Dear Dr. van der Meer,

Thank you for your helpful comments and feedback on our manuscript "Interplay of community dynamics, temperature, and productivity on the hydrogen isotope signatures of lipid biomarkers." We have revised the manuscript in accordance with your suggestions and the feedback from the two external reviewers.

There are two major changes to the revised manuscript. First, following your suggestion, we have expanded our focus to consider the influence of heterotrophic sources of fatty acids. We have reframed the introduction to focus on the use of generic fatty acid hydrogen isotopes as a proxy for net community metabolism, as well as the use of phytoplankton-specific biomarkers as a water isotope proxy. We have rearranged the discussion to focus first on the effect of lipid source on our observed $\alpha_{Lipid\text{-}Water}$ values (new section 4.1) and then on the effect of environmental gradients on $\alpha_{Lipid\text{-}Water}$ in phytoplankton (section 4.2). To accommodate the expanded discussion of heterotrophic fatty acids, we have condensed the discussion about light availability, which is unlikely to have had a major effect in lake surface waters during spring/summer.

The other major change is that we have removed the data and discussion pertaining to the sediment trap and core top samples. Following the advice of Reviewer 1, we initially tried to incorporate values from the remaining fatty acids, which we did not have good data for at the time of the original submission. There are interesting trends here, especially the ^{2}H-enrichment of the core top fatty acids relative to those in sediment traps (see figure in updated response to reviewer 1). However, the additional discussion necessary to explain these trends expanded the paper's scope and reduced its focus. We believe it is more appropriate to include these data in a second manuscript we have been preparing concurrently, which also includes newly obtained sediment trap and core top data from 8 additional lakes in central Switzerland. Please see our updated response to Reviewer 1 for more of this data and the rationale for removing the sediment data from this submission.

We have made a number of smaller changes to the text, in line with the suggestions of the two reviewers. These are detailed in our updated responses to the two reviewers. We have also measured $\delta^{2}H$ values of phytol, and updated the figures and discussion to include these results.

We believe that all of these changes have strengthened the manuscript considerably, and appreciate the time you and the reviewers have spent to improve this paper.

Best wishes,

Nemiah Ladd, on behalf of all co-authors

Revised Response to Reviewer 1

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 the changes we made in the revised manuscript. In a few cases, we disagreed with the suggestion, and have explained our reasoning below each point.

Thanks again for your time and feedback,

Nemiah Ladd

Summary: Ladd et al. describes the lipid composition and hydrogen isotopes of particulate OM through a time series from alpine two lakes and relates observed variability to temperature and nutrient availability. The work is strengthened by concurrent labeled incubations to get at lipid production rates and comparison to recent sediments. This work is robust, the conclusions are generally well supported, and the paper is well written.

I would like to see more data from the sedimentary samples and a revision in terms used to describe fractionation, but otherwise recommend that this manuscript be accepted for publication.

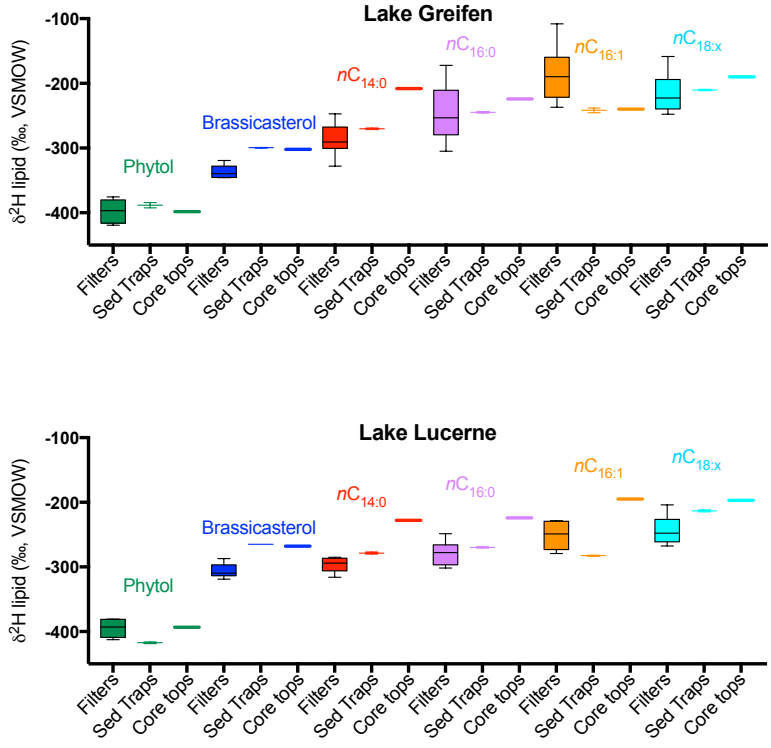
Larger comments:

- Probably my largest criticism of this manuscript is in somewhat confusing usage of terms that descript the magnitude and direction of isotope fractionation. The most common usage in this manuscript is 'increase/decrease in hydrogen isotope fractionation' which is inherently vague because it doesn't indicate direction. Describing alpha values as high and low is equally problematic as a very low alpha describes a very large fractionation (albeit a very large negative fractionation or depleted lipid signature). I would suggest converging on a single nomenclature and describing pools as more or less D-enriched or D-depleted and fractionations as positive, negative, more negative etc, rather than using term like higher and lower and increased and decreased. Section 4.1.1 is a good example of where you switch fluidly between XX permil per degree C to alpha in 0.001 and back with concurrent usage of increase, decrease, etc.
    - We have revised the text and now refer to changes in fractionation as increases or decreases in $\alpha_{Lipid\text{-}Water}$, and referenced these as decimals instead of ‰. We have also described changes in d2H values of specific pools as more ^{2}H-depleted or ^{2}H enriched.

- Please differentiate the cyanobacteria from eukaryotic algae. Lumping the two is a vestige from the time prior to DNA sequencing technologies that allowed for easy differentiation. This is imprecise and unnecessary for this manuscript. Please revise.
    - We have modified the text to refer to "cyanobacteria and eukaryotic algae" in place of the more generic "algae". In some cases, especially when comparing organisms with different core metabolisms, we found

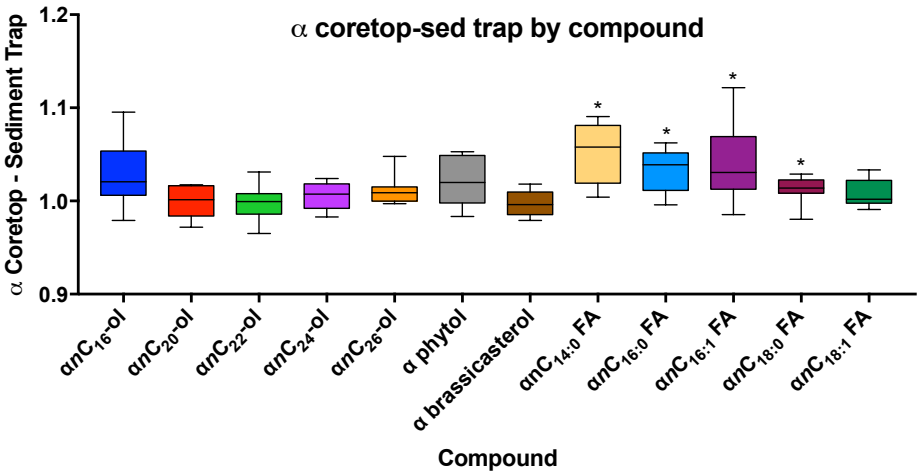
it less clunky to lump these two groups together, but have used the terms “phytoplankton” or “photoautotrophs” instead of “algae.”

- p10 Section 3.3 – Why do we only get to see data for the c16:0 FA for the sedimentary lipid section? Do the other lipids agree? I would also like to see if the sediment traps and the core top data agree with one another. Given the accumulation rate and sampling procedure the differences might suggest seasonal signals vs. annual integration. Generally the level of data and detail for the sedimentary samples should be as robust as the particulate (given that you are comparing the two and the later is the signal that most people look at).
    - When the sediment samples were originally analyzed, the concentrations were optimized for $n\mathrm{C}_{16:0}$. In many cases, the peak areas were not appropriate for other fatty acids, and the standard deviations of those measurements were high. We have rerun these samples on the GC-IRMS again at a more suitable concentration in order to be able to get good sediment data from all of the compounds discussed from the filters. The results show a more complicated picture than what was apparent when we had only measured palmitic acid, and pulling out the core top data from the sediment traps adds more complexity. Here is what the new data look like:

