**Biogeosciences
Discussions**

# Interactive comment on "A model for variable phytoplankton stoichiometry based on cell protein regulation" by J. A. Bonachela et al.

**J. A. Bonachela et al.**

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Dear Editor,

Please consider below the reply to the first referee report for the paper "A model for variable phytoplankton stoichiometry based on cell protein regulation", of which we are the authors.

Response:

We want first to thank the referee for raising these points, which allow us to clarify both here and the text some important aspects of the nutrient uptake sub-model.

As the referee points out, we use plausible physiological mechanisms to build our model. Based on the previous regulatory model published in (Bonachela et al., 2011), we put the highest emphasis on the mechanisms responsible for protein regulation. To this end, we use essential and well documented links between protein synthesis and the internal content of the nutrients for the cell. We encode these well known processes by means of mathematical functions whose specific shape is, as explained in the manuscript, not determinant of the emergent phenomenology of the model. That phenomenology is, in addition, in qualitative agreement with the expected (and observed) behavior of phytoplankton cells under different environments. In summary, we think that the use of essential processes as foundations for the model, and the replication of most of the experimental results that can be found in the literature, ensures the mechanistic character, and plausibility and realism of our model, respectively.

We reply below to the specific questions of the referee, in order to dissipate his doubts on the mechanistic content of the model. We have also improved the sub-model description in the manuscript to clarify these points:

1. The uptake sub-model is basically an extension of the expressions mathematically deduced from (Bonachela et al., 2011), which are in agreement with those in (Armstrong, 2008). In the latter, the exact expression the referee mentions is presented, Eq.(9), and the approximation $\sqrt{1+x} \sim 1 + x/2$ is used to deduce an expression in the shape of the extended Michaelis-Menten functional form.

   Because we are mostly interested in qualitative behavior under different environmental conditions, as in (Bonachela et al., 2011) the use of the approximate expression does not offer a disadvantage but rather has important advantages that are enhanced by the dynamic regulation of the maximum uptake rate ($V_{max}$) present in our model. For instance, the dynamics of $V_{max}$ are eventually translated in a dependence of $V_{max}$ with the external nutrient concentration; for one single generic nutrient $X$, the observed behavior in the extreme cases $[X] \to 0$

and $[X] \to \infty$. make the uptake rate V take well known mathematical forms ($V = 4\pi D_X r_s[X]$, and $V = V_{max}^{lo}$ in the notation of our paper, see Eqs.(3)-(6), or Eq.(4) in (Bonachela et al., 2011); the deduction can be found in the Supplementary information of that paper). Without any loss of generality, the sub-model is able with these expressions to reproduce qualitatively the theoretical expectations and experimental observations of seminal papers such as (Gotham and Rhee, 1981) or (Morel, 1987).

These two points have been made clearer in the text, concretely in the description of the effective half-saturation constant expressions, just before the dynamics of $V_{max}$ are introduced, using the paragraph:

*The final expressions obtained after replacing Eqs.(5) and (6) into Eqs.(3) and (4) result from approximating the solution of a second order polynomial for $V_{max}$ (see (Armstrong,2008; Bonachela et al., 2011) for detailed derivations). The actual performance of the approximation and the exact term has been compared in (Armstrong 2008; Fiksen et al. 2013), but the dynamic character of the number of proteins –and, therefore, $V_{max}$ (see below)– has not been taken into account in those studies. This approximation has proven to work well for both small and large phytoplankton cell sizes (Armstrong,2008), while the dynamic equations for the number of uptake sites introduce an effective dependence on size intended to compensate for the coarser performance observed for intermediate sizes. The use of these "approximate" expressions has, on the other hand, important advantages. For instance, it facilitates the description of two well-known extreme results using one single expression: the scarce nutrient limit provides an uptake rate independent of the number of uptake sites, while the large nutrient concentration extreme provides the maximum uptake rate measured in typical (bulk) experiments. These two cases are usually referred to as diffusion and kinetic limitation regimes (see (Bonachela et al., 2011) for a mathematical derivation, and references therein).*

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The note, just after "*...independent of the number of uptake sites...*" in that paragraph:

*Specifically, $V = 4\pi D_X r_s[X]$, with rs the radius of a protein that takes up nutrient $X$.*

And the comment, in the Discussion section:

*The dynamic character of these proteins is eventually translated into an effective dependence of $V_{max}$ on nutrient concentration, which allow the general mathematical expressions presented with this model behave in a very different way under, e.g. diffusion versus kinetic limitation for the cell.*

2. The half-saturation parameters presented in the table correspond to the standard (i.e. bulk) parameters. This can be seen by using Eqs.(5) and (6) in the manuscript and taking into account that $V_{max}$ decreases with nutrient concentration. For large nutrient concentrations, $V_{max}$ becomes small, the second term becomes small compared to 1, and the effective half-saturation constant approaches its bulk value.

