Appendix A: Physical properties of the cells.

The modelled cells are considered spherical. The total volume V (μm^3) is:

$$V = \frac{4}{3}\pi r^3 \tag{A.1}$$

where r is the equivalent spherical radius (excluding the shell). The volume of the vacuole V_V the plasma membrane V_{MO} and the inner membrane V_{MI} are:

$$V_V = v^3 V = \frac{4}{3}\pi (rv)^3 \tag{A.2}$$

$$V_{MO} = \frac{4}{3}\pi \left[r^3 - (r - t_M)^3 \right] \tag{A.3}$$

$$V_{MI} = \frac{4}{3}\pi \left[(rv + t_M)^3 - (rv)^3 \right] \tag{A.4}$$

where v is the radius fraction of the vacuole and t_M is the thickness of the membranes. Finally, the volume of the cytoplasm V_c (excluding vacuole, membranes and shell) is:

$$V_C = \frac{4}{3}\pi r_0^3 = \frac{4}{3}\pi \left[(r - t_M)^3 - (rv + t_M)^3 \right] \tag{A.5}$$

where r_0 is the radius of a sphere without vacuole. This can also be written in terms of the cell's carbon mass x as

$$r_0(x) = \left(\frac{4\pi}{3} \right)^{-1/3} \left(\frac{x}{\rho_C} \right)^{1/3} \tag{A.6}$$

where ρ_C is the carbon density in the cytoplasm.

The composition of cytoplasm and membranes are different with the density of membranes (ρ_M = 0.6 × 10^{-6} µg µm^{-3}) being higher than density of the cytoplasm (ρ_C = 0.11 × 10^{-6} µg µm^{-3}) (Strathmann 1967; Raven 1987). Thus, the total carbon content of the cell x_{tot} is:

$$\begin{aligned} x_{tot} &= V_C \rho_C + (V_{MI} + V_{MO}) \rho_M \\ &= \frac{4}{3}\pi \left[r^3 (1 - v^3) \rho_C + \left[(r - t_M)^3 - (rv + t_M)^3 \right] \rho_M \right] \end{aligned} \tag{A.7}$$

By solving Eq. A.7, we can derive r from x_{tot} and v (Fig. A.1). The exact solution including the thickness of the membranes is a complex function and is most readily found numerically. In the limit when the membrane thickness is small compared to the cell dimension, r can be approximated as:

$$r(x) \approx \left(\frac{3x}{\rho_C} \frac{1 - v^3}{4\pi} \right)^{1/3} \tag{A.8}$$

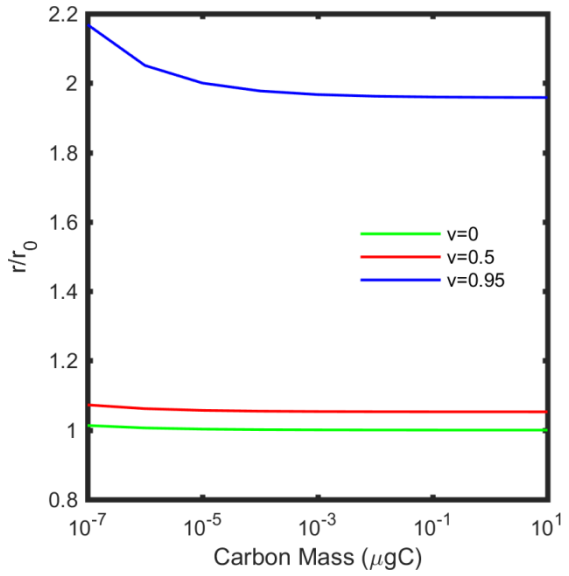

Fig. A.1. The cell radius r of a vacuolated cell compared to the radius r_0 a non-vacuolated cell a cell of the same carbon mass (x_{tot}) at 3 different vacuole size (v).

Diatoms are surrounded by a silicate shell with a thickness t_S and a volume V_S. The relation between the thickness of the shell and the total volume of the cell V is given by equation A.9 derived from data of size dependent silicon content in diatoms (Brzezinski, 1985; Eppley et al., 1967; Harrison et al., 1977 and Parsons et al., 1961): (Harrison et al. 1977; Brzezinski 1985)

$$t_S = 1.62V^{0.24} \tag{A.9}$$

$$V_S = \frac{4}{3}\pi\left(r + t_S\right)^3 - \frac{4}{3}\pi r^3 \tag{A.10}$$

Therefore, the volume of the shell, the corresponding silica content in the shell and the Si:C ratio depend on both the carbon mass x and the size of the vacuole v (Fig. A.2).