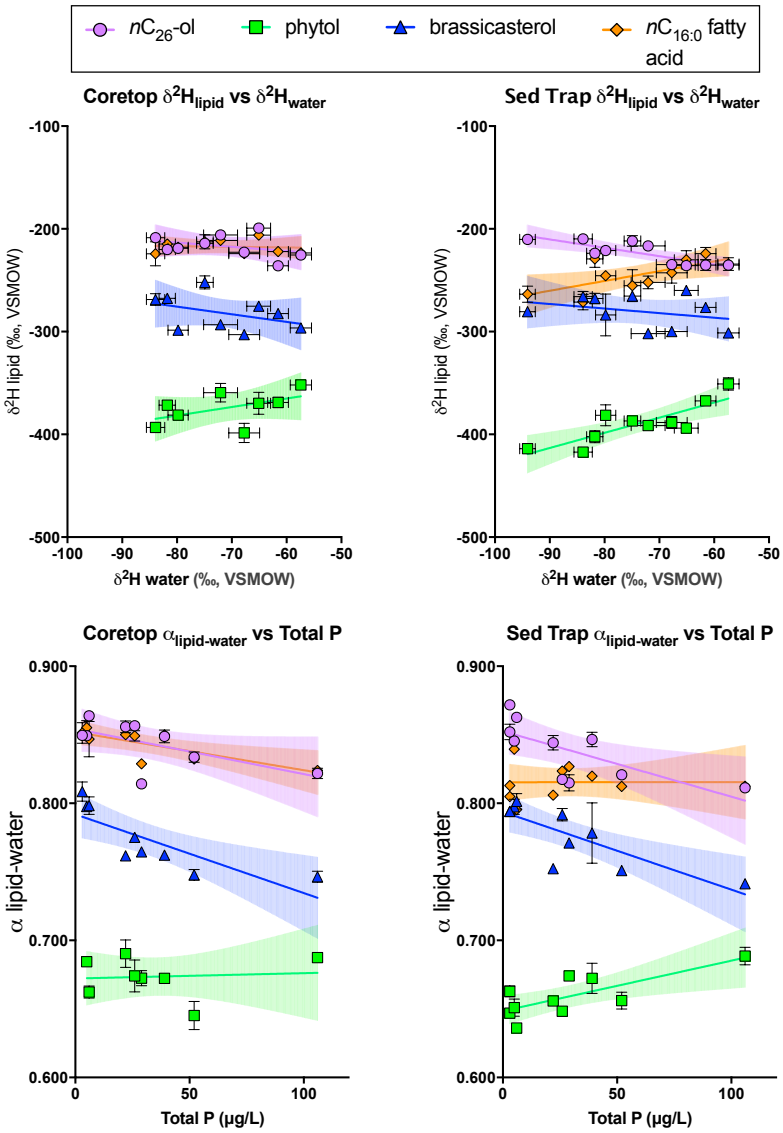


These have a number of interesting features, including ^{2}H enrichment of most coretop fatty acids relative to sediment traps, and relatively good agreement between phytol δ2H values in POM and core tops. However, discussing possible reasons for these trends added quite a bit of text to the revised manuscript, and reduced its focus. Since submitting the first version of this paper to *Biogeosciences*, we have also measured δ2H values of multiple lipids from coretops and sediment traps in 8 additional lakes in central Switzerland. These samples were also collected in the

spring of 2015, but there is no corresponding time series of particulate organic matter from surface waters in these lakes. The full set of samples from ten lakes allows for a more robust discussion about differences in δ2H values between traps and core tops, and for differences in δ2H values among different types of lipids. Here is a summary figure of the fractionation between lipids in sediment traps and coretops:



The scatter plots below show the relationships between δ2H lipid and δ2H water for a subset of these lipids, as well as changes in $\alpha_{\text{Lipid-Water}}$ with total phosphorus concentrations:



Rather than trying to include all of the sediment trap/core top data in the present submission, which would result in a rather unwieldy manuscript, we think it makes more sense to remove the limited sediment data that was in the original submission and keep the focus on the data from the filters. We have a manuscript in preparation presenting and discussing all the sediment data, and hope to have this ready for submission soon.

- p12-15 – As you have both cell counts and lipid yield data you should be able to normalize for cell counts in this discussion. You note at the end of the paragraph that cell counts agree, but it seems like you can take it a step farther.
    - A problem with doing this is that the cell count samples were collected on different days than the lipid samples. We can linearly extrapolate between the monthly cell counts to come up with an estimate of the number of cells on our sampling dates and normalize the lipid yields to these, but given the potential for the cell count sampling to miss short lived blooms of specific taxa makes us hesitant to use this data quantitatively and we prefer to keep the discussion here qualitative.

- p15-7 – I don't buy this explanation and you definitely haven't demonstrated that this is a general feature of isoprenoids in your samples. Presumably there is plenty of phytol in your extracts, what does that look like?
    - We had phytol in our extracts. It eluted in the first fraction of the silver nitrate columns we performed on our alcohol fraction (third fraction collected during our silica gel columns). We had not measured phytol δ^2H values from these samples when we originally submitted the manuscript, but have done so now and have included this data in the revised paper.
    - As for brassicasterol, $\alpha_{Lipid\text{-}Water}$ for phytol was not correlated with temperature. If part of the change in $\alpha_{Lipid\text{-}Water}$ for fatty acids is due to temperature, this suggests that the responsible mechanism does not have as strong an effect on isoprenoids. All relationships between temperature and $\alpha_{Lipid\text{-}Water}$ from cultures that have been reported in the literature are for acetogenic lipids. We have expanded the discussion here to include that point.

- p16-1 – Can you calculate and compare a weighted average isotope value (as in Osburn et al., 2016) between these two?
    - We are generally not in favor of calculating weighted average isotope values. The differences in δ^2H values among different lipids are not trivial and can be related to real information about environmental conditions, lipid sources, and physiological state. This information is lost when computing weighted averages, so our preference is to report all compound specific isotope values individually.
    - Calculating weighted averages can be useful for comparing δ^2H values of fatty acids (or *n*-alkanes) produced by two different organisms in culture when the distribution of homologous compounds differs by taxa and the source of each lipid is known. For field samples representing a mixed source from diverse organisms, calculating a weighted average masks effects of different relative contributions from different taxa. In the case of our samples, it is quite likely that the source of each fatty acid is not evenly distributed among all fatty acid producers, because the relative concentrations of the fatty acids change over the course of the season. For example, the concentration of $nC_{16:1}$ relative to that of $nC_{16:0}$ in Greifensee varies from ~5% to ~100%.
    - Even when the source is known and constant, different processes within the cell can affect the δ^2H value of fatty acids of different chain lengths in variable ways. For example, in continuous cultures of *T. pseudonana*, Sachs et al. (GCA, 2017) observed increasing δ^2H values with increasing light availability for $nC_{14:0}$ fatty acid, but no trend for $nC_{16:0}$ or $nC_{16:1}$ fatty acids, with possible biochemical implications for these differing responses. This information is lost with a weighted average.

- I am generally curious about what other compounds were present in this dataset that can be compared between the particulate and the sediments. In particularly the midchain fatty acids as these are often used to describe paleolake water.
    - In addition to the compounds measured in this study, the filters

contained very small amounts of relatively short (~C17) *n*-alkanes, but concentrations were not high enough for H isotope measurements. Other prominent compounds in the alcohol fraction were phytol, cholesterol, stigmasterol, ergosterol, and β-sitosterol. Phytol eluted cleanly in the first $AgNO_3$ fraction. The other sterols either split between fractions, or still had issues with coeluting peaks after the $AgNO_3$ columns. We spent quite a bit a time trying to modify and develop a protocol that would obtain clean peaks for all sterols, but in the end were forced to optimize the method for one, brassicasterol.
    - We have now measured δ^2H values of the phytol, and included these data in the revised manuscript.
    - There were only trace amounts of fatty acids longer than C_{18}, and none of the longer chain fatty acids or *n*-alkanes associated with leaf waxes were collected on the filters. Saturated C_{18} fatty acids were also only present in very small quantities.

- Figure 3: I am not sure all of these subfigures are useful. A bigger stacked figure showing all compounds from a single lake might be more informative.
    - We have modified figure 3 to a condensed version with all alpha values (including new phytol data) from each lake plotted in a single panel, and have moved the old version of figure 3 to the supplemental material, in case anyone wants to look more closely at the individual relationships.

- Figure 4: Divide out sediment traps and surface sediment data then report all measured rather than just C16:0
    - We have now measured additional fatty acids and phytol from sediment traps and core top samples, and added this data to the figure shown in our response to your earlier point. There are interesting differences in the δ2H values between the core tops and sediment traps. However, for reasons explained above, we believe it makes more sense to explore these relationships in our manuscript in prep, which includes sediment data from 8 additional lakes.

Smaller comments

- p4-26-32 – How permeable were the incubating carboys to light and to air? It seems like the experiment is meant to replicate growing conditions, but doesn't really account for change associated with different light conditions or lack of oxygen. We can't really evaluate how comparable the incubations were to normal conditions.
    - The carboys were made from transparent plastic, and we will add this qualifier to the text.
    - They were not gas permeable, and oxygen concentrations were not measured at the end of the incubations. However, we expect six hours was not a long enough incubation period to have had a large effect on the oxygen concentrations.

- p5-33 – This is cooler and a shorter time than I have seen before. Is there reference for this? If not, are you sure that this condition was sufficient to quantitatively extract all lipids?

- When we first bought our microwave extraction system, our lab manager performed a number of tests of different extraction programs using replicates of a large homogenized sediment sample from Lake Zug in central Switzerland. These included a range of temperatures up to 100°C, and extraction times up to 40 minutes. All samples were extracted twice, and the replicate extractions were analyzed as well in order to assess whether the first extraction had successfully recovered the lipids from the sample. In this test, extracting at higher temperatures than 70°C and for longer times than 5 minutes did not have a significant effect on lipid yields.
- We have added a reference to a recent paper from our lab, Randlett et al., that used the same microwave extraction program.

- p6-5-8 – You don't report compositional information for other lipids. This is ok, but is there anything noteworthy?
  - Please see above response to 7th bullet point in the larger comments.

- p6-15 – What compound classes are eluting in each of these fractions?
  - We updated this sentence to read: "The first fraction, containing *n*-alkanols and phytol, was eluted with 20 mL of 4:1 hexane/DCM, the second fraction, containing stanols and singly unsaturated sterols (such as cholesterol) with 20 mL of 1:1 hexane/DCM, the third fraction, containing most doubly unsaturated sterols including brassicasterol, with 16 mL of DCM, and the remaining compounds with 4 mL of ethyl acetate."

