Anonymous Referee #3

Received and published: 11 November 2020

In the manuscript, "Modelling Silicate – Nitrate - Ammonium co-limitation of algal growth and the importance of bacterial remineralisation based on an experimental Arctic coastal spring bloom culture study" by Vonnahme et al. the authors present a new model development for diatom co-limitation of nutrients. Based on experimental data they expand the classical model by Geider et al. (1998), which remains its feasibility for larger (ecosystem) models, while improving the representation of algae growth. Improving biological parameterizations in ecosystem models is important and contributes to improving their predicative capability. However, the authors should address some points listed below.

We want to thank the reviewer for the positive and very helpful review and addressed the points raised as described below.

The authors report that “With the onset of the stationary phase in the bacteria-enriched cultures about 30% of the cells formed biofilms on the walls of the cultivation bottles (estimated after sonication treatment).” (line 230). The formation of such biofilms has occurred in other experiments before and cannot always be avoided. However, it does potentially have a huge impact of microbial dynamics and interactions. Therefore only reporting (and discussing) it is insufficient, if one is to compare experimental results with a new modelling approach. I would suggest to run a model sensitivity analysis specifically targeting this.

We agree that the biofilm formation can have a substantial impact on some microbial dynamics and the identity of different carbon pools. Since, the biofilm only contributed to 30% of the cell counts we are still confident that the model is well suited to represent the dynamics of the experiment and coastal Arctic spring blooms. The current model has various dynamics changing after silicate limitation, especially in the presence of bacteria and NH4 regeneration. As outlined in the response to reviewer 2, we changed the reduction of photosynthesis after Si limitation from a hard-wired number (80%) to a tuneable parameter. Since Si limitation corresponds with the timing of biofilm formation, we assumed that the silicate limitation parameters (in particular Si_{PS} – reduction of photosynthesis after Si limitation) could describe the changed dynamics.

To test if this assumption holds true and to deepen the discussion of biofilm related dynamics, we suggest 3 potential dynamics which are likely different in the biofilm. Since the biofilm formation corresponds with Si limitation, we modelled changed dynamics after Si limitation to represent specific changes known to be differen in biofilms.

1) DOC coagulation to EPS as part of the POC pool
   a. $dPOC_{EPS}/dt = x_f * x_{eps} * C$
   b. (x_{eps} – fraction of coagulation excreted DOC)
2) Increased DOM excretion in the stationary phase
   a. IF (Si < lim){ $x_f = x_{f2}$ } else { $x_f = x_{f1}$}
   b. (x_{f1}- excretion before Si limitation, x_{f2} –excretion after Si limitation)
3) Increased remineralization of excreted DON in the stationary phase
   a. IF (Si < lim){ $rem = rem_2$ } else { $rem = rem_1$}
   b. (rem_1- remineralization rate before limitation, rem_2 –remineralization rate after Si limitation)

We extended the model as described above and compared the new model output with the original fit. We changed tuned the new parameter manually until the model output showed substantial differences (approx. > 10% in POC, PON, Chl, or DIN). Eventually, we tested the effects of 100% DOM coagulation ($x_{eps} = 1$), 1000x higher remineralization rate after Si limitation (rem2 = 10000, rem1=10), 2x higher DOM excretion after Si limitation (xf1 = 0.06, xf2 = 0.12). The order of magnitude of perturbations needed to get changes of 10% of at least 1 output variable gives an indication of the parameters sensitivity. We then tried to tune the new model again to the initial fit by changing the Si_{PS} parameter. For each case, we were overall able to return to the original fit with less (1 & 2) or equal (3) perturbations of the SiPS parameter than was perturbed in the added parameters. This shows that SiPS is more sensitive and collinear (unidentifiable) with the added parameters, which shows clearly that the 3 suggested model extensions would not improve the model without additional data (e.g. EPS measurements). We added this discussion in a shortened way to the manuscript. More details are given below:

