Dear Dr. Smittenberg,

Thank you for your comments and the helpful feedback. We have responded to each of your points in line with your text below. Our responses begin with the open bullet points. For the most part we agree with your suggestions, and have indicated our plans for a revised manuscript, which we will complete after the discussion period ends. In a few cases, we think that further dialogue would be helpful, either because we are unclear about the intent of your comment, or because we have a different perspective. We have also added some points of clarification to questions you have.

We have highlighted text below in blue where we think additional feedback from you would be helpful as we complete our revisions to the manuscript.

Thanks again for your time and feedback,

Nemiah Ladd

The authors measured the hydrogen isotopic composition (d2H) of lipid biomarkers, in particular short-chain fatty acids (C14-C18) and brassicasterol, from particulate organic matter filtered on a monthly basis from the surface water of two Swiss lakes, over the course of the algal 'growing season' of 2015. They combined these measurements with estimates of productivity using 13C labeling and data of community assemblage, and other environmental data like temperature and trophic status of the two lakes - one eutrophic and the other oligotrophic. This study gives useful insights in the hydrogen isotopic fractionation during biosynthesis of these lipids through time, the factors that influence this fractionation. The study is a welcome expansion of similar efforts performed on algal cultures, and aids in the assessment under what conditions biomarker d2H can potentially be used as a sedimentary proxy for past hydrological changes, and/or what the limitations are. The study is set up and executed properly, and the paper is well written. I have, however, some remarks, comments and questions I like the authors to address.

- Page4 Line26. I need to assume the carboys were made of clear plastic to allow photosynthesis?
    - We will add the word “transparent” here to clarify

- P8L15 increased from April to July? Or levels were low from April to July? Write more clearly.
    - We will change this sentence to read: “Lipid concentrations increased significantly in Lake Greifen from April to July, and then declined slightly from July to September.”

- P9L24-25 "When analyzing.. Table 2)". Unclear sentence, rewrite.
    - We will change this sentence to read: “Regressions for individual fatty acids typically had higher R^2 values than the pooled correlation for all short-chain fatty acids.”

- P10L31-34 "The slope.... " Not clear, rewrite.
    - This sentence actually just repeats the same information that is included in the above bullet point from the results section. We will delete it from the revised version of the manuscript.

- P11L30-33. This part appears out of place and fits better within the next section
    - Based on this comment and the following one, we think it would be a good idea to reorganize the discussion slightly. We will keep sections 4.1.1 about temperature and 4.1.2 about light availability. We suggest adding a short section about trophic status/nutrient availability (4.1.3), and then a section about productivity (4.1.4). This section will briefly explain how the three environmental factors (temp, light, nutrients) relate to productivity and lipid production rates, and then discuss the lack

of correlation between production rates and ^{2}H fractionation factors. As part of this rearrangement, we will move the information on these lines to the new section 4.1.4 about productivity.

- P12. Section 4.1.3. Lipid production rate. - I suggest to rename this section to 'trophic conditions' or 'nutrient availability', which is a primary environmental factor similar to temperature and light - with all three bearing on productivity and related fractionation. At the moment the discussion appears a bit mixed, nutrient availability and growth rate are somewhat used interchangeably.
    - We agree that it would be more precise to rename this section, and to clearly split the discussion about trophic status and lipid production rates. For the revised manuscript, we will restructure the discussion as described in response to the previous comment.

- About the source of the fatty acids: The authors appear to only consider algae, or at any case aquatic organisms, as their source. However, fatty acids may also come from terrigenous sources, and this potential source may change over time. For example, surface runoff during early spring may bring in relatively large amounts of terrestrial organic matter at a time that lake primary productivity is still low.
    - It is true that vascular plants also produce large amounts of short chain fatty acids. However, our samples were collected from surface water in the middle of the lake, and there is not a good mechanism to transport large amounts of terrestrial material to such a location. Our incubations indicate that short-chain fatty acids are produced rapidly relative to the standing stock of lipids in the surface water, as indicated by the short residence times reported in Table 3. This result suggests that the vast majority of the short-chain fatty acids collected on our filters are produced in the lake water. Additionally, this result argues against large contributions of fatty acids from heterotrophs, since it would take more time for the ^{13}C label to be consumed after fixation by photoautotrophs, and our incubations only lasted for six hours.
    - Another reason why we think it is unlikely that there were significant contributions of short-chain fatty acids from non-aquatic sources is the absence of long-chain *n*-alkanoic acids, long-chain *n*-alkanes, and other biomarkers for higher plants on our filters.

