

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 #2,

we thank the reviewer for his/her very helpful comments on our manuscript and highly appreciate the suggested changes. We address all the valuable comments in a point-by-point response below, and carefully considered these points in our revised manuscript. We hope that our revised manuscript sufficiently addresses all the comments and critiques and is now suitable for publication in Biogeosciences.

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The major issue raised by Reviewer #2 is based on the use of “non-natural” sources which makes it difficult to mimic anything that is happening in nature. We do not fully agree that these DOC sources cannot be found in nature and now give more information on the DOC sources and their occurrence. However, we agree with the Reviewer #2 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) The authorswith increasing phytoplankton DOC. And they conclude that chemical quality rather than source of DOC determines bacterial DOC turnover. I found that this conclusion is rather an open door and their results may not be directly translated to lakes as they are based on the DOC sources used which may not represent the natural DOC sources (see below). In lakes, the main sources terrestrial and phytoplankton have specific DOC compositions, which is thought to determine their fate in lakes. So, there is a direct relation between source and composition or quality.

Response 1) We do not fully agree with this statement. In this study we wanted to mainly focus on leaf litter which directly enters small lakes and hence provide a constant source of fresh leaf leachates. Therefore, we have better defined this important topic in the manuscript:

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.

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Comment 2) There are issues with the two sources of DOC were used: namely leached leaf litter representing allochthonous DOC and “algal” hydrolysate for autochthonous DOC. There is relatively little information on the procedures used to make the two sources. It is for instance not stated if the leaves were green fresh or brown senescent, which may have important implications for the composition of the leached DOC.

Response 2) Thanks for this remark. We now included a more detailed description of the production of the two DOC sources used in the experiments. For example, we added the information that we used brown leaves. These leaves were directly collected with a net after they fell down from the tree. Although we agree that our experimental set-up does not necessarily “mimic natural conditions”, we wanted to use the leaves in the same state as they would fall down from the tree. To avoid an unpredictable change via microbial activity in the soil, we prevented leaf processing on the soil by direct collection of the leaves (in a dry state) after they fell down from the trees.

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 3) Also, the methods used to extract DOC from the algae are poorly described/lack information and look rather severe (a combination of dissolution in distilled

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water, autoclave, and ultrasonication). The methods description for algal DOC should be improved: why a mixture of 2 cultures and what was the ratio between these two cultures; why was the treatment so harsh and different from the leaf DOC extraction; how much of the biomass was extracted as DOC; how much material was used per volume of water. It also seems to me that this harsh treatment will not likely extract DOC of similar composition as released by natural phytoplankton, which may hamper the translation of the results to the natural environment. For instance, the phytoplankton DOC contained a high amount of aromatic HS (humic substances), which seems rather unusual for natural autochthonous DOC from phytoplankton. And, DOC leaf was more available to bacteria than DOC phytoplankton in single source incubations, which is not the case for natural source materials. Given that the composition of the two DOC sources may not be representative natural autochthonous and allochthonous DOC inputs into lakes, it is difficult to tell to what extent the results can be directly translated to bacterial carbon cycling in lakes. The authors should fully acknowledge this in the paper.

Response 3) We now added further information on the procedures used to generate both DOC sources in the methods and cite papers where similar sources were used for their incubations. For example, Kritzberg et al. 2006 (FEMS Microbiol. Ecol.) used a similar procedure to extract phytoplankton DOC and also leaf leachate DOC.

“The leaf leachate (DOCleaf) was produced by leaching ^{13}C -enriched beech leaves (*Fagus sylvatica*, L.) from trees grown under a $^{13}\text{CO}_2$ atmosphere in greenhouses in Nancy, France. The leaves were harvested after they fell off the tree, dried and stored at room temperature. We leached brown beech leaf disks (diameter 1.1 cm) for 48 hours in double distilled water at 4°C in the dark. The phytoplankton lysate (DOCphyto) was extracted from a cyanobacterium (*Aphanizomenon flos-aquae*, L. SAG 31.87) and a green algal (*Desmodesmus* sp.) culture by centrifugation, dissolution in double distilled water, hydrolysis (20 min at 120 bar and 121°C) and ultra-sonication (1.5 min at 10 W). The phytoplankton cultures were both not axenic and are representative for

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those lakes (Yamamoto, 2009; Wu et al., 2011). Both DOCleaf and DOCphyto were filtered through a pre-rinsed 0.2 μm polycarbonate filter (Whatman, Dassel, Germany) before inoculation, both procedures are similar to the ones described in Kritzberg et al. (2006).”