1) The POC can include not only algae biomass, but also EPS that holds the biofilms together. For estimating the potential importance of this POC pool, we added a model run, where all the excreted carbon (given by x_f * POC) is coagulating to EPS and thereby contributing to the POC pool. As shown in Fig 1 the outcome are ca 30% increased POC values in the stationary phase, which is in accordance with our estimate from the sonication treatment. However, it is unlikely that all excreted DOM aggregates to EPS (Schartau et al., 2017) and earlier studies describe much lower proportions of EPS being part of the EPS pool (up to 7% of an biofouling diatom biofilm, Khandeparker and Bhosle, 2009), with the highest fraction after nitrogen and silicate limitation in the stationary phase. A potential model extension to account for EPS aggregation that contributes to the EPS pool would be the approach, described by Schartau et al. (2017) who model carbon excretion (3 different DOC pools) and aggregation to TEP (transparent exopolymeric substances). However, since we did not measure cellular C and extracellular EPS separately, we argue that the extension requiring 11 additional parameters and 3 additional state variables of TEP (EPS) and 3 different DOC pools would i) not be in line with our goal to develop a simple model, and ii) would not be justified by the measured data, making the tuning process rather speculative (overfitting issue). Nevertheless, we acknowledge that this process needs to be discussed and we add the figure below to the supplement to show the maximum potential importance of EPS aggregation (assuming immediate aggregation of excreted DOC).

   For estimating the importance of considering EPS aggregation, we also tested an extended model where a fraction of the excreted carbon is coagulated to EPS (xeps), contributing to the POC pool. We added following equation:

$$POC_{EPS} = x_{EPS}\, x_f\, POC$$

   with a start value of 50% aggregation and no constraints (values between 0 % and 100 %). The difference between the extreme values of 0 % and 100 % are shown in Fig. 1 below and lead to 30 % difference in maximum POC. This makes the parameter quite insensitive. In fact the SensFUN of the FME package defines the sensitivity of the added xeps parameter close to 0. We also suggest, that the effect of xeps could be compensated by the SIPS term of the EXT model (% reduction in photosynthesis after Si reduction), leading to a very similar fit, indicating collinearity. This is mainly the cause since, EPS aggregation only has a major role with a linear response in the stationary phase when also Si is limiting. Thus, an additional xeps term would be unidentifiable with the current set of measured data.

   We will add a more thorough discussion of the approach by Schartau et al. (2017) and the suggestion for a more simplified model extension described above (adding the xeps parameter), for experiments were EPS data are available, but with its limitations for the current model due to collinearity/unidentifiability issues.

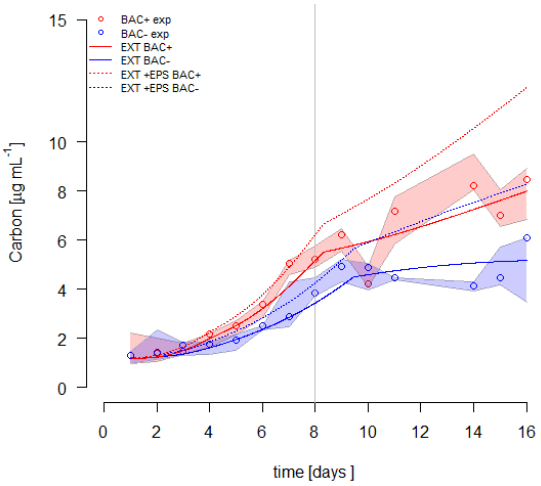

Figure 1. POC concentrations of the measured data and model, including a model run showing POC as originally modelled POC + excreted DOC assumed to aggregate immediately and completely to EPS (dashed line).

2) Another finding by Khandeparker and Bhosle (2009) is an increased DOM excretion after Si and N limitation, which is not yet part of our model. Hence, we added a second excretion term xf2 after silicate limitation. A doubling of the excretion rate after silicate limitation leads to slightly reduced POC and PON values (Fi. 2), but no changes in Chl and only small extra NH4, due to the higher importance of the ambient DON for NH4 regeneration. The lower POC values can be completely compensated by doubling the the Photosynthesis after Si limitation (SiPS 0.2 -> 0.4) parameter. The lower PON value can almost be compensated with the same parameter.
This little difference indicate, that a modelling approach with changing xf rates after Si or N limitation is not necessary, at least not in our model system with high refractory DOM concentrations. It may however, become important in open-ocean systems with less terrestrially derived DOM.