- The non-existent correlation between fractionation factor and growth rate is likely due to the surprising low growth observed at day 220 at lake Greifen (why so much lower than at day 180, do the authors have an explanation?), and with just 5 measurements over the entire period this is bound to give bad statistics. I therefore wonder if there is no other information available about algal productivity, possibly the data from the long-term monitoring program at EAWAG could be used? Have the authors considered a more simple method of estimating productivity like chlorophyll concentration? How dependable and reproducible is the labeling-incubation method? What if the productivity data point at day 220 (and even the concentration of FA) from lake Greifen is compromised - would a higher rate at day 220 suddenly result in a good correlation?
    - We also find the low production rates from day 223 in Lake Greifen to be a bit confusing and unexpected, but they may have to do with the weather from that day. We tried to restrict our sampling to fully sunny days in order to minimize confounding effects from light availability. Unfortunately, day 223 on Lake Greifen ended up being partially cloudy, and these incubations represent the only ones that were not carried out in full sunshine, which may account for the lower production rates.
    - It is true that one questionable value could skew small number statistics. We checked the correlations between $\alpha_{Lipid\text{-}Water}$ and lipid production rate for Lake Greifen without the sample from day 223. There were not significant correlations for any of the five lipids. The R^2 value for $nC_{16:1}$ was still the highest, but declined from 0.84 to 0.81. The correlations for the other lipids improved slightly, but the R^2 values remain relatively low.
    - We agree that it would be good to supplement our lipid production rates with chlorophyll concentrations. We have additional filters that have been stored frozen since collection since each sampling day, and are currently analyzing them for pigments. We hope to have these data to include in the revised manuscript. The

abundance of various pigments associated with specific taxa of algae may also be a helpful constraint for the algal community side of the story.

- Finally, with regards to the comment about the reliability of the ^{13}C incubations, the method has been successfully employed several times in marine settings (e.g. Popp et al., 2006, doi:10.1029/2005PA001165, Prahl et al., 2004, doi:10.1016/j.dsr.2004.12.001, Wolhowe et al., 2014, DOI: 10.1016/j.pocean.2013.12.001). Precision between our replicate incubations from the same day averaged 11 ± 7% of the production rate for Lake Greifen and 15 ± 9% of the production rate for Lake Lucerne. These uncertainties are shown in the error bars on the lipid production rates in Figure 2.

- To what extent do turnover time and export of dead organic matter (or lack thereof) may have an influence on the bulk hydrogen isotopic compositions measured? How much algal biomass is taken up by heterotrophs and recycled, thereby partially keeping the original isotopic signature? How much particulate OM is alive? In other words, how much 'memory' does the system have over the season leading to attenuation of the isotopic signal? If there is such attenuation, then the instantaneous productivity at a given point in time, especially at a later stage when it is going down, may be ever more unrelated to the accumulated particulate OM and lipid stock. Note that lake temperature has a large inertia thus will automatically correlate well with any other parameter with a slow response time.
  - Part of the reason why we did the incubations to measure lipid production rates was to address this question. For the most part, lipid production rates are high relative to the lipid concentrations, indicating quick turnover. Residence times for each target lipid are reported in Table 3, and for most cases are less than one day. The fatty acid with the longest residence times is $n\mathrm{C}_{16:1}$, but even these never exceed three days. Admittedly, the production rates are based on incubations conducted during daylight hours only, so they are likely to average to a lower rate over a full 24-hour period. However, they suggest that most of the fatty acids in the lake are produced within the past week at the most.
  - The compound with the longest residence times is Brassicasterol (Table 3). This is also the compound whose hydrogen isotope fractionation shows the smallest correlation with temperature, suggesting that the correlation between fatty acid ^{2}H fractionation and temperature is not an artifact caused by two parameters both with slow response times.