By using two phytoplankton cultures, we wanted to include a broader range of phytoplankton species and we mixed the same amount from both species. For our experiments, however, DOC extraction efficiency was not important since we added the DOC sources in well determined quantities to assure full microbial DOCphyto availability in all of our experiments. The severe algae dissolution was required to get them lysed in form of DOCphyto. We are aware that the phytoplankton lysate does not necessarily represent the chemical quality of exudates from phytoplankton and, therefore, we did not write this in the manuscript. Our intention was to supplement the allochthonous DOC source with DOCphyto which in the natural system can also stems from lysis of phytoplankton biomass and not exclusively by algal exudates. HS are not unusual components of phytoplankton origin (in McKnight and Aiken, 1998, Sources and age of aquatic humus. In Hessen and Tranvik (book) Aquatic humic substances). And finally, the point that DOCleaf is more available is one of the major findings in our manuscript. Although we step back from the difference between the two sources to acknowledge the comment on the “non-significant results”, they are both well available which has been tested in separate experiments. Thus, the general view that allochthonous DOC is less available can not be applied for DOCleaf. See the papers from Berggren et al. (2007, Global Biogeochem. Cycles; 2010, Ecol. Lett.) who also show that there are some allochthonous DOC sources with a high microbial availability.

We now fully acknowledge the rather unusual way of extracting the phytoplankton DOC and step back from the direct transfer of our data to natural systems.

Comment 4) DOC algae is used for the autochthonous DOC, but this is a bit odd as it was extracted from a mixed green algae and a cyanobacterium culture. Clearly a cyanobacterium is not an algae, and it should be renamed to DOC phytoplankton or

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similar. There are other issues with acronyms: HS, LMWS, HMWS are only explained in the supplement, and BR and BP (page 14279) are not explained anywhere as far as I could tell.

Response 4) Thanks for the comment. We changed the DOC nomenclature accordingly and now use phytoplankton lysate or DOCphyto. In addition, we introduce each abbreviation in each section separately.

Comment 5) Bacterial community structure was determined by both PLFA and DGGE analysis. Both show that community structure was similar when individual DOC sources were used and that a shift was only observed with mixed DOC. This last observation makes sense, but the similarity in community structure between single source incubations is difficult to explain given the difference in composition between leaf and phytoplankton DOC. Also, what was the initial community composition of the lake water inoculum? Was this determined and was it similar to the single source incubations? It seems that at least the PLFA data are available based on the Fig 4 y-axis. Finally, 4 data points are shown for the leaf DOC incubations, whereas triplicate incubations were used?

Response 5) This is a good point. We think that there are differences between the single source incubations (only DOCleaf and only DOCphyto). However, it might be possible that the method we used (DGGE) is not sensitive enough to resolve for all differences in BCC. In another experiment in chemostats with similar DOC sources (also DOCleaf from alder leaves and DOCphyto) we found more pronounced differences between both DOC sources when using next-generation sequencing. We now acknowledge these method caveats in the discussion.

“Surprisingly, we found no differences between the two single source incubations. This could be due to the fact that the DGGE technique might only reveal the abundant bacterial taxa. Thus, this result needs to be further tested with, for example, next generation sequencing with a higher resolution.”

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We determined the bacterial source community in the PLFA but not in the DGGE analysis because we used the same inoculum for all incubations. Furthermore, our hypotheses were not related to the initial source community but to the final community composition and, thus, we did not include this aspect in our analyses of both nMDS. In addition, the inclusion of the source community increases the stress factor and thus decreases the resolution of our analysis.

We had four replicates for the leaf DOC incubations. This is also explicitly mentioned in the description of the experimental set up. We did not want to randomly exclude one of them to have the same number of replicates in all treatments.

Comment 6) I felt that the discussion lacks clear structure and various issues are discussed repetitive. The authors should have another go at the structure of the discussion to improve clarity. Also, a substantial part of the discussion and conclusions appears to be based on non-significant effects. Why? This should be treated with a lot of care eg. this should not make it to the abstract.

Response 6) Thanks for these remarks. We reworked on the discussion and now relate it to clear hypotheses and removed repetitions. Additionally, we reformulated the parts with non-significant results and also deleted them from the abstract.

Comment 7) These publications also studied the effects of DOC source on bacterial community structure and may be included: Roika et al. 2012 Aquatic Sciences 74: 513 Jones et al 2009 Environm. Microbiol. 11: 2463 And Kritzenberg et al 2005 Aquatic Microbial. Ecol. 38: 103 studied the relationship between DOC source and growth efficiency.