The carbon density of the cell (ρ_{tot} µgC µm^{-3}) is:

$$\rho_{tot} = \frac{V_C\rho_C + \left(V_{MI} + V_{MO}\right)\rho_M}{V_C + V_{MI} + V_{MO}} \tag{A.11}$$

The silicate density of the shell ρ_S is fixed 10.3 × 10^{-6} µgSi µm^{-3} (Hansen and Visser, 2019), and the Si:C ratio (µgSi µgC^{-1}) is thus:

$$c_{Si:C} = \frac{\rho_S}{\rho_{tot}} \frac{V_S}{V_C + V_{MI} + V_{MO}} \tag{A.12}$$

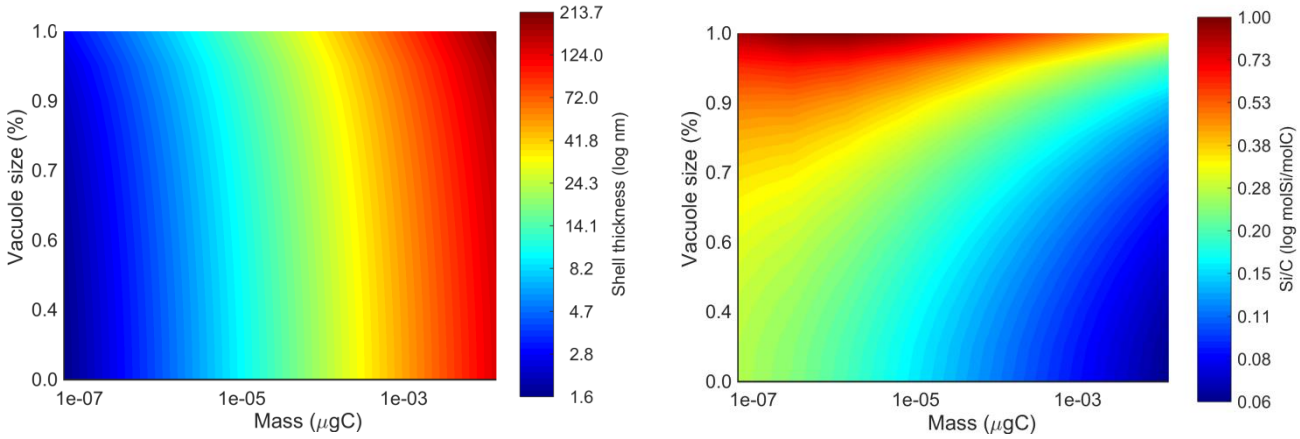

Figure A.2: Shell thickness (nm) (a) and Si:C ratio (b) according to the carbon mass $\boldsymbol{x}$ (x-axis) and the vacuole size v (% of radius) (y-axis).

Appendix B: Investment in membranes.

The creation of membranes requires a fraction ϕ_M of the carbon produced by the cell. The fraction of carbon used for membranes depend of membranes related to the total carbon content of the cell:

$$\phi_M = \frac{\left(V_{MI} + V_{MO}\right)\rho_M}{V_C\rho_C + \left(V_{MI} + V_{MO}\right)\rho_M} \tag{B.1}$$

The remaining carbon for uptake processes: $\phi_L + \phi_F = 1 - \phi_M - \phi_{struct}$ depends on the radius of the cell and the size of the vacuole (Fig. B.1). With larger vacuoles, the 'cost' of membranes may be important for small vacuolated cells as a large fraction of cell's carbon is located in vacuole's membrane.

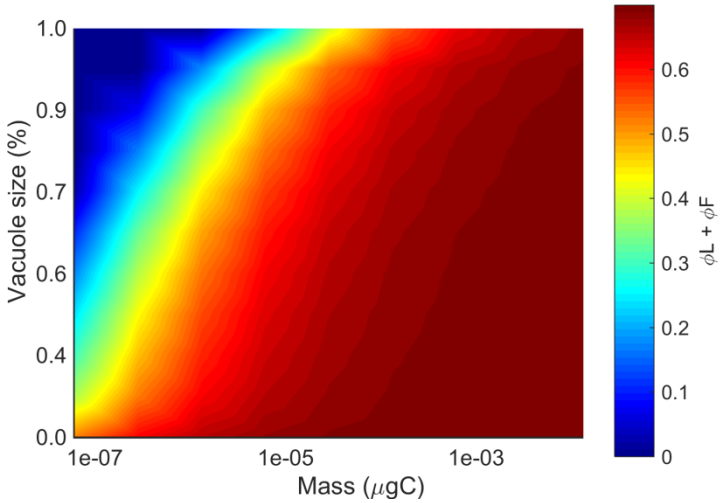

Figure B.1: Maximum investment in resources $\phi_L + \phi_F = 1 - \phi_M - \phi_{struct}$ with regards to the carbon mass x (x-axis) and the vacuole size v (y-axis).

