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Interactive comment on "Increasing addition of autochthonous to allochthonous carbon in nutrient-rich aquatic systems stimulates carbon consumption but does not alter bacterial community composition" *by* K. Attermeyer et al.

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Dear reviewer #1, we thank the reviewer for his/her valuable comments on our manuscript and appreciate that s/he finds the manuscript informative. We now have carefully revised the manuscript and considered all comments. We hope that our revised manuscript sufficiently addresses all the comments and critiques and is now suitable for publication in Biogeosciences. In the following, we give a detailed, point-by-point response to all comments and remarks. Our respective changes in the revised manuscript are given below. The central issues raised by Reviewer #1 relate to the fact

that the study rests to a large extent on mimicking "something that is happening in nature - pulsed inputs of phytoplankton derived DOM as blooms crash for example.". We agree with the Reviewer #1 that it may be problematic to claim that our study is mimicking the nature. In the revised manuscript we excluded this transfer (see also comments below).

Comment 1) This manuscript presents and ambitious attempt to understand how bacterial turnover and community structure responds to pulsed additions of fresh autochthonous organic matter. The manuscript is data rich and a large number of methods have been applied. In general, the paper is clearly structured and the language reads well.

Response 1) We appreciate that the reviewer emphasizes our extensive data set and applied methods in the manuscript.

Comment 2) The overall aim is to mimic something that is happening in nature - pulsed inputs of phytoplankton derived DOM as blooms crash for example - and to study how the bacteria respond to that. I see two problems with this. First, it is to a large extent descriptive. I lack some clearly stated hypothesis that can serve as a backbone throughout the paper.

Response 2) To render our manuscript less descriptive we reformulated our hypotheses and completely reorganized the discussion accordingly. Below we provide the new final section from the introduction including our redefined hypotheses: "We aimed to evaluate the effects of fresh allochthonous and autochthonous DOC on bacterial decomposition and BCC, and to elucidate the degradation of distinct DOC fractions by natural bacterial communities. We incubated the DOC sources in (1) single source incubations and (2) mixed treatments with a fixed amount of leaf leachates (DOCleaf) and added different concentrations of an algal lysate (DOCphyto) together with bacterial communities from the littoral zone of a temperate shallow lake. We hypothesized that (1) both sources irrespective of origin are efficiently decomposed by the bacterial

community. Further, we expect (2) increased DOC degradation with increased addition of DOCphyto and that (3) mixing both C sources results in a shift in BCC."

Comment 3) Secondly, I do not think that the experiments mimic natural conditions well.

Response 3) This is a good point and we agree that our set up is not mimicking all natural conditions very well. However, we believe that the used leaf leachates and autochthonous DOC source are representative DOC sources in freshwaters. To better account for the rather "artificial" lab conditions, we stepped back from the direct transfer of our data to the natural lake conditions. Further, to underline the importance of leaf leachates, we added a more detailed and extended introduction to the revised manuscript (first section in the introduction): "Allochthonous C inputs to aquatic ecosystems can vary in time and quality, e.g. particulate or dissolved forms (Carpenter et al., 2005). In forested watersheds, substantial amounts of leaves can fall into lakes and dissolve into the water column (Gasith and Hasler, 1976; France, 1995; Vander Zanden and Gratton, 2011)."

Comment 4) The allochthonous DOM is made out of leaf leachates that are highly labile to bacterial degradation, but rarely enter the aquatic environment. What enter the aquatic environment are the leftovers after the soil microbes have utilized what they can.

Response 4) How much the allochthonous DOC has been re-mineralized by the soil microorganisms greatly depends on the environmental conditions and its residence time in the soil. In our study, we wanted to concentrate on the leaf litter and its leachates (DOCleaf) which directly enter aquatic systems. Therefore, we provide further information showing the importance of leaf leachates (DOCleaf) in aquatic systems: Inland waters, including the shallow lakes we studied, are often in forests or surrounded by alder trees and the amount of freshly fallen leaves can be substantial. That was also described by Gasith and Hasler 1976 (Limnol. Oceanogr.), France 1995 (Freshw. Biol.)

and Vander Zanden and Gratton 2011 (Can. J. Fish. Aquat. Sci.) who found a negative correlation between terrestrial particulate OM input and lake size. A more recent study by Cottingham and Narayan (2013, Ecosphere) also focuses on the direct release of leaf leachates and their turnover in lakes. These authors are talking about "airborne" fluxes including freshly fallen leaves which can leach fresh DOCleaf into the water column. We now included these references and a better description of the lakes in the manuscript.