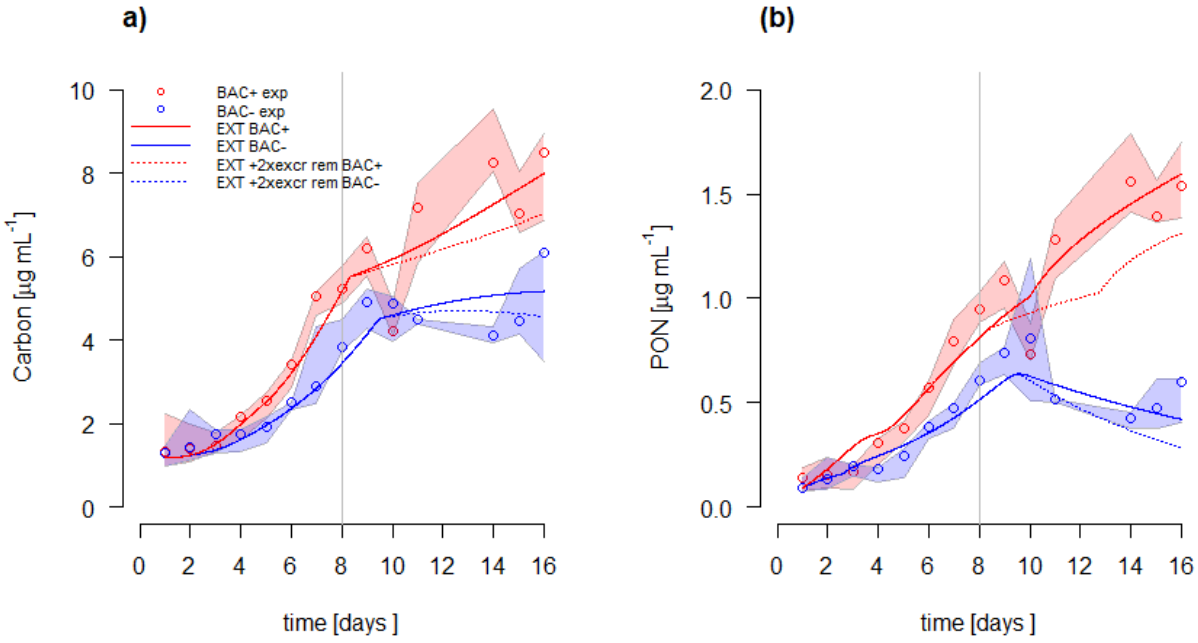

Figure 2. Impacts of a 2 times increased DOM excretion after Si limitation (dashed line).

3) The biofilm can also facilitate interactions between bacteria and algae due to the closer proximity. This increased interactions could be represented in an increased remineralization rate of excreted organic matter (rem) after N or/and Si limitation. A potential model extension accounting for it would include a second higher remineralization rate after Si or N limitation. However, the difference between the EXT model where C is excreted as DOC (xf), or simply lost for maintenance respiration (RC) is minor (Fig. 3).
After adding a second remineralization rate of labile DOM (rem2) an increase of 3 orders of magnitude is needed (rem2 = 10e03 rem) to show any visible effect on N assimilation and NH4 regeneration (Fig. 3), showing that this is a highly insensitive parameter. However, about 4 order of magnitude higher rates appear to bring the modelled NH4 concentrations closer to the measured data (while fits to POC, PON, and Chl become very different form the measured data), hinting that the poor model fit to NH4 may not only be related to immobilized NH4 in the measured data (e.g. NH4 adsorbed or trapped in EPS), but may also be related underestimated DON excretion or remineralization in the model.
However, more than 10% DOM excretion and a DON remineralisation rate 3-4 order of magnitude higher than remineralization of the ambient, likely terrestrially derived DOM is rather unlikely. A likely explanation of the low impact of increased remineralization in the biofilm of our experiment is the high ambient DOM concentrations, which are the main DON source for NH4 remineralization (See difference of the extended model with and without excretion in the manuscript). Since our model is supposed to represent coastal systems, we thus argue that only 2 different remineralization rates related to refractory and labile OM is sufficient. In more open ocean setting with less allochthonous DOM input, increased remineralization rate of algae EPS in the stationary phase, may be a useful addition.