- p6-22 – Do any of the aforementioned methods need citations or are they all original to this study?
  - The methods reported here reflect minor modifications of long-established protocols. For the most part, they are not unique to this study. For example, the saponification and silica gel protocols are identical to those used in several recent papers about lipids from the Biogeochemistry group at Eawag. However, those methods represent small tweaks on long-standing methods (volume of solvent used, brand of silica gel column, etc.) and do not represent a novel innovation. We observe that in many organic geochemical papers, authors cite their previously published work in the portion of their methods about lipid extraction and purification, even though those papers are not the original example of silica gel chromatography or base hydrolysis. We think it is more useful to provide the specifics of our protocols so that the reader can clearly see what we did, without having to search for additional papers.
  - The most recent paper from our group to use this extraction/saponification/silica gel scheme is Randlett et al., 2017, which we have added to the references. However, since there were some small modifications (no phase extraction to remove salt, an additional methanol fraction at the end of the silica gel columns) we kept the details of all our methods in the paper.
  - The specific silver nitrate scheme used to purify brassicasterol in this study is new, but the concept of using silver nitrate impregnated silica

gel chromatography to separate organic compounds based on their number of double bonds is long-standing and well established.

- p7-24 – Please confirm that reported errors include propagated errors from replicate measurements, standards, and derivatization processes.
    - We added the following text after "mass balance": "and reported errors represented propagated errors from replicate measurements and the uncertainties associated with the added hydrogen."

- p8 section 3.1 – this section could probably be condensed, it is quite repetitive.
    - The first two paragraphs of this section have been condensed and combined. Data about phytol has been added.

- p9-2 – lowest here means fastest right? I would suggest going with time designations when referring to residence times
    - We changed "lowest" to "shortest" and agree that it makes the point clearer

- p11, sec 4.1.2. this has been studied in plants
    - The light effect on hydrogen isotope fractionation in plants has been attributed to changes in transpiration (e.g. Yang et al., 2009, doi:10.1007/s00442-009-1321-1), which is not applicable for algae and cyanobacteria.
    - We are familiar with an experiment by Marc André Cormier and Ansgar Kahmen where biosynthetic fractionation in leaf waxes was measured for plants grown under different light conditions. This work has been presented at conferences and workshops, but is not yet published, and is therefore not cited in our revised manuscript.
    - Since we first submitted this paper, a new study was published in GCA by Sachs et al. about the effect of light on H-isotope fractionation in the diatom *T. pseudonana*, with results that are somewhat contradictory to those from the previous studies of alkenones. We have updated this section of the discussion to include these new results, but have also cut back the discussion of light intensity considerably, since it seems unlikely to have been a major factor influencing $\alpha_{\text{Lipid-Water}}$ in the surface waters of the lakes in this study.

- p12-29 – lower alpha is confusing here
    - We have revised to consistently refer to lower/higher alpha values, rather than more/less fractionation.

- p13-2 – The ultimate explanation should be the same for all FAs unless you are suggesting a different source.
    - Given that the nC16:1 FA concentrations do not covary with the others, and that there are significant changes in the ratio of nC16:0 to nC16:1, it seems possible that there is a different source for them, or that only a limited number of the nC16:0 producers also make nC16:1, and that their relative abundance varied throughout the study period. We have added this point to the revised manuscript.

- p13-11 – This is a bit of an overstatement. Just because we don't understand the governing mechanisms of species-specific fraction does not make it random.
    - We changed this line to "…varies by hundreds of per mil among species in ways that are not yet understood."

- p13-27 – Can a statistical analysis help you here? Does any taxa change correlate with changes in fractionation?
    - Unfortunately, the two sample sets were not collected on the same day, since they come from two uncoordinated sampling campaigns.
    - We hoped that we would be able to use pigment data from filters collected on the same days as our samples to help address this point. Unfortunately these samples appear to be degraded after ~1.5 years of storage (see response to reviewer 2 for more detail).

- p13-31&32 – This is a false comparison, there are photoheterotrophs in this paper and they look far more like photoautotrophs than they do heterotrophs. You must at least acknowledge the photoheterotrophs.
    - The photoheterotrophs in the Zhang et al. paper (purple sulfur bacteria) do indeed have similar H isotope fractionation to the photoautotrophs. The large differences in H isotope fractionation were between photoautotrophs and obligate heterotrophs, especially heterotrophs that were grown on TCA-cycle precursors and intermediates.
    - We have revised this section to focus more on the possible late winter/early spring contributions of fatty acids from obligate heterotrophs, and removed the discussion of mixotrophy in golden algae, which, as you point out, is unlikely to have a large isotope effect.

- p14-2 – This hypothesis is also not consistent with an oligotrophic lake either. many algae and cyanobacteria are capable of mixotrophy. Almost all of them do this at night and some under diverse conditions.
    - We have removed this hypothesis from the discussion.

- p14-18 – Be careful, there are plenty of bacteria that make primarily 16:0, 16:1, and 18:1.
    - We acknowledge that heterotrophic bacteria can produce a large amount of 16:0, 16:1, and 18:1, and have modified the discussion considerably to consider the role they may have played in contributing to the relatively ^{2}H-enriched April samples.

- p17-14 – Reporting an offset in permil would be helpful to the paleo community
    - This sentence has been removed from the revised manuscript.

- p17-19 – isoprenoid change to sterol, you haven't made the case for isoprenoids more generally.
    - This sentence is no longer in the revised manuscript.

Minor editorial comments:

- p1-9 – isotope → isotopic
  - Changed

- p1-21 – in situ should be italicized here and throughout the manuscript
  - The style guidelines for *Biogeosciences* indicate that common Latin phrases, including "in situ", should not be italicized, so we will leave this unitalicized.

- p1-22 – increased magnitude of fractionation needs a direction term as the fractionation factor for lipid-water can be both above and below 1.
  - We changed this line to specify increased ^{2}H discrimination at higher temperature.

- p2-22-23 – This is stating the obvious, rephrase or combine with the following sentence.
  - We revised as: "However, a number of variables can influence the offset between the $\delta^{2}H$ values between lipids and source water, which is typically expressed by the fractionation factor $\alpha_{Lipid\text{-}Water} = (^{2}H/^{1}H_{Lipid})/(^{2}H/^{1}H_{Water})$."

- p2-25 – also metabolism see Zhang et al., 2009 or Osburn et al. 2016
  - This is a separate issue from the environmental gradients that affect H isotope fractionation in photoautotrophs, which are discussed in this paragraph. However, metabolism can have large effects on d2H values for compounds such as $n\mathrm{C}_{16:0}$ fatty acid, which are produced by heterotrophs and chemoautotrophs, as well as photoautotrophs. This idea was mentioned briefly in the discussion of our initial manuscript. We have now included it in the introduction, and added the references you mentioned, as well as two relevant papers by Heinzelmann et al. and another paper by Osburn et al. We have also expanded the discussion of community metabolism in the new section 4.1.

- p3-1– I think that should be permil per degree
  - Modified

- p3-5 – I am not sure what 'more' fractionation means
  - We mean that alpha is further from unity; in this case, since the fractionation is more negative, alpha is smaller and that represents more fractionation. We revised this sentence to make it clearer, in line with our response to your first comment.

- p3-13 – remove 'the'
  - We removed it

- p4-10 – What is the depth at this sampling point?
  - The water depth was 96m. This information was added to this section.

- p5-24&25 – something is wrong with these sentences. Perhaps a ‘was’ after ‘sample’
    - We added “was” after “sample water”

- p7-10 – Initially? Were they subsequently converted to a different scale?
    - We deleted the word “initially,” which is not relevant

- p7-31 13 needs superscript
    - We changed 13 to ^{13}

- p7-32 – lipid
    - We deleted the “s” at the end of “lipids”

- p8-15-16 – these numbers are the same (1.0 and 1.1) but the text suggests that one should be lower.
    - The concentrations were the same as each other for the first sampling date in April (both ~1 μg/L), but subsequently, nC16:1 concentrations were much lower than those of nC16:0 (13 μg/L = maximum value for nC16:1, 55 μg/L for nC16:0).
    - After condensing this section in line with your earlier comment, this sentence no longer exists.

- p9-19 – add ‘relative’ after depleted
    - We added this

- p14-26 space before 2010
    - We added this

Revised Response to Reviewer 2

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 the changes we made in the revised manuscript. In a few cases, we disagreed with the suggestion, and have explained our reasoning below each point.

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 added the word “transparent” here to clarify

- P8L15 increased from April to July? Or levels were low from April to July? Write more clearly.
    - We changed 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.
    - This sentence has been removed from the revised manuscript

- 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 deleted 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 feedback from reviewer 1 and the associate editor, we have restructured the focus of the discussion considerably. This specific point is no longer included.

- 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.
    - Based on feedback from reviewer 1 and the associate editor, we have restructured the focus of the discussion considerably. The new section 4.2 is called "Relationships between seasonal environmental gradients and $\alpha_{lipid\text{-}water}$ values" and explores the co-varying trends in temperature, light, and nutrient availability, and the roles they may have had in influencing $\alpha_{lipid\text{-}water}$.

- 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 during the peak summer, 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.
    - However, we only considered the potentially large effect of fatty acid contributions from heterotrophs to the springtime signal, when lipid concentrations were relatively low. We have expanded the discussion to consider this possibility considerably in the new section 4.1.

- 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 $n\mathrm{C}_{16:1}$ 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 had hoped to include concentrations about chlorophyll and other pigments from additional frozen filters that were collected on our sampling days. However, these samples were collected and stored without the intention of analyzing pigments, and were kept at -20 °C for ~1.5 years. They were not stored under nitrogen, and seem to have degraded in these conditions. The chromatograms had lots of chlorophyll a degradation products, and the remaining chlorophyll a concentrations were lower than expected for lake surface water (less than 1 μg/L), so we have opted not to include data from them in our analysis.
- 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 $^{2}\mathrm{H}$ 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 similar comments from Reviewer 1 and the associate editor, we have removed this point.