Appendix C: Light affinity

The absorption of photons Q (µE s^{-1}) by a non-vacuolated spherical cell of radius r is:

$$Q(r,\lambda) = 2\pi r^2 L \int_0^1 \xi \left(1 - e^{-2\lambda r\sqrt{1-\xi^2}}\right) d\xi = \frac{\pi L}{2\lambda^2}\left[\left(1+2\lambda r\right)e^{-2\lambda r} + \left(2\lambda^2 r^2 - 1\right)\right] \quad \text{(C.1)}$$

(Duyens 1956) where L (µE μm^{-2} s^{-1}) is the light intensity (PAR), and λ (μm^{-1}) is the light attenuation coefficient, assumed to be constant throughout the volume of the cell. The light attenuation coefficient can be decomposed as $\lambda = a\,c$, where a (μm^2) is the optical cross section of a single plastid, and c (μm^{-1}) is the concentration of plastids within the cell (Raven 1984). In a diatom, and potentially in other cells, the distribution of plastids is not uniform, but concentrated within a layer close to the cell surface. For our spherical shell model of a vacuolated cell, where no plastids, and hence no absorption occurs in the vacuole, the absorption is:

$$Q(r,\lambda) = 2\pi r^2 L \int_0^1 \xi \left(1 - e^{-2\lambda r \ell(r,v)}\right) d\xi \quad \text{(C.2)}$$

where:

$$\ell(\xi, v) = \mathrm{Re}\left[\sqrt{1-\xi^2} - \sqrt{v^2-\xi^2}\right] \quad \text{(C.3)}$$

(Papageorgiou 1971). Unfortunately, while this can be solved numerically, it does not yield to a simple analytical expression. We can recast the cellular model into a simpler form that captures the effect of vacuolation on light absorption. In particular, we consider a flat cylinder with the same physical radius r as the vacuolated cell, and an equivalent bio-volume. Thus the absorption of light hitting the surface of the cylinder is:

$$Q(r,\lambda) = \pi r^2 L\left(1 - e^{-\lambda h}\right) \quad \text{(C.4)}$$

where h (µm) is the cylinder height. The equivalency of bio volume means that:

$$h = \frac{4}{3}\left(1 - v\right) r \quad \text{(C.5)}$$

This gives a much simpler analytic expression for light affinity A_L [µmol C (µE μm^{-2})$^{-1}$], namely

$$A_L = \pi r^2 q\left(1 - e^{-\lambda 4r(1-v^3)/3}\right) \quad \text{(C.6)}$$

where q is the photosynthetic yield (µmol C μE^{-1}).

Investment in photosynthesis ϕ_L, is linked to the light attenuation coefficient as:

$$\lambda = ac = \vartheta\phi_L \tag{C.7}$$

where an increase in investment, increases the concentration c of plastids; the return on investment is governed by the parameter ϑ (µm^{-1}). Typical values (Raven, 1984) suggest that λ = 0.1 µm^{-1} when about half of the cell's carbon is associated with photosynthesis. This suggests a value of ϑ = 0.2 µm^{-1}. The resulting light affinity as a function of the two-dimensional trait space is shown on Fig. C.1 for both vacuolated and non-vacuolated cells.