We suggest therefore, that a higher remineralization rate is likely, but that a large part of the remineralized NH4 is not available for algae growth due to the biofilm. Thus, the modelled NH4 values represent the available NH4 for algae, which representation is the aim of this study.

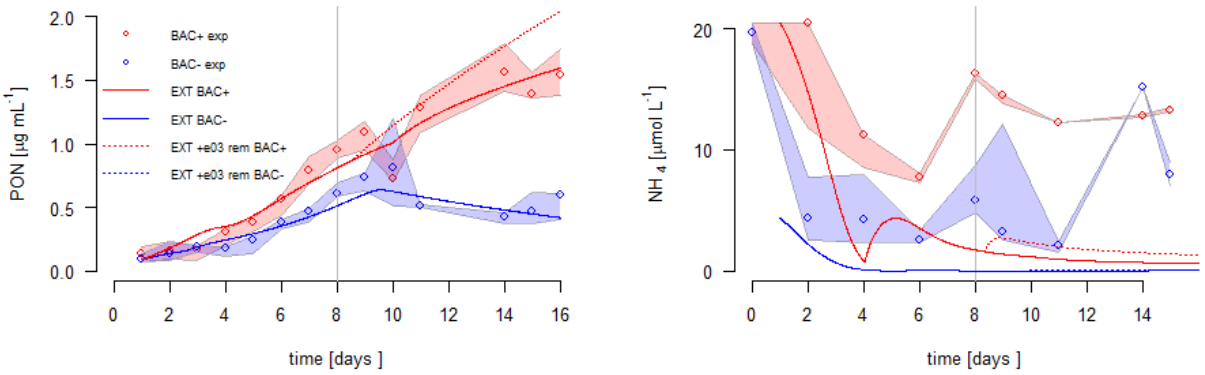

Figure 3. Comparison of the original model and a 3 orders of magnitude increased remineralization rate of excreted DON after Si limitation (dashed line).

We add Fig. 3 to the supplement and discuss the potential of increased bacterial remineralization in biofilms and why this is not quantitatively important in our experiment and model.

4) Another effect of the biofilm may be adsorption of ammonium to the EPS or concentration in pockets, not available for algae growth. In fact, this could be one of the explanation for the consistently high NH4 values in the stationary phase, which are poorly represented in the model (See response to Referee #2).

Response to Referee #2
“Ammonium is most likely immobilized in the biofilm via adsorption to the EPS and accumulation in pockets unavailable to diatoms. These immobile NH4 pools are still part of the measured data. With the model assuming all NH4 being available for algae growth, this is a problem.”

This could, in particular, explain the high values of measured NH4 compared to the model results as shown in Figure 5c. In addition we could add a small discussion of a potential pH dependence

of NH4+ adsorption to the EPS in terms of the pKa values of NH4+ and carboxylic groups, which belongs to the acidic polysaccharides as a fraction of EPS:

- Carboxylic groups have a pKa < 5, i.e. far away from seawater pH ~ 8, which means that they are always in the deprotonized negatively charged form R-COO- in seawater.
- NH4+ has a pKa ~9 closer to seawater pH.
- Thus, the NH4+/NH3 ratio will be higher in more acidic microenvironments (pH ~7.5-8).
- Thus, a lower pH due to bacterial respiration would increase the concentration of NH4+ in comparison to the bulk medium, which results in a higher immobile NH4 pool due to adsorption to the EPS.
- This could explain the higher discrepancy between modelled and measured NH4+ values in the experiments with bacteria (as seen in Figure 5c).