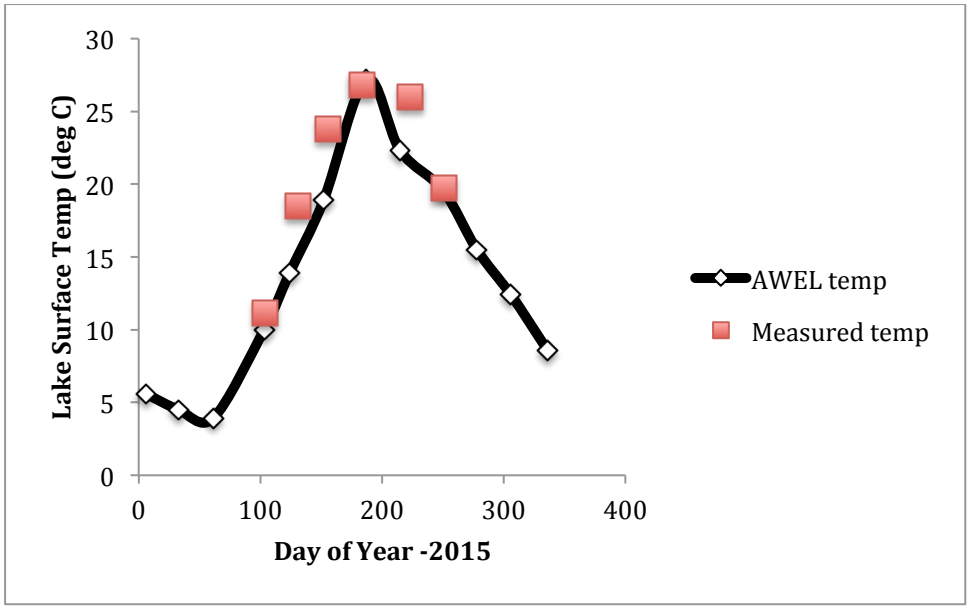
- P13L32. I would be very careful assuming that all heterotrophs have more enriched fatty acids than photoautotrophs based on only one study.
  - Subsequent studies (such as Osburn et al., 2011, doi.org/10.1016/j.gca.2011.05.038, Heinzelmann et al., 2015a, doi: 10.1093/femsle/fnv065, Heinzelmann et al., 2015b, 10.3389/fmicb.2015.00408) have also observed more enriched fatty acids in heterotrophs relative to autotrophs. The importance of net community metabolism was only briefly considered in our original manuscript, but based on feedback from reviewer 1 and the associate editor, we have expanded our focus on this aspect considerably, both in the discussion and in the introduction.

- 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 have added the following text to this paragraph: "Alternatively, the difference in $\alpha_{Brassicasterol\text{-}water}$ values between the two lakes could be due to variable contributions of brassicasterol from different algal sources. While brassicasterol is produced by fewer organisms than short-chain fatty acids, it still has multiple sources (Volkman et al., 1998; Volkman, 2003; Rampen et al., 2010; Taipale et al., 2016). Species-specific differences in hydrogen isotope fractionation have not been observed for sterols, but have been reported for fatty acids and alkenones (Schouten et al., 2006; Zhang & Sachs, 2007), making this an unconstrained possibility that could be responsible for the difference in $\alpha_{Brassicasterol\text{-}water}$ between the oligotrophic and eutrophic lake."

- 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 added surface water temperature curves to Figure 2.


30
25
20
15
10
5
0
Lake Surface Temp (deg C)
0
100
200
300
400
Day of Year -2015
AWEL temp
Measured temp

Interplay of community dynamics, temperature, and productivity on the hydrogen isotope signatures of lipid biomarkers

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Abstract. The hydrogen isotopic composition (δ^2H) of lipid biomarkers has diverse applications in the fields of paleoclimatology, biogeochemistry, and microbial community dynamics. Large changes in hydrogen isotope fractionation have been observed among microbes with differing core metabolisms, while environmental factors including temperature and nutrient availability can affect isotope fractionation by photoautotrophs. Much effort has gone into studying these effects in laboratory studies with single species cultures. Quantifying the natural extent of these changes in freshwater lacustrine settings and identifying their causes is essential for robust application of δ^2H values of common short-chain fatty acids as a proxy of net community metabolism, and of phytoplankton specific biomarkers as a paleohyrologic proxy, yet these remain poorly constrained.

This work targets the effect of community dynamics, temperature, and productivity on $^2H/^1H$ fractionation in lipid biomarkers through a comparative time series in two central Swiss lakes: eutrophic Lake Greifen and oligotrophic Lake Lucerne. Particulate organic matter was collected from surface waters at six time points throughout the spring and summer of 2015, and δ^2H values of short chain fatty acids, as well as chlorophyll-derived phytol and the diatom biomarker brassicasterol, were measured. We paired these measurements with in situ incubations conducted with $NaH^{13}CO_3$, which were used to calculate the production rates of individual lipids in lake surface water. As algal productivity increased from April to June, net discrimination against ^2H in Lake Greifen increased by as much as 148 ‰ for individual fatty acids. During the same time period in Lake Lucerne, net discrimination against ^2H increased by as much as 58 ‰ for individual fatty acids. A large portion of this signal is likely due to a greater proportion of heterotrophically derived fatty acids in the winter and early spring, which are displaced by more ^2H-depleted fatty acids as phytoplankton productivity increases. Smaller increases in ^2H discrimination for phytol and brassicasterol suggest that a portion of the signal is due to changes in net photoautotrophic ^2H fractionation, which may be caused by increasing temperatures, a shift from maintenance to high growth, or changes in the community assemblage. The fractionation factors for brassicasterol were significantly different between the two lakes, suggesting that its hydrogen isotope composition may be more sensitive to nutrient regime than is the case for fatty acids or phytol.

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1 Introduction

Compound specific hydrogen isotope measurements of lipid biomarkers are an emerging tool with diverse applications to microbial community dynamics (Osburn et al., 2011; Heinzelmann et al., 2016), organic matter cycling (Jones et al., 2008; Li et al., 2009), and paleoclimatology (Sachse et al., 2012 and sources therein). The hydrogen isotopic composition of source water exerts a first order control on lipid hydrogen isotopes (expressed as $\delta^2H = ({}^2H/{}^1H_{Sample})/({}^2H/{}^1H_{VSMOW}) - 1$) (Sessions et al., 1999; Sauer et al., 2001; Sachs, 2014). However, a number of variables can influence the offset between the δ^2H values between lipids and source water, which is typically expressed by the fractionation factor $\alpha_{Lipid\text{-}Water} = ({}^2H/{}^1H_{Lipid})/({}^2H/{}^1H_{Water})$.

For short-chain (C < 20) fatty acids, which can be synthesized by a diverse range of organisms, including photoautotrophs, chemoautotrophs, and heterotrophs, core metabolism typically exerts a large control on $\alpha_{Lipid\text{-}Water}$, with variability in δ^2H values exceeding 500 ‰ for organisms grown on the same source water (Zhang et al., 2009a; Osburn et al., 2011; Heinzelmann et al. 2015a; Osburn et al., 2016). These metabolic differences have led to the suggestion that δ^2H values of short-chain fatty acids can be used as an indicator of net community metabolism (Zhang et al., 2009a; Osburn et al., 2011; Heinzelmann et al., 2016; Osburn et al., 2016). This application has previously been assessed in coastal marine settings (Heinzelmann et al., 2016) and hot springs (Osburn et al., 2011), but not in lakes.

The δ^2H values of lipids produced exclusively by photoautotrophs, such as alkenones and certain sterols, have received particular attention as a proxy for past water isotopes (Sessions et al., 1999; Sauer et al., 2001; Huang et al., 2004; Sachse et al. 2012; Sachs, 2014), which is useful for paleoclimatologists seeking to reconstruct changes in temperature, moisture source, and the balance of precipitation to evaporation, all of which influence δ^2H values of water (Craig and Gordon, 1965; Gat, 1996; Henderson and Schuman, 2009; Steinmann et al., 2013). The hydrogen isotopic composition of lipids produced by cyanobacteria and eukaryotic algae is well correlated with those of source water in laboratory and field settings (Sauer et al., 2001; Huang et al., 2004; Englebrecht and Sachs, 2005; Zhang and Sachs, 2007; Sachse et al., 2012), and is stable under near surface temperatures and pressures for carbon-bound hydrogen (Sessions et al., 2004; Schimmelmann et al., 2006). Hydrogen isotopes of biomarkers from eukaryotic algae have been successfully applied to infer changes in past climate using sediment cores from diverse lakes (Huang et al., 2002; Sachs et al., 2009; Smittenberg et al., 2011; Atwood and Sachs, 2014; Zhang et al., 2014; Nelson and Sachs, 2016; Richey and Sachs, 2016; Randlett et al., 2017) and marine settings (Pahnke et al., 2007; van der Meer et al., 2007; van der Meer et al., 2008; Leduc et al, 2013; Vasiliev et al., 2013; Kasper et al., 2014; Vasiliev et al., 2017).

However, among photoautotrophs, there is increasing evidence that $\alpha_{Lipid\text{-}Water}$ is not constant, and can change with variables such as salinity, species, light availability, growth rate, and temperature (summarized in Table 1) (Sachs, 2014 and sources therein; Chivall et al., 2014; M'boule et al., 2014; Nelson and Sachs, 2014; Heinzelmann et al., 2015b; Sachs and Kawka, 2015; van der Meer et al., 2015; Wolhowe et al., 2015; Maloney et al., 2016; Sachs et al., 2016; Sachs et al., 2017). While the

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array of secondary isotope effects may appear daunting, these relationships can provide useful information about past environmental changes in their own right, and developing a thorough understanding of them is important for robust interpretations of δ^2H_{Lipid} values from phytoplankton.