- On page 14, the authors argue against a large contribution of heterotrophic bacteria based on low abundances of iso- and anteiso fatty acids. However, a large amount of heterotrophic biomass might be planktonic and not bacterial, while also not all heterotrophic bacteria will produce exactly those biomarkers - the majority will still predominantly produce C16:0 FA. It is not clear from the text to what extent the presented algal community data reflects only phototrophic algae (it is presented as such), or if these data are more inclusive to all microbial life (in which case heterotrophic plankton is surprisingly absent).
  - In response to a similar comment from Reviewer 1, we have decided to modify the text at the end of the last paragraph of section 4.1.4 (starting at P14L17 of the original manuscript) to the following text: “If the source of so much $n\mathrm{C}_{16:0}$ fatty acids was bacterial, it might be expected to correspond to increased concentrations of lipids associated with heterotrophic bacteria, such as iso- and anteiso-$\mathrm{C}_{15:0}$ and $\mathrm{C}_{17:0}$ (Perry et al., 1979; Volkmann et al., 1980). Since there are not significant amounts of these short-chain odd-carbon branched fatty acids in the particulate organic matter throughout the time series, including in the early spring, it seems less likely that such a large component of the even-carbon fatty acids is be derived from bacterial sources. However, this does not rule out greater contributions from mixotrophic algae relying on heterotrophy in the spring, nor from heterotrophic bacteria species that primarily produce short-chain, even fatty acids.”

- P13L32. I would be very careful assuming that all heterotrophs have more enriched fatty acids than phototrophs based on only one study.
  - Subsequent studies (such as Osburn et al., 2011, doi.org/10.1016/j.gca.2011.05.038, Heinzelmann et al., 2015a, doi: 10.1093/femsle/fnv065, Heinzelmann et al., 2015b,

10.3389/fmicb.2015.00408) have also observed more enriched fatty acids in heterotrophs relative to autotrophs. However, it is true that to date hydrogen isotope fractionation has only been investigated in a tiny portion of the myriad heterotrophic microbes (and photoautotrophs) that exist, and it is possible that there is more diversity in heterotroph hydrogen isotope fractionation than these initially studies indicate. We will modify this sentence to reflect this uncertainty, and to include the additional references.

- P15L11-15. It is very well possible, or even likely, that the different lakes (with quite different trophic status) host different diatom species (or even non-diatoms, who knows..) making brassicasterol. Zhang et al has shown that different species making the same lipid may fractionate quite differently. This may also explain the large difference in fractionation of brassicasterol in the two different lakes.
    - It is true that different species can have different fractionation factors for the same lipid. The species assemblage data collected by Eawag can help us assess how much the diatom community varies between the two lakes. No diatom species were identified in Lake Lucerne in 2014 that were not present in Lake Greifen in 2015. However, some of the prominent diatom taxa from Lake Greifen are not present in Lake Lucerne. The most abundant of these are *Stephanodiscus* sp. (up to 25% of the diatom community in Greifen), followed by *Melosira* (up to 5% of the diatom community in Greifen). We will modify this section of the discussion to indicate that the differences in brassicasterol net fractionation between the two lakes may be due to contributions from different diatom producers. This is almost certainly the case for fatty acids between the two lakes as well.

- It would be useful to plot temperature through the season - not one based on five own measurements, but those from EAWAG or a similar service.
    - In addition to our six measurements from Lake Greifen, the environmental agency for Canton Zurich measured lake water temperature at monthly intervals. Their data is plotted in black in the figure below, and generally agrees well with our data (in red) from slightly different days. We are trying to find similar data from Lake Lucerne, and will add lake surface temperature curves to figure 2 in the revised version of the manuscript.