Since the biofilm formation corresponds with silicate limitation, the reduced photosynthesis might of course be related to either the biofilm or the silicate limitation. But for untangling the effects of biofilm formation and silicate limitation, more experiments or data would be needed. However, only 30% of the culture was part of the biofilm and the best fit of an 80% reduction corresponds very well with an earlier study by Werner (1978), who did not have the issue of biofilm formation. Hence, we suggest that the main cause for the reduction of photosynthesis is related to Si limitation and not the biofilm.

We add this argumentation together with collinearity issues of SiPS with potential model extensions taking the biofilm into account to the discussion. We will also add the figures above showing the impact/sensitivity of potential model extensions to account for the changed dynamics in a biofilm to the supplement.
In the manuscript we add a discussion about the results explaining: i) the potential changes in a biofilm (increased DOM excretion, increased remineralization, trapped NH4), ii) the importance of the biofilm for our model run (POC as EPS instead of algae biomass, differences in fitting and sensitivity for the stationary or exponential phase, considerations of the biofilm being only 30%), iii) we also added a discussion of biofilms or aggregates/marine snow in the environment, which our study aims to represent.

Rakhee DS Khandeparker & Narayan B Bhosle (2001) Extracellular polymeric substances of the marine fouling diatom amphora rostrata Wm.Sm, Biofouling, 17:2, 117-127, DOI:10.1080/08927010109378471

The authors appropriately discuss quota models and their use. A different approach to model celluar nutrient kinetics, that has been argued to be more mechanistic, considers uptake sites for nutrients (Aksnes & Egge, 1991, Mar Ecol Prog Ser. 70:65-72). A good, though slightly technical, paper applying this approach and combining it with variable cellular stoichiometry is Flynn et al., 2018, PLoS Comput Biol 14(4): e1006118. Setting up a model like this for your data could be highly interesting, but beyond the scope of this study. However discussion the approach would provide a very useful context.

We want to thank the reviewer for the interesting suggestion and reference. We added the model by Flynn et al. to Table 1. We also included a discussion of the approach. We argue overall that the model is too complex for the aim of our study, which tries to keep the number of parameters as low as possible allowing scalability (similar to Flynn, 1997; Flynn, 2001), but acknowledge the important role of considering transporter densities, cell size, and mobility. Especially the importance of mobility is an interesting aspect, that we now discussed in the context of diatom sedimentation.

Change:

“The next step to quota based-models is the consideration of more detailed cell based characteristics, such as transporter density, cell size, and mobility, including sedimentation (Aksnes and Egge, 1991). Flynn et al. (2008) discuss a model with detailed uptake kinetics showing that large cells are overall in disfavored over small cells due to higher half saturation constant, but that they may still have competitive advantages due to lower investment in transporter production, and increased sedimentation, increasing the mobility that may offset the disadvantage of a larger size. While this extension is too complex for our aim of a simple model, the dynamics may become important when modelling different algae taxa.”

In the introduction (line 46) and in the discussion the authors mention the role of the impact of climate change on coastal phytoplankton succession, including projected increased DOM inputs via river run off. Several studies have found and/or suggested a delayed bloom due to increase turbidity (e.g. Opdal et al. 2019, Glob Change Biol. 2019;00:1–8), which should be mentioned here.

We thank the reviewer for the suggestion, which is an important clarification to the introduction and in particular for the discussion.

Change in Introduction:

"… stratification in coastal Arctic systems is expected to increase… earlier stratified surface layer in spring, which may lead to an earlier spring bloom (Tremblay and Gagnon, 2009).""However, at the same time, brownification and increased sediment resuspension is already leading to light inhibition in spring, which may lead to a delayed spring bloom (Opdal et al., 2019)."

