Interactive comment on “Competition for inorganic and organic forms of nitrogen and phosphorous between phytoplankton and bacteria during an *Emiliania huxleyi* spring bloom (PeECE II)” by T. Løvdal et al.

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Referee #1

Referee comment: The data presented in this paper feel a bit "over calculated"; and more complex than necessary. For me, it would help to also see some figures with rough ("primary")data before making the step to "secondary" parameters like "affinity", "turnover times", and "biomass specific affinity". For example: I find Fig. 4 far more understandable and interesting than the other Figures. The calculation of these "secondary" data includes quite a few assumptions and conversion factors and extra uncertainty due to the methodological limitations discussed at the end of the discussion.
In the end, the actual conclusions from these data are quite limited (see abstract). I wonder if these conclusions can also be reached in a more basic way (e.g. uptake rates and the related contributions of the different substrates to their summed uptake) and then make the step to affinity for the different substrates (and clearly explain what these parameters represent exactly).

Authors response: - We agree that the data presented in the original manuscript (ms) would be less complex if presented as uptake rates. There are, however, several reasons why we choose to present them as biomass specific affinity. First, reliable uptakes rates can not be obtained without knowing the exact ambient concentrations of the substrate in question. Chemical determination of bioavailable mineral nutrients (here NH4, NOx and PO4) was difficult because they where close to, and sometimes even below, the detection limit, and we do not know how large the fraction of SRP has been that really represents PO4. Previously, it has been cautioned against using chemically determined data to calculate fluxes of nutrients in aquatic environment (Dodds 2003). The ambient concentration of specific organic substrates was not measured. Second, single time point measurements (P-substrates) may be better suited for estimation of turnover times than for estimation of uptake rates. Third, competitive ability can not be assessed without taking biomass into consideration. We have decided to present our data as biomass specific affinity because the goal of the study was to explore the competition between algae and bacteria. The competitive ability of an organism (at low substrate concentrations) is characterized by its biomass specific affinity. Hence, we do not believe that clearer conclusions could have been reached by presenting the data as proposed by referee # 1. References (Thingstad and Rassoulzdegan, 1999; Løvdal et al., 2007) to explain the concept of biomass specific affinity have been included, not only in the discussion, but also in the introduction of the revised ms. We agree that the conclusions in the original ms have been limited because of lack of robustness in our estimates. In the revised ms, much effort has been put on validating the different conversion factors used, and also reducing the amount of such factors (See comments to Referee # 2). Changing biomass estimates did not change the confidence limits of
any statistical test reported in the original ms. More primary data have been included in the revised version of the ms (nutrients, phytoplankton counts, and bacteria counts and size). Chl a has not been used to estimate biomass in the revised ms so it is not included. However, Chl a concentrations will be presented in the paper by Engel et al. to be published in the same issue of Biogeosciences.

Referee comment: I think the use of uptake of a single amino acid (leucine) as a model for total DFAA or even total DON uptake is tricky. Different amino acids may have different uptake pathways and affinities. Also see comment below. In addition, data in Grossart et al. 2006 show that there was a large DCAA pool present, which is not even mentioned in the discussion.

Authors response: - We are aware of the fact that leucine uptake does not represent total DFAA or DON uptake, which has been in fact mentioned in the original ms (pg. 3361, l 2-5). We note that T[leucine] and affinity[leucine] values reported here are estimates of the ambient pool of enzymatically hydrolysable free leucine, and does not encompass all components of the DON pool. Additionally, hydrolysis of the DCAA pool may substantially increase the bacteria’s ability to utilize DFAA (including leucine) and hence N from the amino acids. This notion has been stressed in the revised ms. Free amino acids are however meant to be among the main sources of organic N to phytoplankton and bacteria (Berman and Bronk, 2003), and leucine, believed to constitute a major N-source in the marine food web, is often used as a model DON substrate (Paul, 1983; Billen, 1984).

Referee comment: Page 3345, line 4: Why use the term "osmotrophs" instead of just something like "bacteria and phytoplankton" as used in the title?

Authors response: - Changed as suggested.

Referee comment: Page 3346, line 25: Why talk about cyanobacteria in detail here while these are not relevant for the paper?
Authors response: - This section is deleted.

Referee comment: Page 3350 (M&M uptake 15N compounds): I think the occasion where extra (unlabelled) NH4 was added should be specified more clearly since this is where you measured potential NH4 uptake rates rather than true ambient uptake rates. Specify the NH4+ concentration measurement limit. Later in the paper (page 3353), it is mentioned that NH4+ concentrations were always <0.02 uM, indicating that the detection limit was 0.02 uM or lower. This means that addition of 0.5 uM extra (unlabelled) NH4+ was really high.

Authors response: - Extra (unlabelled) NH4 was added before each measurement of NH4 uptake. The detection limit for NH4 was 0.1 uM. We have clarified this point in the revised ms.

Referee comment: Page 3351, lines 4-5: "uptake rates were estimated from the regression relationship between uptake and time". How is "uptake" defined here and how was it calculated from at%15N?