Response 7) Thanks for these additional references. We included them and also more recent ones from similar experiments (Fonte et al. 2013 Microb. Ecol.) into our discussion.

“However, BCC shifted when both sources were mixed in the treatments. A greater di-

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versity 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.”

Specific comments. (SC)

Thanks for all these very helpful comments. We changed them accordingly (here assigned by an OK).

SC1) The abstract is rather long and contains a lot of introduction.

Response SC1) We shortened the introduction of the abstract. “Dissolved organic carbon (DOC) concentrations - mainly of terrestrial origin - are increasing worldwide in inland waters. Heterotrophic bacteria are the main consumers of DOC and thus determine DOC temporal dynamics and availability for higher trophic levels. Our aim was to...”

SC2) Page 14264 line 15: : : ., which is related to rising CO₂ Response SC2) OK.

SC3) Page 14265 line 7: : : ., the effect of DOC quantity Response SC3) OK.

SC4) 2.1: please move the third paragraph up one paragraph Response SC4) OK.

SC 5) Page 14269 line 12: this paragraph fits as a sub-chapter (Calculations or similar) after 2.5, as 2.5 describes some of the analysis used in the formulas.

Response SC5) OK. We changed the chapter to “Stable carbon isotope analysis” and created now three sub-chapter, one “PLFA analysis and determination of stable carbon

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isotope ratios of PLFA”, the second “Determination of stable carbon isotope ratios in DOC and respired DIC” and the third as “Calculations” according to the suggestions.

SC 6) Page 14269 line 25: Keeling plots are specifically used for atmospheric concentrations; this should be described as an isotopic mixing model.

Response SC6) Keeling plots refer to the situation where CO₂ from one source is mixed with a background signal. The Keeling plot essentially “un-mixes” the respiratory signal from the background. As such they have many applications in ecology and are not limited to “atmospheric concentrations”. Some examples are soil respiration, tree stem respiration, humming bird respiration, bat respiration, among others.

Carleton, Scott A., Blair O. Wolf, and Carlos Martinez del Rio. "Keeling plots for hummingbirds: a method to estimate carbon isotope ratios of respired CO₂ in small vertebrates." *Oecologia* 141.1 (2004): 1-6.

Karlsson, Jan, Mats Jansson, and Anders Jonsson. "Respiration of allochthonous organic carbon in unproductive forest lakes determined by the Keeling plot method." *Limnology and oceanography* 52.2 (2007): 603-608.

Kayler, Zachary E., et al. "Characterizing the impact of diffusive and advective soil gas transport on the measurement and interpretation of the isotopic signal of soil respiration." *Soil Biology and Biochemistry* 42.3 (2010): 435-444.

Maunoury, Florence, et al. "Seasonal, daily and diurnal variations in the stable carbon isotope composition of carbon dioxide respired by tree trunks in a deciduous oak forest." *Oecologia* 151.2 (2007): 268-279.

Voigt, Christian C., et al. "Bat breath reveals metabolic substrate use in free-ranging vampires." *Journal of Comparative Physiology B* 178.1 (2008): 9-16.

SC 7) Inoculum is typically used for microbial additions and not for DOC (heading 3.1).

Response SC7) OK, we changed inoculum to amendment throughout the whole text.

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SC 8) Page 14272 line 4: a better comparison between treatments. Response SC8) OK.

SC 9) Page 14273 first paragraph: this is difficult to follow and repeats the data shown in the figure. Please discuss in terms of major findings.

Response SC9) We changed this paragraph according to the suggestions and focused only on the differences in the HS fractions.

“Analysis of DOC fractions in the treatments with mixed DOC sources revealed changes over time, particularly in the HS fraction (Fig. 3). Overall, all DOC fractions showed a decline in their concentrations after 12 days of incubation. However, this decline in concentration was highest for the HS fraction, as can be seen by their relative proportions (Fig. 3). The inoculated HS fractions were similar in all treatments, which were mainly due to the similar proportion of HS in the two C sources (Fig. 2) but the percentage of decomposed HS increased from treatment 1 to 4. In addition, the chromatographic analysis revealed a change in the proportion of aromatic and unsaturated structures. There was a higher degradation of aromatic structures and high molecular sizes in treatment 3 and 4 compared to that in treatment 1 and 2 (Fig 2). At the end of the incubation, the aromaticity in treatment 3 and 4 was lower than for treatment 1 and 2 despite having added DOCphyto to these treatments, containing the highest amounts and proportions of aromatic structures.”