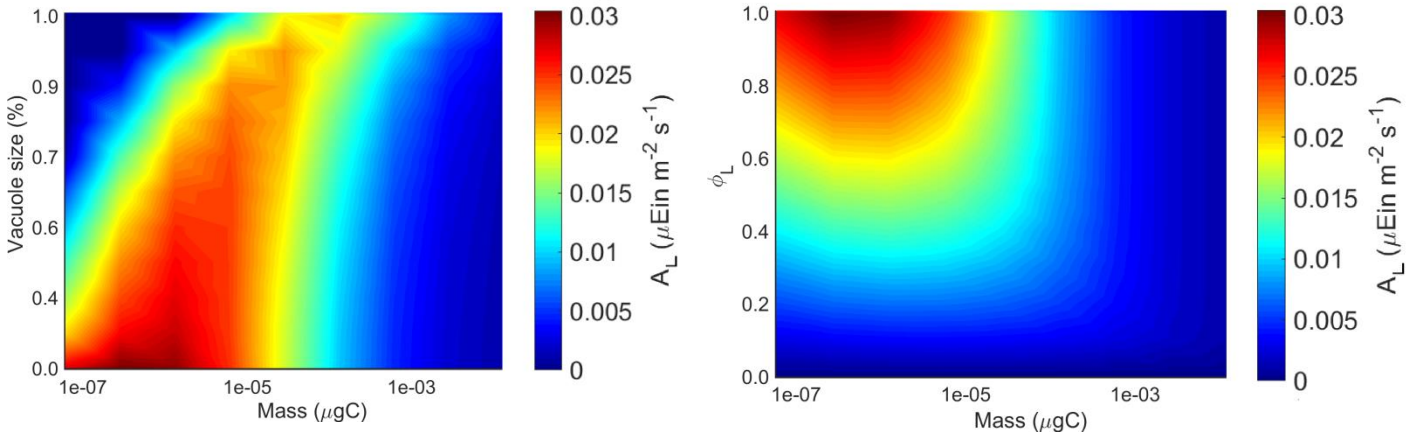

Figure C.1: Light affinity (µE m^{-2} s^{-1})$^{-1}$ d^{-1}) as a function of carbon mass $\boldsymbol{x}$ (x-axis) and (a) the vacuole size $\boldsymbol{v}$ (y-axis) for vacuolated cells or (b) investment in light $\boldsymbol{\phi_L}$ for non-vacuolated cells.

Appendix D: Liebig's law of the minimum, down regulation and leakage

We assume throughout that uptake of resources are constantly in stoichiometric balance with the requirements of cellular growth, where the cell stoichiometry is determined by its investment traits (see above). In this, net growth follows the usual constraints set out by Liebig's law of the minimum. There are, however a few issues that complicate this calculation. Firstly, uptake of resources require energy, paid for in terms of fixed carbon, i.e. as a portion of the incoming carbon flux. In order to maintain energy efficiency, the uptake of inorganic nutrients (N and Si) should be down regulated. Secondly, ingestion of particulate organic matter comes with inorganic nutrients that can be in excess to requirements. These nutrients should be excreted back into the environment. Finally, we assume no imposed limit on photosynthesis when nutrients are limiting; the cell continues to produce carbohydrates where that which is in excess to requirements (i.e. limited by inorganic nutrient supply) is leaked into the environment as dissolved organic carbon (DOC). This leakage of DOC has no

direct impact on the model formulation as reported here, but can be important when we implement osmotrophy as an addition unicellular feeding strategy.

The harvesting of light (photosynthesis) J_L (µgC d^{-1}) and uptake of nitrogen J_N (µgN d^{-1}), silicon J_S (µgS d^{-1}) and food J_F (µgC d^{-1} or µgN d^{-1}) have corresponding metabolic costs, β_R (µgC µgC/N/S^{-1}), that are all paid in carbon (Table 1). In order to maintain the stoichiometric balance between carbon and nitrogen $c_{C:N}$ and between carbon and silicon ratio $c_{Si:C}$ given by the shell/carbon biomass ratio in the case of vacuolated cells, the uptake of nutrient has to be down-regulated when carbon is limiting, *i.e.*, when potential nutrient fluxes exceed net carbon flux into the cell. It is assumed that the cells are unable to downregulate the amount of carbon taken up and that it must photosynthesize according to the amount of available sunlight but they can reduce their uptake rate of dissolved nutrients. The down-regulation of nutrient uptake prevents the cell from taking-up (and paying the additional cost for) nutrient that cannot be used for growth.