Most previous investigations into variability in $\alpha_{Lipid\text{-}Water}$ in algal lipid biosynthesis have been done with controlled axenic cultures of eukaryotes in laboratory settings. While similar relationships between salinity and $\alpha_{Lipid\text{-}Water}$ have been observed for eukaryotic algal and cyanobacterial lipids in both laboratory (Schouten et al., 2006; Chivall et al., 2014; M'boule et al., 2014; Heinzelmann et al., 2015b; Maloney et al., 2016; Sachs et al., 2016) and field calibrations (Sachse and Sachs, 2008; Sachs and Schwab, 2011; Nelson and Sachs, 2014), the temperature and growth rate effects observed in cultures have yet to be assessed in lacustrine settings where photoautrophic δ^2H_{Lipid} values are likely to be applied to reconstruct past hydroclimate. In contrast to cultures, lake water contains a diverse and dynamic community of phytoplankton, most of whom contribute lipids to the sediment that cannot be attributed to one particular species. The culturing data that exist are limited to a few species, many of which are only found in marine environments.

In order to evaluate the significance of temperature and growth rate effects on the hydrogen isotopic composition of algal lipids produced in lakes, we collected monthly samples of particulate organic matter in two lakes in central Switzerland throughout the spring and summer of 2015. Both lakes experience similar changes in surface water temperature during this time period, but one of them (Lake Greifen) is characterized by high nutrient availability and increasing algal productivity and biomass throughout the spring and early summer. The other lake (Lake Lucerne) is oligotrophic and had relatively low, constant rates of algal productivity throughout the study period. We paired measurements of hydrogen isotope fractionation with in situ incubations designed to determine lipid production rates, allowing us to distinguish between the effects of productivity and temperature on hydrogen isotope fractionation.

In addition to measuring δ^2H values of brassicasterol (24-methyl cholest-5,22-dien-3β-ol) and phytol, lipids that are produced exclusively by photoautotrophs, we also analysed short-chain fatty acids ($nC_{14:0}$, $nC_{16:0}$, $nC_{16:1}$, $nC_{18:1}$), which, although they are typically the most abundant lipids in algal and cyanobacterial cells, are also synthesized by heterotrophic and chemoautotrophic microbes. The time series of fatty acid δ^2H values from an oligotrophic and eutrophic lake presented here is the first opportunity to assess how changes in net community metabolism might be recorded by these compounds in lakes.

2 Methods

2.1 Site description

Lake Greifen (Greifensee) is a small perialpine lake, located in the eastern fringes of the Zurich metropolitan area at 47° 21' N and 8° 40' E (Fig. 1). The lake has a surface area of 24 km^2, and a maximum depth of 32 m. The lake is fed by three small brooks, and has one main outlet, the Glatt

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Canal. Lake Greifen experienced severe eutrophication in the mid-20th century (Hollander et al., 1992; Keller et al., 2008). Since strict government regulations on nutrient inputs were imposed in the 1970s the water quality in the lake has improved, but its deep water remains anoxic and nutrient levels in the upper water column are still elevated. Winter overturn in the lake brings additional nutrients to the surface water, resulting in large phytoplankton blooms in the spring and summer as temperature and light availability increase (McKenzie, 1982). All samples from Lake Greifen were collected from the northern part of the lake, near a permanent platform maintained by Eawag (at 47° 21.99' N, 8° 39.89' E).

Lake Lucerne (Vierwaldstättersee) is a large perialpine lake, located in central Switzerland at 47° 0' N and 8° 30' E (Fig. 1). The lake, which has a total surface area of 116 km^2, is formed of seven distinct basins, of which the deepest is 214 m. The lake is fed by four alpine rivers: the Reuss, Muota, Engelberger Aa, and Sarner Aa, and its primary outflow is Reuss river from the northwest tip of the lake. Although Lake Lucerne experienced a mild eutrophication event in the 1970s, it is oligotrophic today (Bürgi et al., 1999; Bührer and Ambühl, 2001; Thevenon et al., 2012). All samples from Lake Lucerne were collected from the center of Kreuztricher basin (near 8° 21' N, 47° 0' E), with a water depth of 96 m.

2.2 Sample collection

Particulate material in each lake was collected at approximately monthly intervals throughout the spring and summer of 2015 (mid-April through early September). Surface water (~0.5 m water depth) was filtered onto a pre-combusted 142 mm diameter GF/F filter (0.7 µm pore size) using a WTS-LV Large Volume Pump (McLane, Massachusetts, USA). Pumping began at 7 L/min and continued until the flow rate decreased to 4 L/min or until 25 minutes had passed. All filters were collected at midday on sunny or mostly sunny days. Filters were wrapped in combusted aluminum foil and stored in a cool box on ice until transport to the laboratory, where they were stored at -20 °C until analysis.

Water samples were collected from surface water before and after pumping began. Samples were collected in 4 mL screw cap vials, sealed with electrical tape, and stored at room temperature prior to analysis. Depth profiles of temperature, conductivity, pH, turbidity, and dissolved oxygen were collected for the upper 20m of the water column each sampling day at the beginning and end of filtration using a multiparameter CTD probe (75M, Sea & Sun Marine Tech, Trappenkamp, Germany).

On the morning of each sampling day, 4 x 12.5 L of surface water was collected in acid-rinsed, autoclaved, transparent carboys for in situ incubations. In two of the four carboys, 1 mL of concentrated $NaH^{13}CO_3$ solution was added. The other two carboys were unlabeled. Carboys were mixed and attached to a fixed, floating line so that they stayed in the upper 50 cm of lake water throughout the day. After 6 hours, they were retrieved and the contents were filtered onto a pre-combusted 142 mm diameter GF/F filter using a peristaltic pump. Water samples for DIC analyses were collected in 12 mL exetainers prior to isotopic labeling, after labeling but before incubation, and

after incubation. These samples were sterile filtered through a 0.2 µm syringe filter and stored in the dark at 4 °C prior to analysis.

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2.3 Water isotope measurements

Surface water isotope samples were filtered through a 25 mm syringe filter with a 0.45 µm polyethersulfone membrane to remove particulate matter. Water δ^2H and $\delta^{18}O$ values were measured by Cavity Ring Down Spectroscopy (CRDS) on a L-2120i Water Isotope Analyzer (Picarro, Santa Clara, CA, USA) at ETH-Zurich. Each sample was injected seven times in sequence, and the first four values were discarded to avoid any memory effects from the previous sample. Three water standards with known δ^2H values of ranging from -161‰ to 7‰ and $\delta^{18}O$ values ranging from -22.5‰ to 0.9‰ were injected at the beginning and end of each sequence, as well as after every 10 samples. These standards were used to correct measured values to the VSMOW scale and to account for any instrumental drift over the course of the sequence. Average standard deviations were 0.4‰ for hydrogen isotopes and 0.06‰ for oxygen isotopes.

2.4 DIC concentrations and $\delta^{13}C$ measurements

DIC concentrations were measured on a TOC-$L_{CSH/CHN}$ Total Organic Carbon Analyzer (Shimadzu, Kyoto, Japan). Solutions with DIC concentrations ranging from 5 mg/L to 100 mg/L were injected at the beginning of the sequence to form a calibration curve, and one standard of 50 mg/L was run after every five samples. Samples were analyzed in triplicate.

3.7 mL exetainers were prepared for $\delta^{13}C$ measurements of DIC by adding 100 µL of concentrated H_3PO_4 and filling the headspace with He. 1 mL of sample water was added with a syringe through the septa of the exetainer. Samples were allowed to equilibrate overnight before analysis. Carbon isotope values were measured on an Isotope Ratio Mass Spectrometer (IRMS) (Isoprime, Stockport, United Kingdom). A standard of known isotopic composition was analyzed after every 6 samples. All samples were measured in duplicate.

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2.5 Lipid extraction and purification

An internal standard containing nC_{19}-alkanol, nC_{19}-alkanoic acid, and 5α-cholestane was quantitatively added to freeze-dried filters, which were extracted in 30 mL of 9:1 Dichloromethane (DCM)/Methanol (MeOH) in a SOLVpro Microwave Reaction System (Anton Paar, Graz, Austria) at 70 °C for 5 minutes (Randlett et al., 2017), centrifuged, and the supernatant containing the total lipid extract (TLE) was poured off and evaporated under a gentle stream of N_2. The TLE was saponified with 3 mL of 1 N KOH in MeOH and 2 mL of solvent-extracted nanopure H_2O for 3 hours at 80 °C (Randlett et al., 2017), after which the neutral fraction was extracted with hexane. Subsequently, the aqueous phase was acidified to pH = 2, and the protonated fatty acids were extracted with hexane.

Neutral fractions were further purified using silica gel column chromatography, following a scheme modified from Randlett et al. (2017). The sample was dissolved in hexane and loaded onto a 500 mg / 6

mL Isolute Si gel column (Biotage, Uppsala, Sweden). *N*-alkanes were eluted in 4 mL of hexane, aldehydes and ketones in 4 mL of 1:1 Hexane/DCM, alcohols in 4 mL of 19:1 DCM/MeOH, and remaining polar compounds in 4 mL of MeOH. The alcohol fraction was acetylated with 25 µL of acetic anhydride and 25 µL of pyridine for 30 minutes at 70 °C. The δ^2H and $\delta^{13}C$ values of the added acetyl group were determined by analyzing acetylated and unacetylated nC_{12}-alkanol.

