

## ***Interactive comment on “A $^{13}\text{C}$ labelling study on carbon fluxes in Arctic plankton communities under elevated $\text{CO}_2$ levels” by A. de Kluijver et al.***

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We thank the reviewer for the time to review the manuscript and the comments that helped improving the manuscript. The suggestions and comments raised are answered below.

General comments:

Referee: This is a very interesting study that aims at describing carbon flow in a planktonic food web during a mesocosm study with  $\text{CO}_2$  perturbation. The study uses  $^{13}\text{C}$  organic and inorganic carbon pools (DIC, DOC, POC) to follow the transfer of  $^{13}\text{C}$  added to the mesocosm system at the start of the experiment.  $^{13}\text{C}$  labeled biomarker (polar lipid fatty acids) were used to discriminate between plankton groups from bacte-

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ria to zooplankton. Results obtained from the  $^{13}\text{C}$  analyses are compared to a NPZD-model, the technical details of which are described in a separate manuscript (van Engeland et al, BGD, submitted). It has to be acknowledged that the authors attempt to draw a holistic picture of the very complex development of a planktonic ecosystem. However, the  $^{13}\text{C}$  approach as well as the applied model include many assumptions and derive knowledge from an indirect approach. The study would clearly benefit from a more detailed comparison to data on carbon cycling and ecosystem development that were directly obtained during the same mesocosm study (e.g. Leu et al., Czerny et al., Brussaard et al., Engel et al., Piontek et al., Niehoff et al.). I therefore cannot recommend publication of the present study in BG without a major revision.

Reply: Most of these papers were not available to us at the time of submission. Now that they are available, we will extend the comparisons and add them to strengthen our discussion. The comparison is not always straightforward because our data were directly obtained from the mesocosm study, whereas some of the other data relate to mesocosm water incubated outside the mesocosm under modified conditions. Moreover, some of these studies focus on concentration measurements/stock assessments while we directly quantified transfer of  $^{13}\text{C}$ : i.e. a flux. In addition, most of the studies focus on the period after nutrient addition, while we focus with the model on the period prior to nutrient addition. An elaborated comparison of the  $^{13}\text{C}$  method for total (particulate) primary production to other methods ( $^{14}\text{C}$ ,  $\text{O}_2$  production, DIC budget) is presented by Tanaka et al. and Engel et al., both in this issue.

Detailed comments:

Referee: Introduction: the authors should give more information on the suitability of PLFA as biomarker in general and for the chosen taxa in particular; what is the variability of conversion factors applied to calculate organic carbon from PFLA biomass? Since the chosen groups Phyto I and II include a variety of species, I would assume that the PFLA:OC ratio is highly variable. How is this accounted for when estimating production rates?

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Reply: We agree with the reviewer that PLFA:OC conversion to biomass (as with any other marker) has the potential to introduce systematic errors. We assumed a constant PLFA:OC conversion for phyto I and phyto II, so we didn't account for variability in PLFA:OC ratios among phytoplankton groups. However, the conversion is rather constrained, because phytoplankton biomass cannot exceed POC. Moreover, PLFA:OC ratios are rather constant because PLFAs are structural components and not functional (Chl a) or reserve compartments that are more variable and depending on environmental or physiological conditions. We will add a small introduction on the use of PLFA as biomarkers and a discussion on the uncertainty in conversion factors and the effect on biomass and production rates. We note that the conversion factor do not affect the temporal dynamics in  $^{13}\text{C}$  biomass and production rates for the different phases and  $\text{pCO}_2$  levels, but only the amplitude (absolute values). We don't agree that considering larger groups of phytoplankton increases the error in conversion factors, but rather reduces them (similarly as chlorophyll a). None of the markers are unique for taxa, making it difficult to attribute taxa to one species and there can also be variability in taxa due to biological and environmental conditions.

Referee: Results: The authors observed no  $\text{CO}_2$  effect on Phyto I including autotrophic dinoflagellates (as derived from PFLA). Leu et al (same issue) observed a positive effect on autotrophic dinoflagellates when using polyunsaturated fatty acids; how can this be explained?