Comment 5) Also, I am guessing that there was bacterial growth in the phytoplankton cultures that were used to provide autochthonous DOM. If the research question was not so focused on natural conditions, this would not be a problem. I do think this data can answer some relevant question, but I think it requires a retake on the study as a whole.

Response 5) The phytoplankton cultures were indeed not axenic and s/he is absolutely right with the assumption. Thus we have added this important detail to the description of the phytoplankton DOC (now DOCphyto) production to the method section. In our opinion, this way of culturing is more natural than axenic cultures which do not exist in nature. Autoclaving killed all bacteria and prevented the DOC from further microbial changes or utilization before the amendment. We adopted this approach for supplying an autochthonous DOC source from the literature. For example, Kritzberg et al. 2006 (FEMS Microbiol. Ecol.) used a similar procedure to extract phytoplankton DOC and also leaf leachate. Farjalla et al. 2005 (Microb. Ecol.) did not autoclave, but used a freeze and heat method for DOC extraction. Therefore, we added these citations to our now more detailed description of the procedure. Altogether, these are the reasons why we think our approach is not purely artificial and hence suitable to use for such kind of experiments.

Comment 6) Line 15 in introduction. This reference is not a good one to support that there is increasing amounts of terrestrially derived DOM in freshwaters. It is an experimental manipulation.

Response 6) According to his/her suggestion, we replaced Monteith et al. 2007 by Evans et al. 2006 (Glob. Change Biol.) and Hansson et al., 2013 (Ecology) , both manuscripts indeed provide more field data.

Comment 7) Line 21 Only bacteria and some osmotrophs can directly use DOM.

Response 7) Thanks for this hint. We reformulated this sentence: “DOC serves as an important substrate and energy source for heterotrophic bacteria in pelagic systems (Azam et al., 1983).”

Comment 8) Line 22 There are other processes that are important in controlling DOM turnover, and other factors than concentration and quality that influence bacterial DOM degradation. I don’t think it is necessary to point that out in the text, but you cannot write like this. I don’t really agree with your interpretation of Langenheders paper, and that has relevance for how you interpret your own data too. That the source of the inoculum is more important than the DOC sources, does not meant that the latter is NOT important.

Response 8) We thank the reviewer for his/her valuable remarks. We totally agree and revised this section about BCC in the introduction and deleted the Langenheder et al. paper. The reformulated section is: “DOC serves as an important substrate and energy source for heterotrophic bacteria in pelagic systems (Azam et al., 1983). Metabolic activities of the pelagic bacterial community thus can control DOC turnover, which in turn, can be inter alia determined by DOC concentrations and quality. Although it is well known that bacterial production positively correlates with DOC concentrations (Cole et al., 1988), little is known how the ratio between allochthonous and autochthonous C sources and the chemical quality of DOC influences bacterial activities in aquatic systems. In addition, the bacterial community composition (BCC) may also be crucial for DOC turnover. It has been suggested that DOC as a C source can act as a strong control of BCC (Judd et al., 2006; Docherty et al. 2006). In marine systems it has been shown that different phylogenetic groups consume specific dissolved organic

compounds (Cottrell and Kirchman, 2000). Van Hannen et al. (1999) and Grossart et al. (2007) related changes in BCC to organic matter quality. Therefore, different DOC sources can support different bacterial communities but the knowledge about the effect of mixing different DOC sources on BCC remains equivocal. To our knowledge there is just one study that evaluated the response of a natural bacterial community to the mixing of DOC sources from different origin (Fonte et al., 2003).”

Comment 9) Page 2 line 18. I think this is not a relevant reflection - how could they be.

Response 9) We deleted this sentence.

Comment 10) The experimental design is well explained by figure 1! The way you have designed the experiment makes it difficult to separate the effect of DOM quantity versus quality, since you change both at the same time.

Response 10) We are pleased that the reviewer found the experimental design well explained by figure 1. Thus, to better distinguish between DOC quantity and quality we performed an additional pulse experiment (see Appendix A). In this experiment, we have supplied only one source which allowed us to following exclusively quantitative effects. We measured an increase in DOC decomposition with increasing DOC concentrations. This is in accordance with the main experiment.