Further purification was necessary in order to obtain base line separation of brassicasterol for δ^2H measurements. This was achieved by loading the acetylated alcohol fraction onto 500 mg of Si gel impregnated with $AgNO_3$ (10% by weight, Sigma Aldrich) in a 6 mL glass cartridge. The first fraction, containing *n*-alkanols and phytol, was eluted with 20 mL of 4:1 hexane/DCM, the second fraction, containing stanols and singly unsaturated sterols (such as cholesterol) with 20 mL of 1:1 hexane/DCM, the third fraction, containing most doubly unsaturated sterols including brassicasterol, with 16 mL of DCM, and the remaining compounds with 4 mL of ethyl acetate.

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Fatty acid fractions were methylated with 1 mL of BF_3 in MeOH (14% by volume, Sigma Aldrich) for 2 hours at 100 °C. After methylation, 2 mL of nanopure H_2O was added to the sample and the fatty acid methyl esters (FAMEs) were extracted with hexane. The δ^2H and $\delta^{13}C$ values of the added methyl group were determined by methylating phthalic acid of known isotopic composition (Arndt Schimmelmann, Indiana University).

FAMEs and brassicaterol were quantified by gas chromatography – flame ionization detection (GC-FID) (Shimazdu, Kyoto, Japan). Samples were injected by an AOC-20i autosampler (Shimadzu) through a split/splitless injector operated in splitless mode at 280 °C. The GC column was an InertCap 5MS/NP (0.25 mm x 30 m x 0.25 µm) (GL Sciences, Japan) and it was heated from 70 °C to 130 °C at 20 °C/min, then to 320 °C at 4 °C/min, and held at 320 °C for 20 minutes. FAMEs were identified by comparing their retention times to an external standard (Fatty Acid Methyl Ester mix from Sulpelco, Ref 47885-U). Brassicasterol and phtyol were identified by comparing their retention times to those obtained by analyzing a subset of samples by gas chromatography – mass spectrometry (GC-MS) under identical conditions. In order to determine how much of the compound was in the original sample, peak areas were normalized to those of the internal standard. Peak areas were quantified relative to an external calibration curve in order to determine suitable injection volumes for isotopic analysis.

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2.6 Lipid δ^2H and $\delta^{13}C$ measurements

The stable isotope values of individual FAMEs and brassicasterol were measured by gas chromatography – isotope ratio mass spectrometry (GC-IRMS). A GC-1310 gas chromatograph (Thermo Scientific, Bremen, Germany) equipped with an InertCap 5MS/NP (0.25 mm x 30 m x 0.25 µm) (GL Sciences, Japan) was interfaced to a Delta Advantage IRMS (Thermo Scientific) with a Conflow IV interface (Thermo Scientific). Samples were injected with a TriPlusRSH autosampler to a PTV inlet operated in splitless mode at 280 °C. The oven was heated from 80 °C to 215 °C at 15

°C/min, then to 320 °C at 5 °C/min, and then was held at 320 °C for 10 minutes. Hydrogen isotope samples were pyrolyzed at 1420 °C after they eluted from the GC column. Carbon isotope samples were combusted at 1020 °C after elution.

Raw isotope values were converted to the VSMOW (hydrogen) and VPDB (carbon) scales using Thermo Isodat 3.0 software and pulses of a reference gas that was measured at the beginning and end of each analysis. Sample δ^2H and $\delta^{13}C$ values were further corrected using the slope and intercept of measured and known values of isotopic standards ($nC_{17,\ 19,\ 21,\ 23,\ 25,\ 28,}$ and $_{34}$-alkanes, Arndt Schimmelmann, Indiana University), which were run at the beginning and end of each sequence, as well as after every 6 to 8 sample injections. Offsets between measured and known values for these standards were used to correct for any drift over the course of the sequence or any isotope effects associated with peak area or retention time. The standard deviation for these standards averaged 4‰ and the average offset from their known values was 2‰ for hydrogen isotopes. For carbon isotopes, the average standard deviation of isotopic standards was 0.4‰ and the average offset from known values was 0.1‰ over the period of analysis.

An additional standard of nC_{29}-alkane was measured three times per sequence, corrected in the same way as the samples, and used for quality control. The standard deviation of these measurements was 4‰ for hydrogen and 0.5‰ for carbon over the period of analysis. The H_3^+ factor was measured at the beginning of each sequence and averaged 3.6 ± 0.3 during the analysis period. Samples were corrected for hydrogen and carbon added during derivatization using isotopic mass balance, and reported errors represent propagated errors from replicate measurements and the uncertainties associated with the added hydrogen.

2.7 Calculated lipid production rates

Lipid production rates were calculated using Eq. (1) (modified from Popp et al. 2006):

$$Production\ rate = (\delta^{13}C_l - \delta^{13}C_n)/(\delta^{13}C_{DIC} - \delta^{13}C_n) * (C_l/t) \quad (1)$$

where $\delta^{13}C_l$ is the $\delta^{13}C$ value of the target compounds from labeled incubations, $\delta^{13}C_n$ is that from unlabeled incubations, $\delta^{13}C_{DIC}$ is the $\delta^{13}C$ value of DIC, C_t is the concentration of the lipid at the end of the incubation, and t is the duration of the incubation. Residence times – assuming a steady state, the amount of time needed to replace all molecules of a given lipid – were calculated by dividing C_t by the production rate, which reduces to Eq. (2):

$$Residence\ time = t*(\delta^{13}C_{DIC} - \delta^{13}C_n)/(\delta^{13}C_l - \delta^{13}C_n) \quad (2)$$

2.8 Statistics

PRISM software (Graphpad Software Inc., La Jolla, CA, USA) was used to carry out all statistical analyses. Ordinary least squares regression was used to determine relationships between fractionation

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factors and temperature, fractionation factors and lipid production rates. Regression lines are only shown where the slope of the regression was significantly different from 0 at the $p < 0.05$ level. The results of all linear regression analyses are presented in Table 2. Differences between the slopes of various regressions were assessed using a two-tailed test of the null hypothesis that both slopes are equal. Differences in the mean values of replicate measurements were determined using an unpaired, two-tailed t-test, and were considered significantly different for $p < 0.05$.

3. Results

3.1 Lipid concentrations and production rates

Lipid concentrations increased significantly in Lake Greifen from April to July, and then declined slightly from July to September, except for phytol and nC$_{16:1}$ fatty acid, which had increasing concentrations into the late summer (Fig. 2a). nC$_{16:0}$ fatty acid had the highest concentrations, while those of nC$_{16:1}$ fatty acid were usually the lowest, except in April-May, when nC$_{14:0}$ was the least abundant fatty acid. Brassicaterol concentrations were 1-2 orders of magnitude smaller than those of fatty acids and phytol concentrations were intermediate (Fig. 2a). Lipid concentrations in Lake Lucerne were generally an order of magnitude lower than in Lake Greifen (Fig. 2b). Fatty acid concentrations increased significantly from April to May in Lake Lucerne, and were then relatively stable throughout the rest of the time series (Figure 2b). Again, nC$_{16:0}$ fatty acid had the highest concentrations and nC$_{16:1}$ was the least abundant fatty acid. Phytol concentrations were typically an order of magnitude lower than those of fatty acids in Lake Lucerne, and increased slightly over the course of the time series. Brassicasterol concentrations were an order of magnitude lower still, and reached a maximum in June (Fig. 2b).

In both lakes, fatty acid production rates were highest for nC$_{16:0}$, followed by nC$_{18:x}$ (unsaturated C$_{18}$ fatty acids, primarily nC$_{18:1n9c}$, or oleic acid), nC$_{14:0}$, and nC$_{16:1}$ (palmitoleic acid) (Fig. 2c and 2d). Phytol and brassicasterol production rates were 2 - 3 orders of magnitude lower in both lakes than those of fatty acids (Fig. 2c and 2d). Lipid production rates were up to three times higher in Lake Greifen than in Lake Lucerne (Fig. 2c and 2d). Lipid production rates generally increased from May to July and then remained high in Lake Greifen, while in Lake Lucerne they were relatively constant throughout the study period (Fig. 2c and 2d).

Residence times – or the amount of time necessary to replace all molecules of a given compound assuming steady state – of individual lipids were calculated according to Eq. (2) (Sect. 2.7) and were typically shortest for nC$_{14:0}$, nC$_{16:0}$, and nC$_{18:x}$ fatty acids, with values as low as 9 ± 1 hr in Lake Greifen in May, and as low as 12 ± 3 hr in Lake Lucerne in August (Table 3). Of the fatty acids, nC$_{16:1}$ had the longest residence times, reaching 60 ± 13 hr in Lake Greifen in May and 62 ± 27 hr in Lake Lucerne in June (Table 3). Brassicasterol residence times were the longest of any lipid in the first part of the time series, but were exceeded by phytol for the last two sampling dates (Table 3).