SC 10) Page 14273 line 25: Only bacterial and total PLFA are discussed. However incubations were done with a day/night cycle; were any phytoplankton derived poly-unsaturated PLFA detected? And how would this influence the results.

Response SC10) We only measured significant amounts of PLFA for the heterotrophic bacterial PLFA (i + a 15:0) and for PLFA that account for both phytoplankton and algae. Unfortunately, no results were obtained for the main diatom biomarker PLFA, 20:5w3, and the main green algae marker, 18:3w3 (Boschker et al. 2005).

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If we would have measured high concentrations of phytoplankton derived poly-unsaturated PLFA would indicate that there is fixation of inorganic carbon and thus a third source of C despite our two amendments (DOCleaf and DOCphyto).

SC11) Page 14274 line 4: : : . efficiency (BGE) was Response SC11) OK.

SC12) Page 14274 line 4: Please state for how many incubations respiration could not be estimated.

Response SC 12) We added a new sentence to the results section 3.4 Carbon flow:

“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.”

SC13) Page 14275 line 8: Delete “To answer this inquiry,”. Response SC13) OK.

SC 14) Page 14275 line 24: If differences were not significant, why are the data discussed extensively here and why are some of the conclusions based on these data? See also page 14279 line 14 and page 14280 line 19.

Response SC14) This is a good point and we reduced the discussion of non-significant results and focused on our significant findings.

SC15) Page 14276 line 13: Delete the However. Response SC15) OK.

SC 16) Page 14277 line 21: FAME should be changed to PLFA throughout the manuscript. Bacteria do not contains FAME but PLFA. Response SC16) OK.

SC 17) Page 14278 line 11. I don't get this sentence: Degradation of DOC supported high bacteria biomass, which in turn led to higher degradation of DOC? It is one or the other.

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Response SC17) We deleted this sentence.

SC 18) Page 14278 line 20.: . Besides nutrient limitation, Response SC18) OK.

SC 19) Page 14279 line 1: : : :.. the question remains Response SC19) OK.

SC 20) Page 14280 line 24: a higher influence? Should read a larger influence and explain to which this is compared Response SC20) OK.

SC 21) Fig 1. The DOC amounts in the hatched bar are difficult to read.

Response SC 21) We improved the readability of figure 1. For demonstration, it is uploaded as figure 2.

SC 22) Fig 2. Molecularity should read Molecular weight. Response SC 21) OK.

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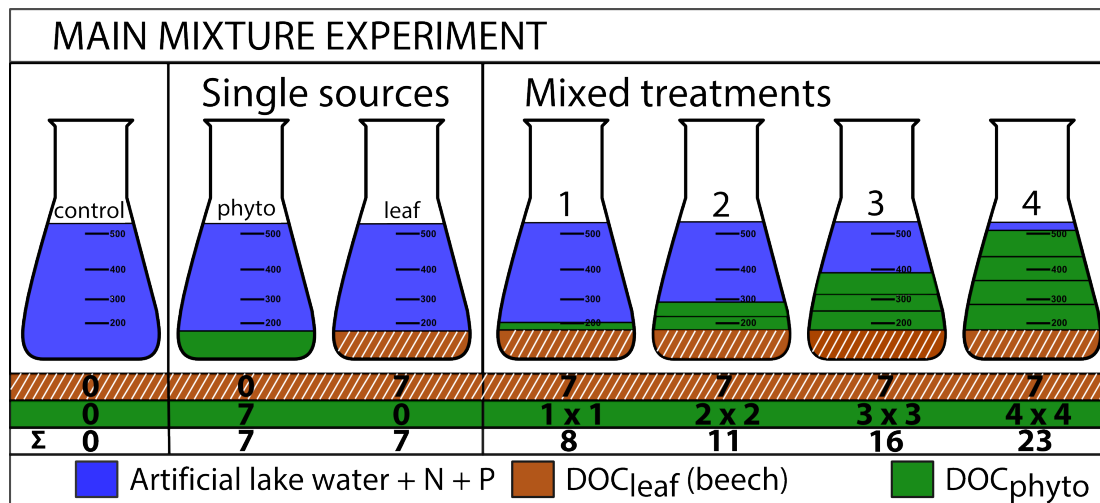


Fig. 1. Fig. 1 Experimental set-up

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