Re: update manuscript Biogeosciences Discuss., 11, 6119–6149, 2014

Dear Editor,

Please find attached the updated manuscript. Our response to the individual points raised by each of the three reviewers has already been uploaded to the site of BGD. In the present revision we have taken great care to change the text, references and information as required. In particular, the following changes have been made, next to smaller changes in text and references:

We took the main comments ("Problems with the casting of the paper " and "these experiments can answer global questions is thus much too simplistic and needs to be toned down") into account by changing the title of the paper: "Disparities between in situ and optically-derived carbon biomass and growth rates of the prymnesiophyte *Phaeocystis globosa"*

In the abstract and in the discussion we now put more emphasis on the fact that this experiment shows results for *Phaeocystis* only. Together with more references to old and more recent work on the use of the fluorescence and quantum efficiency as proxy for growth, a more balanced message is conveyed by this manuscript.

We did not start an evaluation of all literature mentioned by the three reviewers and decided to stick with the results of our experiment and show the usefulness of optical proxies from remote sensing estimates of growth rate. In our opinion, most proposed literature is indeed a cornerstone of the present work and is mentioned. However, it seems that the field of fluorescence and interpretation is more complex than can be handled in this manuscript: For example, reviewer 3 refers to the work of Behrenfeld et al. (2009): for the claim that "the idea that the fluorescence quantum yield (φ_{ph} here) should tell you anything about growth rate or production seems far-fetched as well". We disagree with this interpretation of the article.

A second major change is that all three reviewers insisted on a better description of the analysis of the spectra and derived quantities, despite the fact that this was already published extensively in the LOM paper. We therefore have removed the info from the appendix and wrote a more extensive section 2.3 that covers the experiment, measurements and analysis of the spectra.

Finally, we changed the annoying mistake in the last Figure (now 7C).

We hope that by now the ms is satisfactory for publication.

Kind regards,

Hans van der Woerd
Klaas Timmermans
Louis Peperzak

Texel, 16-1-2015

0Q. We are willing to include references to the (old) literature provided by the referees (H1) and a few sentences in the introduction and discussion to put this experiment in more perspective.

Anonymous reviewer #1

General comments:

1Q. *This reviewer wants to know the question we want to address.*
A. It simply is: how applicable are potentially suitable proxies for remote sensing derived primary production estimates (4th paragraph of Introduction)? We do this by comparing such proxies directly with those derived from standard oceanographic techniques (1st paragraph of Introduction). This question is very much alive in the scientific world: see for example the work of Saba et al. (2010) "Challenges of modelling depth integrated marine primary productivity over multiple decades: A case study at BATS and HOT". *Glob. Biochem. Cycles*, 24: GB3020, doi:10.1029/2009GB003655.

2Q. *I do not know of any proposed application of φ as a measure of growth rate nor of anyone using it as such (the authors do not cite appropriate literature for their statement).*
A. We may not have cited all available literature and there may be misunderstanding about the wording of the ms (we do not say φ is equal to Fv/Fm, we say it is equivalent i.e. as a physiological proxy and show this is so with data) but in our view the goal or casting of the paper is clear. We rewrote this section, including some more literature, see letter to the editor.

3Q. *Methods are extremely succinct and refer extensively to a previous paper.*
A. In order to provide a recipe for other scientist to replicate this type of experiment, we have published a detailed description of the set-up and processing of optical data in a separate paper in L&O Methods. More information of the LOM paper is now included to make the BG article more comprehensive.

4Q. *How was the Fv/Fm measurement carried out?*
A. The Fv/Fm measurement was made on a dark acclimated sample for >20 min.