In the case of a generalist mixotroph, the nutrient uptake J_N is reduced by a factor ε that restricts the nitrogen uptake so as not to exceed the net carbon flux into the cell (after removal of respiration $J_{resp} = \beta_0 x$ (µgC d^{-1}) and carbon uptake costs). ε is obtained by equating the carbon and nitrogen net fluxes (from different sources) (in carbon unit):

$$J_L - \beta_L J_L - J_{resp} + J_F - \beta_F J_F - \beta_N \varepsilon J_N = c_{C:N} \varepsilon J_N + J_F \tag{D.1}$$

which can be solved for ε:

$$\varepsilon = \max\left[0, \min\left[1, \frac{J_L - \beta_L J_L - J_{resp} - \beta_F J_F}{\left(\beta_N + c_{C:N}\right) J_N}\right]\right] \tag{D.2}$$

After downregulation of nutrient uptakes, the light harvesting, nutrient uptake and food fluxes are combined following Liebig's law to compute the effective uptake (Eq. 10). This may lead to further leakage of nutrients (from food uptake) that are in excess to stoichiometric requirements compared to carbon when costs are implemented. The excess nitrogen flux η_N that is leaked by the cell and released back to the nitrogen pool is:

$$\eta_N = \max\left[0, \varepsilon J_N - \frac{1}{c_{C;N}} \max\left[0, J_L\left(1-\beta_L\right) - J_{resp} - \beta_F J_F - \beta_N \varepsilon J_N\right], c_{C:N} \varepsilon J_N\right] \tag{D.3}$$

When nitrogen is in excess and growth is carbon-limited (light is limiting), this term is equal to the difference between nitrogen uptake (after down-regulation) and carbon uptake (second term of Eq. D.3).

In the case of diatoms, we introduce three downregulation factors ε_{L} ε_{N}, and ε_{S}, such that the cell does not take up more nutrients that it can use for stoichiometrically balanced growth. We solve for the three reduction factors by setting all fluxes equal (with appropriate $c_{C:N}$ and $c_{C:Si}$ factors), thereby assuming co-limitation of the three elements.

$$\varepsilon_L\left(1-\beta_L\right)J_L - J_{resp} - \varepsilon_N\beta_N J_N - \varepsilon_S\beta_S J_S = c_{C:N}\varepsilon_N J_N \tag{D.4a}$$

$$\varepsilon_L\left(1-\beta_L\right)J_L - J_{resp} - \varepsilon_N\beta_N J_N - \varepsilon_S\beta_S J_S = c_{C:Si}\varepsilon_S J_S \tag{D.4b}$$

$$c_{C:N}\varepsilon_N J_N = c_{C:Si}\varepsilon_S J_S \tag{D.4c}$$

As three equations with three unknowns cannot be solved immediately, we solve this system over three times: when carbon is limiting $\varepsilon_L = 1$, when nitrogen is limiting $\varepsilon_N = 1$ and when silicon is limiting $\varepsilon_S = 1$. Thereby we have three different solutions for the three reduction factors. The condition when $\varepsilon_L = 1$ and $\varepsilon_N, \varepsilon_S < 1$ occurs when light is limiting. As only one substrate will be limiting, only one of the three solutions fulfil the previous condition at each set of traits under each set of environmental conditions. In case where one of the nutrients (N or Si) are limiting, ε_L is set it to 1, as the photosynthetic uptake cannot be reduced. The value $1-\varepsilon_L$ will determine the leaked carbon, in the form of carbohydrate DOC, that cannot be used for biosynthesis because of the limited nutrient availability.

Liebig's law of minimum in the case of diatoms is given by Eq. 9. Excess fluxes of nitrogen and silicon (η_{N} and η_{S}) when other elements are limited are found as the difference between the uptake of the limited element and nitrogen/silicon uptake:

- When carbon is limiting:

$$\eta_N = \varepsilon_N J_N - \frac{1}{c_{C;N}}\max\left[0, \left(1-\beta_L\right)J_L - J_{resp} - \varepsilon_N\beta_N J_N - \varepsilon_S\beta_S J_S\right] \tag{D.5a}$$

$$\eta_S = \varepsilon_S J_S - \frac{1}{c_{C:Si}}\max\left[0, \left(1-\beta_L\right)J_L - J_{resp} - \varepsilon_N\beta_N J_N - \varepsilon_S\beta_S J_S\right] \tag{D.5b}$$

- When nitrogen is limiting:

$$\eta_N = 0 \tag{D.6a}$$

$$\eta_S = \varepsilon_S J_S - \frac{\varepsilon_N J_N c_{C:N}}{c_{C:Si}} \quad \text{(D.6b)}$$

- When silicon is limiting:

$$\eta_N = \varepsilon_N J_N - \frac{\varepsilon_S J_S c_{C:Si}}{c_{C:N}} \quad \text{(D.7a)}$$

$$\eta_S = 0 \quad \text{(D.7b)}$$

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