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3.2 Lipid δ^2H and $\alpha_{lipid\text{-}water}$ values

In both lakes lipid δ^2H values typically decreased over the spring and summer (Fig. 2e and 2f). This effect was most pronounced for fatty acids in Lake Greifen. For example, nC$_{16:0}$ fatty acid δ^2H values declined by 133 ‰ (from -172 ‰ to -305 ‰) from April to August in Lake Greifen, while they only declined by 53 ‰ (from -249 ‰ to -302 ‰) over the same time period in Lake Lucerne (Fig. 2e and 2f). During the same time period water δ^2H values increased slightly in Lake Greifen (from -73 ‰ to -65 ‰) and were relatively constant in Lake Lucerne (fluctuating between -82 ‰ and -86 ‰) (S2). Changes in the fractionation factor between fatty acids and surface water ($\alpha_{lipid\text{-}water}$) were therefore primarily due to changes in fatty acid δ^2H values. In Lake Greifen, $\alpha_{lipid\text{-}water}$ for nC$_{16:0}$-fatty acid decreased from 0.891 to 0.743 from April to August (Fig. 2g), while in Lake Lucerne, it decreased from 0.821 to 0.763 (Fig. 2h). Similar patterns were observed for $\alpha_{lipid\text{-}water}$ for nC$_{14:0}$, nC$_{16:1}$ and nC$_{18:X}$ fatty acids (Fig. 2g and 2h). Values for $\alpha_{lipid\text{-}water}$ were less variable for phytol and brassicasterol than for fatty acids, although they also declined from April to May. Brassicasterol was always ^{2}H-depleted relative to fatty acids in Lake Greifen, and was depleted in ^{2}H relative to all fatty acids except nC$_{14:0}$ in Lake Lucerne (Fig. 2g and 2h). Phytol δ^2H values were the most ^{2}H depleted of any lipid measured in either lake (Fig. 2g and 2h).

Overall, fatty acid $\alpha_{lipid\text{-}water}$ values were negatively correlated with lake surface temperature in both lakes (R^2 = 0.32, p = 0.004 in Lake Greifen; R^2 = 0.24, p = 0.01 in Lake Lucerne) (Table 2; Fig. 3; Supp. Fig. 1). The slope of the relationship was significantly steeper (p = 0.03) in Lake Greifen than in Lake Lucerne (m = -0.006 ± 0.002 in Lake Greifen and -0.003 ± 0.001 in Lake Lucerne). Significant correlations were observed between lake surface temperature and $\alpha_{lipid\text{-}water}$ values for most fatty acids, but not for nC$_{16:1}$ in Lake Greifen and nC$_{14:0}$ in Lake Lucerne (Table 2; Fig. 3a; Supp. Fig. 1). Significant relationships between lake surface temperature and $\alpha_{lipid\text{-}water}$ values were not observed in either lake for brassicasterol or phytol (Fig. 3; Table 2).

Fatty acid production rates were not correlated with $\alpha_{lipid\text{-}water}$ values in either lake (Table 2). Among individual fatty acids, only nC$_{16:1}$ fatty acids from Lake Greifen had a significant negative correlation between $\alpha_{lipid\text{-}water}$ values and production rate (R^2 = 0.84; p = 0.03) (Table 2). Brassicasterol and phytol production rates were not correlated with $\alpha_{lipid\text{-}water}$ in either lake (Table 2), although brassicasterol $\alpha_{lipid\text{-}water}$ values from Lake Lucerne cluster as a significantly higher group than in Lake Greifen (p = 0.0002).

4. Discussion

In both lakes, the most striking feature of the lipid δ^2H values in the particulate organic matter (POM) is the significant decrease that occurs for most lipids during the spring (April – June) (Fig. 2e and 2f). As the lake water δ^2H values increased slightly (Greifen) or remained constant (Lucerne) throughout the summer, this trend indicates increased hydrogen isotope fractionation and a decrease in $\alpha_{lipid\text{-}water}$ (Fig. 2g and 2h). There are a number of factors that could contribute to this decline in $\alpha_{lipid\text{-}water}$, but

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they generally group into two categories: changes in lipid source or changes in environmental variables, such as temperature, light, and productivity.

4.1 Potential changes in lipid source

Hydrogen isotope fractionation for short-chain fatty acids varies significantly among organisms with different metabolisms (Zhang et al., 2009a; Osburn et al., 2011; Heinzelmann et al., 2015a). In general, fatty acids from heterotrophs grown on tricarboxylic acid (TCA)-cycle precursors are most enriched in ^{2}H, followed by heterotrophs grown on sugars, then photoautotrophs, and finally chemoautotrophs (Zhang et al., 2009a; Osburn et al., 2011; Heinzelmann et al., 2015a). This variability can be greater than 500‰, and has led to the suggestion that the δ^2H values of ubiquitous compounds such as palmitic acid can be used as a proxy of net community metabolism. For example, at a coastal site in the North Sea, fatty acid chain-weighted average δ^2H values declined by more than 40‰ during the spring phytoplankton bloom, which was attributed to increased contributions from photoautotrophs (Heinzelmann et al., 2016).

In Lakes Lucerne and Greifen, large decreases in fatty acid δ^2H values from April to May coincide with increases in fatty acid concentrations of 1-2 orders of magnitude (Fig. 2). It is therefore possible that the ^{2}H-enriched April samples represent a wintertime background of mixed heterotrophic and autotrophic derived compounds. As phytoplankton productivity ramped up with warmer temperatures, water column stratification, and longer daylight hours in the spring, newly produced fatty acids from photoautotrophs could have overwhelmed the heterotrophic signature, causing the net fatty acid δ^2H values to decrease. The increase in phytoplankton cell density (Fig. 4) from April onward in both lakes is supportive of increased contributions of fatty acids from phytoplankton as the study period progressed.

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For Lake Greifen, a simple isotopic mass balance indicates that 31% of the total nC$_{16:0}$ fatty acid would need to come from heterotrophs with $\alpha_{lipid\text{-}water}$ values of 1.200 (the maximum observed by Zhang et al., 2009a) in mid-April if the remaining nC$_{16:0}$ fatty acid was derived from phytoplankton with $\alpha_{lipid\text{-}water}$ values of 0.750 (assuming mid-summer $\alpha_{lipid\text{-}water}$ values represent the phytoplankton end member). Similar calculations suggest that 16% of nC$_{16:0}$ fatty acid would need to come from heterotrophic bacteria in mid-April in Lake Lucerne in order to account for the 50‰ decrease in nC$_{16:0}$ fatty acid δ^2H values over the course of the summer. These calculations assume that all heterotrophic bacteria use the highest $\alpha_{lipid\text{-}water}$ value ever observed for heterotrophs, and that they primarily use the TCA-cycle, rather than glycolysis. If, as is likely, there is diversity in $\alpha_{lipid\text{-}water}$ values for short-chain fatty acids produced by the heterotrophic bacteria, and at least some of the heterotrophs are relying primarily on glycolysis, the portion of these fatty acids from heterotrophic sources in April would need to be even higher than the values calculated above. This would necessitate a proportionally larger winter heterotrophic contribution of fatty acids than was observed in the coastal North Sea (Heinzelmann et al., 2016), and it seems likely that other variables may contribute to the springtime decline in fatty acid δ^2H values.

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Contributions from heterotrophs are also an improbable explanation for ^{2}H-enriched brassicasterol and phytol in April. Brassicasterol is a sterol that is commonly used as a biomarker for diatoms, although it has also been detected in some non-diatom eukaryotic phytoplankton (Volkman et al., 1998; Volkman, 2003; Rampen et al., 2010; Taipale et al., 2016) and occasionally in plant oils (Zarrouk et al., 2009). Since brassicasterol is not produced by bacterial sources, it seems improbable that the 25‰ (Greifen) and 19‰ (Lucerne) decreases in its δ^2H values from April to May could be due to heterotrophic contributions during the winter, as suggested for fatty acids. The 46‰ April – May decrease in δ^2H values of Lake Greifen phytol, the side-chain of chlorophyll molecules, is also unlikely to be caused by heterotrophic contributions in the early spring. Although phytol is produced by some photoheterotrophs, these typically have similar $\alpha_{lipid\text{-}water}$ values to photoautotrophs (Zhang et al., 2009a).

However, seasonal changes in the phytoplankton community composition alone could be a significant source of variability in $\alpha_{lipid\text{-}water}$ over the course of the study period. Hydrogen isotope fractionation for nC$_{16:0}$ fatty acid has been demonstrated to vary by over 100‰ among five different species of freshwater green algae grown in laboratory batch cultures (Zhang and Sachs, 2007). Such variations may be due to different enzymes involved in lipid synthesis among different species, or to the colony-forming behaviour of the two species with higher $\alpha_{lipid\text{-}water}$ values. Smaller species-dependent variations in $\alpha_{lipid\text{-}water}$ have been observed in cultures of haptophytes (~30 ‰ offset between alkenones in *G. oceanica* and in *E. huxleyi*; Schouten et al., 2006). Since there is limited data from culturing experiments, it is not possible to say how widespread such interspecies variability is. It is possible that most phytoplankton display similar magnitudes of hydrogen isotope fractionation during lipid synthesis under similar conditions. However, it is equally possible that lipid synthesis varies by hundreds of per mil among species in ways that are not yet understood.

Given this uncertainty, and the significant changes in abundance of different phytoplankton taxa in Lake Greifen over the course of 2015 (Fig. 4a), contributions of short-chain fatty acids from different species of algae with different magnitudes of hydrogen isotope fractionation could account for some or all of the seasonal variability in $\alpha_{lipid\text{-}water}$. A comparable data set of algal species counts does not exist for Lake Lucerne from 2015, but bi-monthly data has been compiled from 2014 (Fig. 4b). Some changes in relative distributions of taxa are similar between the two lakes; for example, both Lake Greifen in 2015 and Lake Lucerne in 2014 experienced a peak in golden algae (*Chrysophyceae*) in June, and elevated abundance of cyanobacteria in late summer and late autumn (Fig. 4). Other trends differ starkly between the two lakes. Green algae (*Chlorophyceae*) are largely absent from Lake Lucerne, while they make up a significant portion of the algal community in Lake Greifen. Notably, the relative abundance of green algae steadily increased from May to September in Lake Greifen (Fig. 4), during which time $\alpha_{lipid\text{-}water}$ values declined at a greater rate for most compounds than they did in Lake Lucerne (Fig. 2). If green algae tend to have lower $\alpha_{lipid\text{-}water}$ values than other algal taxa, their greater

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abundance in Lake Greifen throughout the summer could account for the greater decline in $\alpha_{lipid\text{-}water}$ over the course of the time series than in Lake Lucerne.