Comment 11) Please state explicitly what is the purpose of the controls. Is "control" really the correct term here, I wonder.

Response 11) This is a good point and we changed the word “control” to “single source incubations”. The “single source incubations” were done to reveal differences in the utilization of the single DOC sources (comparison DOCleaf vs. DOCphyto) and to reveal differences in BCC between “single DOC source incubations” and mixed treatments. These two important points we have also incorporated into our reformulated hypotheses. Please see our answer to the first comment.

Comment 12) Also, I think it would be more clear if inoculum refers only to the addition

of bacteria and that addition of DOM is called "amendment" or similar.

Response 12) We changed it accordingly.

Comment 13) The manuscript is rich in acronyms. I can see it is required, but make it easier for the reader to follow along by reminding us what they mean in results and discussion sections.

Response 13) We kindly acknowledge this comment and introduce the used abbreviations in every section separately.

Comment 14) I think the quality analyses of the two DOM sources illustrates very clearly that this is not mimicking natural conditions.

Response 14) We changed this direction of the manuscript and deleted the part where we state that this set up is mimicking natural conditions. Please see also our answer to the second comment. We now describe that these sources represent examples from a variety of allochthonous and autochthonous sources and we intend to exemplarily show the interactions between these two DOC sources.

Comment 15) There are many clever graphs, but I lack a basic one where one can actually follow DOC loss over time. It is fundamental to the understanding of this paper.

Response 15) We are pleased that s/he likes our figures. And we agree that the illustration of the DOC loss is hard to see in Figure 3. We now split this figure into two graphs for separately demonstrating the total DOC loss and the quality analysis. See below: See the uploaded "Fig. 3 (new)". New legend: Percentage of DOC decomposed (A) and inoculated and final (t12) dissolved organic carbon (DOC) fractions (B) in leachate (L) and phytoplankton (P) single incubation and mixed treatments (1-4). According to Huber et al. (2011) the fractions are humic-like substances and building blocks (HS), low molecular weight substances (LMWS), and high molecular weight substances (HMWS). In A we plotted the mean $\pm$ standard deviation for the DOC decomposition.

Comment 16) I don't follow in the section 3.4 what data you have and don't have. This makes it hard to judge the conclusions made on this data. I don't understand how you separate what is being respired and what is used for biomass.

Response 16) This section now includes the information about the data we have for both (assimilation & respiration) analyses. The distinction between respired vs. assimilated C was done with two different methods, but both used the stable isotope approach (keeling plot for respiration & PLFA analysis for assimilation) and subsequent calculation of two separate mixing models. This is now described in the method section in more detail. We added some further detailed information and references concerning the methods used. "Unfortunately, we were not able to determine the stable C isotope composition of dissolved inorganic carbon (DIC) for each treatment due to methodological problems (two are missing in treatments 1 and 2, and one is missing in treatments 3 and 4). In these samples we did not measure a decrease in DIC concentration and could not calculate the Keeling plot."

Comment 17) A general comment for the results and discussion is that it is very data rich and since it is not focused around clearly stated hypotheses it becomes very descriptive and it is hard to see the context. What is the theory that you want to test?

Response 17) Please see our comment to the first critique. We focused our discussion on three new hypotheses (see answer to first comment) and reduced the data given in the results section.

Comment 18) The discussion is to some extent a deeper presentation of the results. I lack the connection to clear questions/hypotheses and reference to literature in large parts. The discussion is not focused around the question you introduced in the introduction, but discusses results that are not strongly related to that question. For example, the effect on bacterial community is 10 lines in the discussion and lacks references.

Response 18) We fully acknowledge this comment and kindly point to our answer to

the first comment. We changed the BCC discussion and added more references.

Comment 19) Discussion line 10. This is really a non-statement. Be specific.

Response 19) To avoid any misunderstanding, we deleted the whole sentence.

Comment 20) Line 16. This relates to aging and is not applicable here. I guess you have fresh allochthonous DOM but aged autochthonous DOM? If there was bacterial growth in the phytoplankton cultures?