Change in Discussion:

"An earlier temperature driven water column stratification may also lead to an earlier bloom. However, due to increasing river and lake brownification and sediment resuspension, the spring bloom may be delayed (Opdal et al., 2019)." "With decreased light, carbon overconsumption as described by Schartau et al. (2007) may become less important due to decreased photosynthesis. An earlier, or later phytoplankton bloom can lead to a mismatch with zooplankton grazers (Durant et al., 2007;Sommer et al., 2007), which could decrease the fecal pellet driven vertical export and thereby increase the residence time of POM in the euphotic zone and the potential for ammonium regeneration, making the incorporation of bacterial recycling into ecosystem models even more imporatant as also evident from our experimental data and model output."

The authors mention both nitrate and ammonium as nitrogen sources. Additionally, urea can be a relevant nitrogen source in some systems. I am not sure how much of a role this plays in arctic ecosystems, but it should either be discussed or mentioned why it does not play a significant role.

We agree with the reviewer that urea may be another important nitrogen source, especially under nitrate limitation. In some Arctic systems it may reach concentrations of 2 uM. While bacteria may produce urea by ON degradation, the main source of urea is attributed to zooplankton excretion (Conover and Gustavson, 1999). Hence, it does not play a role in our experiment, but may play a role in nature. We added a discussion of urea as potential nitrogen source to the discussion of zooplankton NH4 excretion.

Change in introduction:

"Zooplankton may also release some ammonium and urea after feeding on phytoplankton, but we suggest that this process is likely far less important than bacterial regeneration (e.g. Saiz et al., 2013). Previously measured ammonium excretion of Arctic mesozooplankton is typically low compared to bacterial remineralization (Conover and Gustavson, 1999), with the exception for one study in summer in a more open ocean setting (Alcaraz et al., 2010). In some Arctic systems urea, excreted by zooplanotn may be an important N source for regenerated algae production (Conover and Gustavson, 1999)."

Change in discussion:

"Another potentially important N source for regenerated production may be urea (Harrison et al., 1985), which would lead to an even higher importance of regenerated production as suggested by our study."

Conover, R. J., & Gustavson, K. R. (1999). Sources of urea in arctic seas: zooplankton metabolism. Marine Ecology Progress Series, 179, 41-54.

Harrison, W. G., Head, E. J. H., Conover, R. J., Longhurst, A. R., & Sameoto, D. D. (1985). The distribution and metabolism of urea in the eastern Canadian Arctic. Deep Sea Research Part A. Oceanographic Research Papers, 32(1), 23-42.

Line 168: ": : :but the growth rate can be reduced (Hildebrand, 2002; Gilpin, 2004)". How can the growth rate be reduced? What can lead to this reduction?
We realized that growth rate is not the best term here and changed the sentence as follows:

Change:

"N and Si metabolism have different controls and intracellular dynamics, with N uptake fueled by photosynthesis (as PCref in G98) and Si mainly fueled by heterotrophic respiration (Martin-Jezequel et al., 2000). In general, we assume that nitrogen metabolism is not directly affected by silicate limitation (Hildebrand 2002, Claquin et al., 2002), but we expect cellular ratios to be affected by reduced photosynthesis and chlorophyll synthesis under Si limitation (Hildebrand, 2002; Gilpin, 2004)."

We also suggest to add more recent references on the effects of Si limitation on photosynthesis.

Gilpling (2004) only described the relationships of C,N,Chl production/assimilation under N and Si limition, but didn't give a physiological explanation.
Lippemeier et al. (1999) found a direct inhibition of the PSII reaction centre due to increased photochemical quenching, which is part of the explanation, but still rather descriptive. Our study confirmed lower efficiency of PSII (via Quantum yield measurements) after Si limitation, which is in accordance with Lippemeier et al. (1999) and supports our approach of reduced photosynthesis after Si limitation. Thus, we added a reference to this study in our discussion of the Quantum yield.
Another recent study by Liu et al. (2020) investigated gene expression patterns for C fixation related genes, and found reduced exression under Si limitation, but not under N or P limitation. The most detailed study has probably been done by Thangaraj et al. (2019), who used a metaproteomics approach and found not only downregulated photosynthetic proteins after silicate limitation, but also distracted protein production for mitochondria-chloroplast interactions, chlorophyll synthesis, and mechanisms compensating for disruption in electron transfer.

