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## ***Interactive comment on “Effect of ocean acidification on the fatty acid composition of a natural plankton community” by E. Leu et al.***

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Answer to general comments of both referees:

The presented article is part of a special volume dealing with the complex responses of a natural plankton community to ocean acidification under Arctic field conditions. In this given context it was unavoidable to refer to other publications in preparation /submitted that are part of the same volume. All of these references are now online, and will be published along with our own manuscript. Also, as fatty acid data of a natural community cannot be understood by themselves without the necessary background information on taxonomic composition, nutrient limitation, and other characteristics of the investigated community, it is essential to include data from other participants in this publication for interpreting the observed results in fatty acid composition. This has

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been the intention of the study from the start. Due to the size and the complexity of the experiment it is also self-evident that not a single (small) group of authors can have the ownership of all these data. Criticism directed towards the general experimental setup (30 days duration, lack of replicates - see referee #2) is common for all manuscripts in this special volume and should be addressed in the discussion of the contribution describing the experimental setup (Riebesell et al. , subm.). The mere amount of original data presented in the current article is rather large, as we analysed the community fatty acid composition every other day during the critical phase of the experiment (see extensive tables in the online supplementary material). There are not many studies I am aware of that show such a high temporal resolution for such a long experiment with respect to fatty acid data. Since we furthermore see good correlations between the taxonomic and biochemical changes occurring during the course of the experiment, we are also convinced that the duration of the experiment addresses an appropriate time frame of interest for investigating phytoplankton responses towards increased pCO<sub>2</sub>. The shortcomings in the description of methodological details as commented on by referee # 2 will be clarified in more detail in the revised version. The discussion will be partly re-written in order to focus more on the CO<sub>2</sub> effects on plankton fatty acid composition.

Answers to specific comments by referee #1: - Cbm will be changed to m3 - We will mention in which mesocosms CO<sub>2</sub> was not added (3 and 7) - The statement about the 'nanoplanktonic community utilizing predominantly organic nutrients' was based upon findings from other contributors of the experiment, and will be confirmed by cross-references to the respective papers demonstrating this - Criticism about cross-references in Results chapter: see answer to general comments above! - P.8181, l. 22: 'the C18 n3 PUFAs' will be replaced by '18:4n3 and 18:5n3' - L. 25: we will start a new paragraph with 'The overall PUFA content. . .' - Discussion: we will replace POM with 'planktonic community' - Discussing the characteristics of the blooms that developed during the experiment based upon information we obtained from other manuscripts within this special volume is essential for interpreting our results and was intended to

be like this - The discussion will be re-structured, with a greater emphasis directed towards the aim of the study (relating the community FA composition to different levels of pCO<sub>2</sub>) - The conclusions will also be modified in order to stay in line with that - Tables: will be modified according to suggestions - Figs: Fig. 1: Chl<sub>a</sub> values from the fjord will be removed as they are not discussed in the current ms - Fig. 3: we believe that the size of the stars should be sufficient, therefore, no colours are necessary in this figure - Fig. 2/4: the colour coding for the different treatments was, of course, meant to be consistent. We will change Fig. 4 so that low CO<sub>2</sub> treatments are shown in blue, and intermediate in grey

Answers to specific comments by referee #2: - The ecological significance of heterotrophic PUFA production is extremely small, as it is much more favorable for them to take up those metabolites via their diet. - Comments on general experimental setup should be clarified by the Riebesell et al. ms in the same volume. - The detailed description of how samples were obtained will be added to the ms: For each sample 3 L of seawater were filtered on a pre-combusted GF/F-filter (Whatman), that was immediately put into a 8 mL glass vial filled with dichloromethane:methanol (2:1, v/v), and frozen at -20 °C. – No replicate within one mesocosm was measured, as this would have been pseudo-replication only. - The phrase 'Most other parameters' (in: most other parameters were sampled daily (as presented in the other articles of the same special volume) refers to Chl<sub>a</sub>, particulate stoichiometry, flowcytometry... However, since fatty acid composition samples require a rather large volume, daily sampling of this parameter was not feasible. - Use of 23:0 as internal standard: Certainly this FA could be found in some traces in water samples, however in growin phytoplankton cultures, the total amount of this fatty acid is not really important. Generally, both external and internal standards, could be used for the quantification of analytes. We compared the area/amount of our internal standard with the area of our analytes (fatty acids), in order to gain a specific amount of a component in mg. - We carried out linear regression analysis as we consider the response in fatty acid composition to be the dependent variable, while pCO<sub>2</sub> constitutes the independent variable. Still, results

from this type of analysis give us information about whether or not the variability in the dependent variable could be (significantly) explained by the independent variable – in this sense we used the word ‘correlated’ in the description of our results. If necessary, however, we can try to re-phrase parts of this description in order to emphasize clearer which type of analysis was used. - The three phases (as defined under statistical analyses) were based upon the observed development of three distinct bloom events during the experimental period (see fig. 1). - The PCA was run on the entire fatty acid dataset (including all FAs > 1%, corresponding to the tables shown in the appendix); however, due to reasons of clarity, only selected fatty acids were displayed in the plot. The description of this procedure will be added to Mat&Met - Cross-references to results from the same study (same special volume) cannot be avoided completely (as explained before), but will be kept to a minimum - Development of 16:0 over time will be described with more caution – we meant to say that there was no clear trend visible over time. - The scales of the figures was chosen to show the occurring changes in the best possible resolution - Table 1: 3 and 7 were control mesocosms – this info will be added - Table 2: As we encountered different situations in the three different phases of the experiment, we decided to do linear regression analyses for each of the three distinct phases separately. Therefore, the dependent variables were averaged for these periods. By comparing means of a certain phase we ask the question if the relative (or absolute) amount of a specific fatty acid was on average higher or lower in one treatment than another during this specific phase. - Table 2, cont.: we will remove R2, slope and intercept for the not-significant regressions - Fig. 2: we will remove n3 fatty acids from the plot, as they are part of the overall PUFAs. - Discussion: most problems mentioned by the reviewer again are related to the issue of cross-referencing to other articles from the same study (dealing with CHEMTAX- and particulate stoichiometry results, including methods for Cpart analyses). - Discussion, cont.: method description for analysis of cirriped larvae fatty acid composition will be added to methods section - Linguistic improvements as suggested in the last paragraph of referee#2 will be made

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