c) C cell quotas of *C. pyrenoidifera* on cell basis (pg cell^{-1}) in the experimental flasks after 24 h in response to a P gradient in the medium. Value for preculture (open circle) is also shown. Vertical bars represent the propagation of errors of the nutrient cell quotas of algae calculated from the standard deviations of the particulate P, N and C and cell density (Bevington, 1969). The relationships between cell quotas and P-input fitted a logarithmic equation. Determination coefficients (r^2) and P-level are shown.

tration in the medium was in excess to demand in our study. Although luxury uptake is typical of the normal metabolism of microalgae (Rhee, 1973; Sterner and Elser, 2002), it has seldom been documented in Cryptophyceae (Bi *et al.,* 2012) and this is the first study that document N and P luxury uptake in *C. pyrenoidifera*, a species of phytoplankton widely used as food in experimental studies of food quality effects on zooplankton growth (Lürling and van Donk, 1997; De Lange and van Reeuwijk, 2003; Kagami *et al.,* 2011). In this short time period, P uptake rates per cell in our *C. pyrenoidifera* strain are higher than the highest P uptake rates observed in other studies for *Cryptomonas ovata* var. *palustris* (Cloern, 1977) and the maximum P uptake rate assumed in phytoplankton stoichiometry models (Klausmeier *et al*., 2004).

On the other hand, results are in accordance with Hall's observations (2005), and support that physiological limits to nutrient storage capacity may reduce the ability of producers to adjust their stoichiometry in response to changes in elemental supply ratios. Thus, at more extreme N:P supply ratios, producers became saturated with P (at low N:P supply) or N (at high N:P supply); in both cases, once the producers were saturated, their N:P content no longer responded to a more extreme N:P supply ratio (Andersen, 1997), implying that the responses of the C:N:P stoichiometry of autotrophs along nutrient gradients are not necessarily linear (Persson *et al.,* 2010; Hillebrand *et al.,* 2013; Nifong *et al*., 2014). This bending of the relationship between available and incorporated N:P when nutrients are in excess of demand indicates the presence of some active internal regulatory process constraining stoichiometric variability in aquatic autotrophs (Nifong *et al.,* 2014). This experimental study is the first to validate the theoretical predictions of Nifong's model (2014) which describe that, when resource supply saturates demand, N:P ratios converge on the value of $Q_{max,N}$:$Q_{max,P}$. This value (11:1) is particularly informative for *C. pyrenoidifera* because it represents the optimal species-specific cell stoichiometry and it may provide a reference point to infer the grade of nutrient-limitation (Nifong *et al.,* 2014). In this study, $Q_{max,P}$ in *C. pyrenoidifera* was 15.65 mmol P mol^{-1} C, higher than previously described

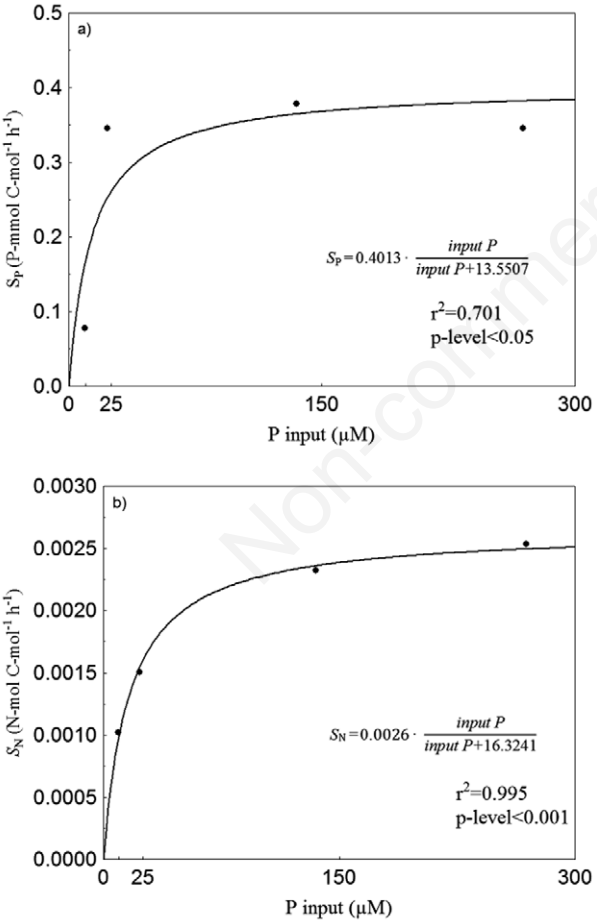

Fig. 3. Behavior of (a) P storage rate (S_P) and (b) N storage rate (S_N) of *Cryptomonas pyrenoidifera* in response to a P gradient in the medium. The relationships between S_P and S_N and P-input fitted a saturation function, based on the Michaelis-Menten model of enzyme substrate interaction, defined by equation 1. Determination coefficients (r^2) are shown.