Reply: Although we both analyzed fatty acids, they were applied in different ways: we used it to quantify  $^{13}\text{C}$  uptake in phytoplankton and bacteria and they used it to study food quality. The results should not be compared directly, since 1) Leu et al. (this issue) looked at the relative composition of fatty acids (%), while we looked at absolute amounts and 2) they looked at total fatty acids, while we looked at polar lipid fatty acids, 3) the markers they used for dinoflagellates were presented in the phyto I and phyto II in our study (due to different labelling patterns). If we look into relative distribution, an increase in these markers is also seen, which we explain as a shift from mixotrophy to

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autotrophy (see discussion in the discussion paper).

Referee: Page 8583, line 10-13: DOC production was  $<0.06 \mu\text{mol C L}^{-1}$  but estimated to be  $<6.2 \mu\text{mol C L}^{-1}$  during the first 11 days and  $<11 \mu\text{mol C L}^{-1}$  during d14-28: According to the estimated production rate, DOC production during the two phases should be much lower, shouldn't it?

Reply: The reviewer is correct that the values mismatch. The production rates were calculated from linear regression and showed very little changes in  $^{13}\text{C}$ -DOC. The latter (cumulative production) values presented a cumulative build-up of  $^{13}\text{C}$ -DOC, which had rather high concentrations due to high background concentrations of DOC. If we only look at the cumulative change, the sum would match with the low production. Because both values likely represent under- and overestimations of DOC production, this section will be removed from the revised manuscript

Referee: Discussion: page 8590 line 16-17 'The addition of nutrients did not increase phytoplankton and bacterial biomass in the mesocosms.' This statement seems to be inconsistent with the findings of Leu et al, Brussaard et al., Czerny et al., (BGD, same special issue). Again, a more direct comparison to other data of the same study would be necessary.

Reply: We see a similar pattern of build-up, decline and build-up again as shown with the other manuscripts, but the maximum concentrations of phytoplankton and bacterial biomass were similar before and after nutrient additions. Most of our findings are consistent with the other methods or differences can be explained, and a comparison will be added to the manuscript. We note that Leu et al. (this issue) looked at fatty acid composition (relative abundance) and not at absolute concentrations of fatty acids.

Referee: Model: I do not see the benefit of this model in the present study. The assumptions of the model seem to conflict with the scientific findings of the study (e.g. constant bacterial biomass, no substrate limitation). The sensitivity of the model towards variations in parameter setting was not tested. Moreover, the use of a fixed

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stoichiometry to derive nitrogen fluxes from carbon fluxes surely is inaccurate (see Silyakova et al., same issue) and falls short on the actual data on nitrogen cycling obtained in the frame of the study.

Reply: Even though models by their very nature are approximations of reality, there is a clear benefit of using a model to analyse data. The model was used to quantify parameters and fluxes. The model doesn't show CO<sub>2</sub> effects that weren't already present in the data, but only connects the data. The assumptions of the model were used for simplification and do not influence the <sup>13</sup>C uptake patterns, which determine growth parameters. Bacterial biomass based on fatty acid biomarker concentrations showed little variation during the first phase (Fig. 1 of our manuscript). Sensitivity of the model toward variations in parameter settings is described in the accompanying manuscript of Van Engeland et al. (this issue) and this sections has been extended in their revision. In the accompanying MS, it is described that the sensitivity of the model for half-saturation constants was low and that varying C:N ratios also showed to be of little influence on carbon fluxes. We will add a short section and reference to the revision. We note that our study focuses on carbon cycling, for which we added a tracer and not on nitrogen cycling, for which data were limited. So nitrogen data were only used to constrain the carbon flow model and we don't intend to adequately describe the nitrogen dynamics. We note that Silyakova et al. (this issue) focus on the period after nutrient additions and ignored organic nutrients. Moreover, particulate organic matter had a stoichiometry close to Redfield (Schulz et al. this issue).

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