Lippemeier, S., Hartig, P., & Colijn, F. (1999). Direct impact of silicate on the photosynthetic performance of the diatom Thalassiosira weissflogii assessed by on-and off-line PAM fluorescence measurements. Journal of Plankton Research, 21(2).

Liu, Q., Xing, Y., Li, Y., Wang, H., Mi, T., Zhen, Y., & Yu, Z. (2020). Carbon fixation gene expression in Skeletonema marinoi in nitrogen-, phosphate-, silicate-starvation, and low-temperature stress exposure. Journal of Phycology, 56(2), 310-323.

Thangaraj, S., Shang, X., Sun, J., & Liu, H. (2019). Quantitative proteomic analysis reveals novel insights into intracellular silicate stress-responsive mechanisms in the diatom Skeletonema dohrnii. International Journal of Molecular Sciences, 20(10), 2540.

We added more details and references to the introduction, model description, and discussion.

Figure 6 and figure 7 do not exist.

We corrected the figure references.

Line 660: Table 1 is not the most up-to-date. Especially on the ecosystem model side it would be nice to see more recent developments reflected as well.

For the cultivation based models, we added the study by Flynn et al., 2018 as mentioned above. For the ecosystem scale models, we cited the original reference of the algae growth and potential nutrient regeneration dynamics, which is often rather old, while the full-scale models are mostly updated in terms of mostly physical formulations. We clarified this in the legend and added following more recent references to the ecosystem scale models:

BFM model:_ Smith, K. M., Kern, S., Hamlington, P. E., Zavatarelli, M., Pinardi, N., Klee, E. F., & Niemeyer, K. E. (2020). BFM17 v1. 0: Reduced-Order Biogeochemical Flux Model for Upper Ocean Biophysical Simulations. *Geoscientific Model Development Discussions*, 1-35.

ReCom-2 model: Schourup-Kristensen, V., Wekerle, C., Wolf-Gladrow, D., Völker, C. (2018): Arctic Ocean biogeochemistry in the high resolution FESOM 1.4-REcoM2 model, Progress in Oceanography, 168, 65-81,doi:10.1016/j.pocean.2018.09.006.

MEDUSA model: Henson, S. A., Cole, H. S., Hopkins, J., Martin, A. P., & Yool, A. (2018). Detection of climate change-driven trends in phytoplankton phenology. *Global Change Biology*, *24*(1), e101-e111.

NEMURO model: Anju, M., Sreeush, M. G., Valsala, V., Smitha, B. R., Hamza, F., Bharathi, G., & Naidu, C. V. (2020). Understanding the Role of Nutrient Limitation on Plankton Biomass Over Arabian Sea Via 1-D Coupled Biogeochemical Model and Bio-Argo Observations. *Journal of Geophysical Research: Oceans*, *125*(6), e2019JC015502.

SINMOD model: Alver, M. O., Broch, O. J., Melle, W., Bagøien, E., & Slagstad, D. (2016). Validation of an Eulerian population model for the marine copepod Calanus finmarchicus in the Norwegian Sea. *Journal of Marine Systems*, *160*, 81-93.

NPZD model: Gruber, N., Frenzel, H., Doney, S. C., Marchesiello, P., McWilliams, J. C., Moisan, J. R., Oram, J. J., Plattner, G., and Stolzenbach, K. D.: Eddy-resolving simulation of plankton ecosystem dynamics in the California Current System, Deep Sea Research Part I: Oceanographic Research Papers, 53(9), 1483-1516, 2006.

Especially in the abstract and the introduction there are several long (sometimes convoluted) sentences. To increase readability it would be could to rephrase these (Schachtelsaetze sind im Englischen nicht so hoch angesehen wie im Deutschen ;)).

We splitted the long sentences into shorter easier to read sentence in the revised version.