Authors response: - The sentence should read; "Turnover times were estimated from the regression line between uptake of added 15N substrates and time". After addition of known concentrations of 15N the following calculations have been performed: The consumed fraction R(t) of added 15N (or radio-labelled P substrates) after an incubation time was assumed to follow the theoretical expression (Thingstad et al., 1993): R(t)=(1-e-t/Ta) (1), where Ta=(Sn+Sa)/v is the turnover time in the sample with added and natural concentration Sa and Sn respectively; v is the velocity of the reaction. The amount of 15N ri in fraction i resulting from primary uptake (excluding secondary transfer between compartments due to grazing etc.) then follows: ri(t)=ai(1-e-t/Ta), where ai is the fraction of total uptake going into size fraction i. From experiments with single incubation times (P substrates), the turnover time Ta of substrate in the incubation bottle was computed from the rearranged Eq. (1): Ta = t/-ln(1-R). From experiments with multiple incubation times (N-substrates), Ta was computed using t and R as obtained from
the regression line. We believe that this is more accurate when measuring turnover times of substrates requiring longer incubation times and larger sample volumes.

Referee comment: Pages 3353-3354 (Results nutrients and bloom development): For me it would help to have the nutrient concentrations, bacterial numbers and Chl a concentrations in one figure or table for a good overview and direct comparison with the other figures. These data are now hidden in the text or need to be filtered from Grossart et al. (2006).

Authors response: - We have included figures showing nutrient concentrations, phytoplankton, and bacterial numbers (Figures 1 and 3). Chl a has not been used to estimate biomass in the revised ms, and hence is excluded.

Referee comment: Page 3355, line 25: In my opinion, Table 1 does not really add any additional interesting information that is not already included in Fig. 4.

Authors response: - Table 1 has been deleted from the revised ms.

Referee comment: Discussion: It may be helpful to structure the discussion a bit more. For example by dividing the discussions into subsections (with separate headers) that each deals with one of your research questions.

Authors response: - In the revised ms, the discussion has been structured as suggested.

Referee comment: Page 3359, line 11 to page 3360, line 4 and figure 6: To me, this section feels a bit too much and not essential/relevant for this paper.

Authors response: - This section has been substantially revised and shortened. We have not completely deleted it because it shows the theoretical reduction in biomass-specific affinity with cell size in algae and bacteria in diffusion limited environments, predicting the theoretical outcome of algal-bacterial competition under such conditions.

Referee comment: Page 3360: It makes more sense to discuss the various method-
ological limitations at the beginning of the discussion rather than at the end.

Authors response: - The Discussion has been revised as suggested.

Referee comment: Page 3360, line 20: How long did the filtration take?

Authors response: - For the measurements of 15N-uptake, the filtrations lasted 15-30 min. For 33P-uptake measurements, incubations were terminated by cold chase. Hence, incubation time in 15N uptake studies has been defined as incubation time + \( \frac{1}{2} \) filtration time in 15N uptake studies. This has been outlined in the revised ms.

Referee comment: Page 3361, line 4-5: The Ietswaart et al. paper tested individual amino acids as single N substrates. In that case, growth of the bacteria and algae is dependent on their ability to synthesize other AAs required for production of their biomass (proteins) from these single AAs. It is quite tricky to translate these results to a field situation where the available DFAA pool has a composition that is similar to that of the microbial biomass as thus requires only very little conversion, making it a much more efficient N substrate.

Authors response: - We agree that the study of Ietswaart et al. may not be applied directly to the natural environment, and this has been stressed in the revised ms. The statement made, however, is relevant because Ietswaart et al. show that leucine, compared to other amino acids, is efficiently utilized by both phytoplankton and natural assemblages of bacteria. Bacteria and phytoplankton are able to synthesize some amino acids by themselves, while other essential amino acids must be obtained from the surrounding media. There is no reason to believe that the different amino acids are equally energetically expensive to synthesize or to obtain. As pointed out by referee #1 (see above); "Different amino acids may have different uptake pathways and affinities". When different amino acids are incorporated into protein by the cellular machinery, they may be acquired in certain proportions relative to each other. If they are used as general N-sources, easily bioavailable amino acids may be preferred.
Referee comment: Page 3361: When you discuss DNA and ATP as potential N substrates, then DCAAs should also be discussed. Fig. 2 in Grossart et al. (2006a) shows high DCAA concentrations!

Authors response: - The DON fraction in marine environments is dominated by proteins, nucleic acids and humic-like substances (Berman and Bronk, 2003). An intriguing question is therefore as to which extent nucleic acids and nucleotides are utilized as N-sources. As far as we know, most researchers have used proteins and free amino acids as model substrates in N-uptake experiments. The point we want to stress, is that although phytoplankton seem to have a low affinity for N from leucine (traditional DON model substrate), they may have a high affinity for N from dDNA and ATP (traditional DOP model substrates), similar to that for P from dDNA and ATP. Therefore, the expected superiority of bacteria in the competition for organic N may not be as clear as we have previously thought. In this aspect, it may also be interesting to assess for example phosphoproteins as potential sources for both P and N. In the revised ms, we have added that DCAA are potentially important N sources, as the referee has correctly pointed out. However, in this context, the DCAA pool is not that interesting because it contains little P. Anyway, this closing section was merely meant as a perspective for future research.

All technical corrections have been included into the revised version of the manuscript.

On behalf of the authors, Trond Løvdal

References:


Interactive comment on Biogeosciences Discuss., 4, 3343, 2007.