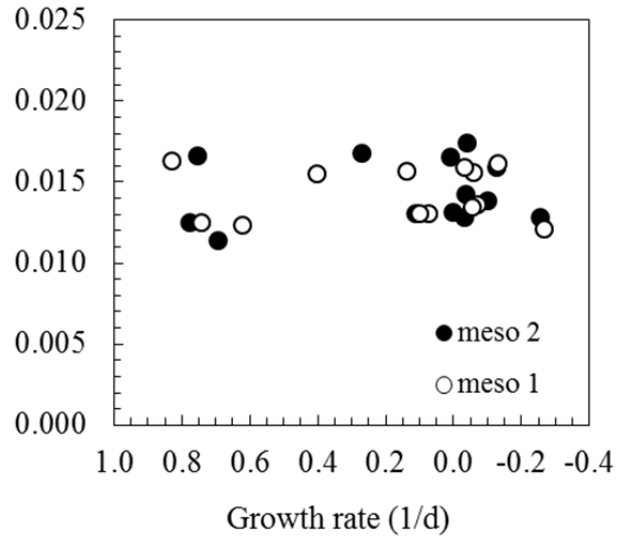
5Q. *In section 3.1 the authors skip over the observation that after day 8, both mesocosms (not only mesocosm 1 where nutrients were added) showed an increase in POC while every other measurement decreased in mesocosm 2.*
A. Carbon fixation might have taken place because Fv/Fm was still >0.4 Fig 2A), not very low as suggested by the referee. Another explanation might be heterotrophic growth (bacteria) but this is unlikely to be significant (see answer to reviewer 2, specific comment 5).

Specific comments:

6Q. *Two different symbols for the fluorescence quantum efficiency*
A. The fluorescence quantum efficiency should always be represented by the symbol φ (See table 1). We will correct this.

7Q. *To me Figure 5 is probably the most interesting result in this paper showing a relatively constant quantum yield even under starvation (nutrient enrichment not having a strong effect).*
A. Thanks for this constructive comment. We will address this point in the discussion with reference to some of the suggested literature.

8Q. *P.6131, line 11. It is not clear at all what the loss of correlation correspond to? There is to my eye a non-linear and fairly nice relationship between □ and growth rate. I note, however, that the quantum efficiency values presented in Figure 6 do not match the values presented on Figure 5.*
A. Sorry for this: we made a mistake. Figure 6C (now 7C in revised ms with φ in %) should be the following one (note: y-axis values are to be multiplied by 100% to match description in text).



Anonymous reviewer #2

General comments:

9Q. *I have to question if the term "mesocosm" is an accurate description of this approach?*
A. The definition is different for the various scientific disciplines. Toxicologists name 1 L beakers with monocultures mesocosms; we use a larger volume. The reviewers of the LOM article had no problems with the term and we define the chosen name.

10Q. *The results are for a single species only.*
A. Correct, our mesocosm is not representative for a natural system. Instead we aimed to reach a controllable experimental set-up that would allow careful comparison of optical and chemical measurements. Note the word "potential" in the (previous) title.

11Q. *The experiment is a short-term batch culture in which nutrient conditions are constantly changing.*
A. Likewise, our mesocosm is not a 'real environment' but an excellent controllable experimental set-up. Nutrient conditions were NOT constantly changing. For the growth-limiting nutrient DIN: it was either replete (indeed declining) or depleted (and spiked once).

12Q. *In my opinion, the authors need to be more realistic about the usefulness of their study for real world applications.*
A. We simply warn that non-linear changes in optically *and chemically* derived growth rate proxies take place, and because optically derived proxies must be used for global carbon production estimates such estimates may be less accurate as we would like (again: note the word "potential" in the previous title). Text has been edited.

13Q. *There is a lack of information provided on cell size and morphological characteristics observed during the experiments*

A. This isolate did not form colonies. Cell size was checked for every sample by the forward- and side-scattering characteristics, as measured by the flow cytometer.

Specific comments:

14Q. *Fv/Fm and φ are NOT equivalent*.
A. Agree. See answer to reviewer 1.

15Q. *More information on the methods must be included to better understand the results.*
A. More Information from the L&O Methods paper is included (see also answers to reviewer 1).