This has been clarified in the text, after the introduction of the effective half-saturation constants, when $K$ is now referred to as "*bulk half-saturation constant*", and the following text has been added:

*In our model, $\widetilde{K} = K$ for large nutrient concentrations (in agreement with the definition of $K$ as a bulk variable), and $\widetilde{K} > K$ when the nutrient concentration is scarce (see below).*

3. As the referee mentions, the half saturation constant is a composite of cell properties such as uptake site radius and handling time. This relation can be found in other sources, e.g. (Bonachela et al. 2011), supplementary information. Because we aim to use well known parameters easy to find in the literature, we used

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the standard $K$ instead of trying to find information for all those parameters. But they could be used if needed, allowing for an even more mechanistic description of the uptake process in terms of more essential cell traits.

We have added, in the same paragraph indicated in point 2, the text:

*...where KN and KP are the standard (i.e. bulk) half-saturation constants (a composite of fundamental traits such as cell radius or nutrient ion handling time (Aksnes and Egge, 1991; Bonachela et al., 2011)) associated with the uptake of N and P, respectively...*

4. The result the referee mentions can be obtained by using the fact that $V_{max}$ grows with decreasing nutrient concentration. Thus, for small $[X]$ (i.e. when the boundary layer appears around the cell), Eqs.(3) or (4) become $V = 4\pi D_X r_s[X]$ (see above) and the uptake rate is not proportional to the number of sites anymore.

5. That is an interesting study indeed, and we thank the referee for pointing it out. However, the differences that are studied in that paper do not take into account the dynamic aspects of $V_{max}$ resulting from the dynamics of nutrient uptake proteins. Actually, those dynamics will be influenced by cell size, relative area covered by these proteins, and external nutrient concentrations, precisely the three variables they explore in that paper to measure the differences between the exact and the approximated expressions (see their Fig.1). Thus, all these aspects –dependent on cell size– are most likely going to compensate for some of those differences.

   We have now cited this study in the paragraph before the sub-section for the dynamics of $V_{max}$, together with the other explanations above:

   *The actual performance of the approximation and the exact term has been compared in (Armstrong 2008; Fiksen et al. 2013), but the dynamic character of the*

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*number of proteins –and, therefore, $V_{max}$– has not been taken into account in those studies.*

   It would be certainly very interesting to repeat that study considering the dynamics of $V_{max}$. For instance, by using our model, or the one-nutrient version presented in (Bonachela et al., 2011).

6. Although thoroughly justified in the paper that introduces that expression for the first time (Bonachela et al., 2011), all the rationale behind the F function can be found in our manuscript. As we explain in the text, experimental results point to the dependence of regulation on the internal content of the nutrient (upregulation when quota is low); the exact shape is justified by the fact that it encodes protein expression, which is normally represented by sigmoidal functions. But, as pointed out in the text, simpler functions decreasing with increasing quota (e.g. a linear function with negative slope) would not change the qualitative behavior we want to achieve.

   We have made some changes to the text to make this point clearer; the description of the F function now reads:

   *We use here a generic sigmoid function; this choice is motivated by the identification of F with processes involving gene expression –traditionally represented by Hill (sigmoid) functions (Alon, 2007); however, the exact mathematical function is not important because other normalized functions with similar trends do not alter the qualitative behavior of the model.*

References:

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Bonachela J.A, Raghib M, Levin SA. 2011. Dynamic model of flexible phytoplankton nutrient uptake. PNAS 108:20633-20638.

Fiksen Ø, Follows MJ, Aksnes DL. 2013. Trait-based models of nutrient uptake in microbes extend the Michaelis-Menten framework. Limnol. Oceanogr. 58:193-202.

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