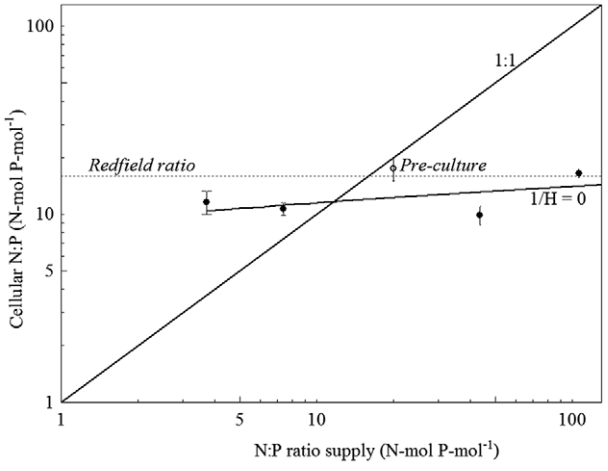

Fig. 4. Immediate homeostatic regulation in the N:P of *Cryptomonas pyrenoidifera* algae as function of the N:P in the medium supplied. N:P of algal cells in the preculture at ecological maximum growth rate is also shown. Vertical bars represent the propagation of errors of the N:P algae calculated from the standard deviations of the particulate N and P (Bevington, 1969). The Redfield ratio is shown as a broken line for visual reference. Both the regression lines and the 1:1 line are plotted. The regression slope 1/*H* was considered zero (see *Methods* paragraph), classifying *C. pyrenoidifera* as 'strictly homeostatic'.

in Cryptophyceae. For instance, in P-enrichment cultures at steady state, $Q_{max,P}$ for *Rhodomonas* sp. was 9.570 mmol P mol^{-1} C (Bi *et al.* 2012).

It is unclear whether $Q_{min,N}$:$Q_{min,P}$ and $Q_{max,N}$:$Q_{max,P}$ ratios are equal. The observed $Q_{max,N}$:$Q_{max,P}$ ratio for *C. pyrenoidifera* is slightly different to the N:P ratio in the preculture growing exponentially before the nutrient pulse. Under the 'optimal uptake assumption' of the Klausmeier´s model (2004) that phytoplankton growing exponentially 'eat what they need' and the ratio of maximal uptake rates for N and P equals the ratio of minimum quotas for N and P (using their symbols, $\nu_{max,N}$ / $\nu_{max,P}$ = $Q_{min,N}$:$Q_{min,P}$), it is thought that within the *C. pyrenoidifera* strain in this study the $Q_{max,N}$:$Q_{max,P}$ ratio is distinct from the $Q_{min,N}$:$Q_{min,P}$ ratio. This slight divergence between the two ratios may indicate different aspects of algae competitiveness (Nifong *et al.,* 2014). The observed deviation between preculture N:P ratio and optimal (*i.e.*, $Q_{max,N}$:$Q_{max,P}$) *Cryptomonas* stoichiometry may indicate conditions of P limitation in our preculture (N:P > $Q_{max,N}$:$Q_{max,P}$).

CONCLUSIONS

In conclusion, our study revealed that *Cryptomonas pyrenoidifera* grown in conditions of nutrient saturation exhibited N:P ratios highly stable during the early acclimation period, which suggests strict elemental homeostasis. We observed that N:P ratio of *C. pyrenoidifera* was strictly homeostatic due to a immediate luxury consumption of both N and P. Moreover, we estimated the ratio of maximum internal cell quotas for N and P (*i.e.*, $Q_{max,N}$:$Q_{max,P}$) under the prevailing experimental conditions. This value (11:1) is particularly informative for *C. pyrenoidifera* because it represents the optimal species-specific cell stoichiometry and it may provide a reference point to infer the grade of nutrient-limitation.

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