Response 20) Thanks for the remark. We deleted the sentence about the size-reactivity continuum model from Amon and Benner here. However, we do not agree that the autochthonous DOC is aged. Although there was bacterial growth during the culturing, the algae were still actively growing, and the newly produced phytoplankton biomass was not processed by the bacterial community. Thereafter, we autoclaved the culture to preventing bacterial organic matter transformation. The ultrasonication further extracted C and the subsequent filtration eliminated any bacterial contaminants. Thus, the final DOCphyto was not in contact with any bacteria before inoculation. Therefore, we believe that both DOC sources can be regarded as “fresh”.

Comment 21) Line 25 "higher bacterial DOC degradation" - what do you base that on?

Response 21) Here we meant a higher percentage of DOC consumption. This point is better visualized in the revised figure 3, which we changed according to the suggestion. However, we decided not to point too much on such small differences as they were not significant and the sentence is now: “The total DOC consumption and bacterial growth efficiency (BGE) was very high suggesting a high availability of the phytoplankton lysate as well as the leaf leachate.”

Comment 22) section 4.2 line 10 "the microbially unprocessed and thus bioavailable DOC was related to DOC quantity" - I find this confusing.

Response 22) We revised this sentence. “Although increasing DOC concentrations are generally not related to higher bacterial DOC consumptions (Basu and Pick, 1997), in

our experiment the consumption of the DOC sources increased with increasing DOC-phyto additions (Fig. 3).”

Comment 23) 4.4. The results on selective utilization bears strongly on findings in Kritzberg et al. 2005 AME and Kritzberg et al. 2006 FEMS and the group around Paul del Giorgio (McCallister) has also studied this.

Response 23) Thanks for these references. We included the literature in the BCC discussion as follows: “However, BCC shifted when both sources were mixed in the treatments. A greater diversity of compounds due to the combination of two or more DOC sources can change BCC (Findlay et al., 2003; Carlson et al., 2002; Fonte et al. 2013). Such shifts in BCC were always accompanied by metabolic changes in these studies. However, most of these studies used a labile and a more refractory C source (Fonte et al. 2013). It was suggested that bacterial taxa partition along a C source gradient with different ratios of allochthonous vs. autochthonous C (Kritzberg et al. 2006; Jones et al. 2009). In our incubations, BCC seems to be more affected by the presence of both labile allochthonous and labile autochthonous DOC sources but not by the different ratios of the two DOC sources.”

Comment 24) Page 14280 line 3 "another reason" it is not clear what the first reason was.

Response 24) We deleted this sentence because this section was drastically shortened due to the restructuring of the discussion.

Comment 25) 5. Conclusions "Our study highlights the importance of DOC quantity for bacterial DOC consumption and DOC quality for BCC" - is this not at odds with the title of the manuscript?

Response 25) Thanks for pointing this out. We changed the title because it can be misinterpreted and we change the title into “Enhanced bacterial decomposition with increasing addition of autochthonous to allochthonous carbon without any effect on

bacterial community composition".

Again, I appreciate the hard work that went into this, and I think it can be informative if you center it around other, clearly formulated, questions. Best of luck!

Interactive comment on Biogeosciences Discuss., 10, 14261, 2013.

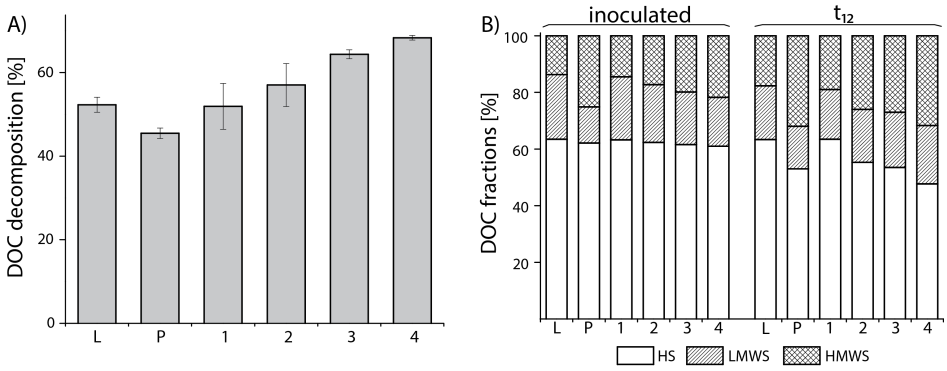

Fig. 1. Fig. 3 (new)