16Q. *The above inquiry regarding mesocosm depth and clarity of the enclosure sides relates to the calculation of "absorption" by phytoplankton.*
A. The reviewer is completely correct that great care must be taken to calculate the absorption spectra of *Phaeocystis*. Water samples were measured with the Integrating Cavity Absorption meter from Hobi Labs. RAMSES irradiance measurements were made at the top and bottom of the mesocosms to monitor the spectral attenuation in the mesocosm. These measurements were combined with a simple spectral attenuation model that incorporates aspects like CDOM absorption and optical thickness. Model and measurements proved to be very robust (see Figures 6 and 8 in the L&O Methods article by Peperzak et al. (2011). Relevant aspects of the methodology were added to the new manuscript.

17Q. *The calculation of phytoplankton quantum efficiency, φ , here and in the appendix needs further elaboration.*
A. See previous answer. The method for calculation is described in great detail in the L&O Methods article, including baseline correction, integration of angles, wavelengths and correction for the re-absorption of emission and air-water interface.

18Q. *All estimates of POC and PON used in growth rate calculations assume that no bacteria or other microorganisms exist in the cultures. Was this verified?*
A. Flow cytometer diagrams did not suggest the presence of other phytoplankton species. We judged the bacteria concentrations as low. The seawater used originated from the Atlantic Ocean, DOC concentrations (as source for heterotrophic growth) are expected to be low.

19Q. *I have a difficult time keeping track of which measurements are used in different calculations and why.*
A. This remark is related to the previous request for more details on the absorption and quantum efficiency calculations. We fully agree that our set-up and calculations should be transparent and reproducible, but hoped to have solved this in the L&O Methods paper. Clearly, from the remarks by 2 out of 3 reviewers we failed and we have taken great care in the updated paper to clarify all these quantities.

20Q. *I do not understand the utility of normalizing POC to Chl a+c.*
A. As is explained in the manuscript, both forms of chlorophyll were present in the same order of magnitude. Adding them increased the precision of the data.

21Q. Fig. 3. Please specify the wavelength range over which these absorption measurements correspond.
A. Reflectance was measured from 400 to 750 nm. The absorption algorithm wavelengths are now given in the text.

22Q. How can the fluorescence emission F be in irradiance units? To be consistent with the described derivation and Table 1, the units should be sr−1.
A. Yes, sr^{-1} is correct.

Anonymous reviewer #3

General comments:

23Q. *The additional change in light environment alone (due to accumulation of chlorophyll biomass during the experiment; Chl goes from ~0 µg/L to ~30 µg/L) would be enough to drive patterns in any of the physiological quantities (e.g., Chl:C). Given this, why is it surprising that none of the physiological parameters correlate with the growth rate?*
A. Thank you for this constructive comment and suggestions for literature that could shed light on our experiments from a different perspective. We noticed in our experiments that rapid changes occur in absorption when N is depleted or spiked and therefore considered nutrient conditions the main driver. Changes in average light conditions are smaller, but still can be important. We will check calculations and suggested literature and include this aspect. PAR up + PAR bottom/2 number Figure 7 LOM article. Indeed, Chl increased in the exponential growth phase but at these µ>0, no clearly discernible trends in the physiological proxies were observed (Figure 6 a-c), indicating that the change in Chl *did not correlate* with growth rate. *New text in discussion.*

24Q. *Why is no attention given to limitation by iron?*
A. A minimum level of Fe- concentration was established by the addition of Fe (1 µM) to the seawater. Concentrations are given in the L&O Methods article and are not growth limiting. Proof can also derived from the fact that the N-spike gave immediate response in mesocosm-2.

25Q. *What is new: if you set aside the optics for a moment, then all we are looking at is a short time-course batch culture and its response to nutrient addition.*
A. Exactly, but we did not “set aside the optics for a moment”. Surprisingly, this type of experiments, where simultaneous measurement of optical and chemical proxies are made are few. This is innovative. See also the general comments in the letter to the editor.