4.2 Relationships between seasonal environmental gradients and $\alpha_{lipid\text{-}water}$ values

Even for lipids produced in axenic cultures of eukaryotic algae, several factors have been shown to influence hydrogen isotope fractionation, including salinity, light availability, temperature, and growth rate (Summarized in Table 1) (Sachs, 2014 and sources therein; Chivall et al., 2014; M'boule et al., 2014; Heinzelmann et al., 2015b; Sachs and Kawka, 2015; van der Meer et al., 2015; Wolhowe et al., 2015; Maloney et al., 2016; Sachs et al., 2016; Sachs et al., 2017). Of these, salinity can be excluded as a source of variability in the freshwater Lakes Greifen and Lucerne. The effect of light availability on $\alpha_{lipid\text{-}water}$ has only been detected at low light levels (below 250 µmol photons m^{-2} s^{-1}) (van der Meer et al., 2015; Wolhowe et al., 2015; Sachs et al., 2017). Although photosynthetically available radiation (PAR) was not measured as part of the present study, all samples were collected from lake surface water at a mid-latitude northern hemisphere site during boreal spring and summer, and it is unlikely that PAR was less than 250 µmol photons m^{-2} s^{-1} at any sampling date (Pinker and Laszlo, 1992), meaning that the effect of light intensity is unlikely to be a source of the observed seasonal variability in $\alpha_{lipid\text{-}water}$ in lake surface water.

Lake Surface Temperature (LST) varied from 11 to 27 °C in Lake Greifen and from 11 to 25 °C in Lake Lucerne over the study period (Fig. 2i and 2j), and therefore may have contributed to the seasonal changes in lipid δ^2H values. In laboratory cultures of eukaryotic algae, $\alpha_{lipid\text{-}water}$ values for acetogenic lipids have been shown to decrease by 0.002 – 0.004 per °C, resulting in more depleted δ^2H values (Schouten et al., 2006; Zhang et al., 2009b; Wolhowe et al., 2009; Wolhowe et al., 2015). Increased hydrogen isotope fractionation at higher temperatures has been attributed to (*i*) changes in the relative activity of different enzymes involved in lipid synthesis at different temperatures, (*ii*) changes in the relative amount of NADPH from the pentose phosphate cycle as temperature changes, and (*iii*) the potential for hydrogen tunneling at higher temperatures as substrate-enzyme complex vibrations increase (Sachs, 2014 and references therein). The relationship between temperature and hydrogen isotope fractionation in cultures is similar to that observed for fatty acids in Lake Lucerne, where $\alpha_{lipid\text{-}water}$ decreases by 0.003 ± 0.001 per °C (Fig. 3; Table 2). The relationship between $\alpha_{lipid\text{-}water}$ and temperature for fatty acids in Lake Greifen (-0.006 ± 0.002 ‰ per °C) (Fig. 3; Table 2) is much steeper than that observed in culturing studies.

If the influence of temperature on hydrogen isotope fractionation is consistent among laboratory cultures and lakes, warmer temperatures can account for the entire seasonal change in $\alpha_{lipid\text{-}water}$ for fatty acids in Lake Lucerne. However, increasing temperatures would only be able to explain part of the decrease in fatty acid δ^2H values in Lake Greifen over the course of the spring and summer. At most, temperature could account for half of the decrease in $\alpha_{lipid\text{-}water}$ in Lake Greifen, assuming a consistent relationship to that observed in cultures.

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Brassicasterol and phytol $\alpha_{lipid\text{-}water}$ values do not have strong relationships with lake surface temperature. There is no correlation between lake surface temperature and $\alpha_{lipid\text{-}water}$ values for phytol in either lake, nor for brassicasterol in Lake Lucerne. In Lake Greifen the two variables are negatively correlated for brassicasterol, although the relationship is not quite significant (Fig. 3f; Table 2). The slope of the relationship between $\alpha_{Brassicasterol\text{-}water}$ and lake surface temperature in Lake Greifen is significantly shallower than that observed for fatty acids (-0.002 ± 0.001 for brassicasterol vs. -0.008 ± 0.002 for nC$_{16:0}$ fatty acid). Given these relatively weak relationships, it seems unlikely that temperature influences hydrogen isotope fractionation of either brassicasterol or phytol. The negative correlations between temperature and $\alpha_{lipid\text{-}water}$ values for fatty acids may therefore be an artefact of the probable increase in photoautotrophically-derived compounds as it became warmer throughout the spring (Section 4.1). On the other hand, the relationship between temperature and $\alpha_{lipid\text{-}water}$ values in cultures has only been observed for *n*-alkanoic acids and alkenones, both of which are acetogenic lipids. It is thus possible that temperature is partially responsible for the decreases in $\alpha_{lipid\text{-}water}$ values as temperature increased in Lakes Lucerne and Greifen, but that the responsible mechanism is specific to lipids produced acetogically, and does not affect isoprenoids, such as sterols and phytol.

Phytoplankton productivity and biomass also increased with temperature in the spring and summer in Lakes Greifen and Lucerne, with a more marked effect in nutrient-rich Greifen (Fig. 2a-d, Fig 4). This trend could also partially explain the increase in ^{2}H fractionation and decrease in lipid δ^2H values that co-occurred with rising temperatures. Increased nutrient availability and higher growth rates have been shown to result in increased hydrogen isotope fractionation during lipid synthesis for eukaryotic algae in laboratory settings, with lipids more ^{2}H depleted relative to source water as growth rate increases (Schouten et al., 2006; Zhang et al., 2009b; Sachs and Kawka, 2015; Wolhowe et al., 2015). This relationship is most likely caused by increased contributions of hydrogen from relatively enriched NADPH from the oxidative pentose phosphate cycle under low-growth, nutrient stressed conditions, at the expense of relatively depleted hydrogen from photosystem I (Schmidt et al., 2003; Sachs and Kawka, 2015). If April samples include a higher proportion of lipids from organisms in a low-growth maintenance phase, they should therefore be relatively enriched in ^{2}H. As light availability and water column stratification became more amenable to photosynthesis later in the spring, relatively ^{2}H-depleted lipids produced with NADPH from photosystem I would be expected to become more abundant, bringing the net δ^2H values and $\alpha_{lipid\text{-}water}$ down.

Bottle incubations to determine lipid production rates were unfortunately not conducted for the first sampling in April, when the most enriched lipid δ^2H values were measured. For the remaining five sampling dates, there were not significant correlations between lipid production rate and $\alpha_{lipid\text{-}water}$, with the exception of nC$_{16:1}$ in Lake Greifen (Table 2). Although lipid concentrations and production rates are not a direct proxy for growth rate, as a higher percentage of algal biomass is typically allocated to lipids under low nutrient and slow growth conditions (Rossler, 1990; Williams and Laurens, 2010), higher lipid production rates for the whole community (rather than on a per cell basis) will also occur under conditions of higher growth rates. The large increase in fatty acid concentrations from April to

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May in both lakes, as well as smaller increases in brassicasterol concentrations, may indicate that the greatest community-wide change in growth rate occurred between those two months, and contributes in part to the decrease in fatty acid, phytol, and brasicasterol δ^2H values from April to May.

4.3 Comparison of mean $\alpha_{lipid\text{-}water}$ in lakes with different trophic statuses

For phytol, nC$_{14:0}$-, nC$_{16:0}$-, and nC$_{18:x}$-fatty acids, there was no significant difference in $\alpha_{lipid\text{-}water}$ between the oligotrophic and eutrophic lake. However, significant differences in $\alpha_{lipid\text{-}water}$ do exist for brassicasterol (0.048 ± 0.008; p = 0.0002) and nC$_{16:1}$-fatty acid (0.058 ± 0.022; p = 0.02) (Fig. 2). For brassicasterol, $\alpha_{lipid\text{-}water}$ is lower in Lake Greifen (0.712 ± 0.006) than in the less productive Lake Lucerne (0.757 ± 0.005). This result would be consistent with decreased hydrogen isotope fractionation (higher α values) for sterols in more nutrient-limited systems, as predicted by culturing experiments (Zhang et al. 2009b; Sachs & Kawka, 2015). The fact that this strong difference in fractionation between the two lakes is observed only for brassicasterol may be because it is the most source specific of the biomarkers that were analysed. Phytol and short-chain fatty acids are produced by all photoautotrophs and may be dominated by phytoplankton who are optimized to grow under the nutrient regimes of each system, while relatively more of the brassicasterol may come from taxa who are nutrient-stressed and relying more on the pentose phosphate pathway than photosystem I.

Alternatively, the difference in $\alpha_{Brassicasterol\text{-}water}$ values between the two lakes could be due to variable contributions of brassicasterol from different phytoplankton sources. While brassicasterol is produced by fewer organisms than short-chain fatty acids and phytol, it still has multiple sources (Volkman et al., 1998; Volkman, 2003; Rampen et al., 2010; Taipale et al., 2016). Species-specific differences in hydrogen isotope fractionation have not been observed for sterols, but have been reported for fatty acids and alkenones (Schouten et al., 2006; Zhang & Sachs, 2007), making this an unconstrained possibility that could be responsible for the difference in $\alpha_{Brassicasterol\text{-}water}$ between the oligotrophic and eutrophic lake. Different sources could also account for the difference in $\alpha_{lipid\text{-}water}$ for nC$_{16:1}$-fatty acid, which displays higher $\alpha_{lipid\text{-}water}$ values in the more productive lake, and therefore cannot be explained by the nutrient effect observed in cultures.

5. Conclusions

We measured δ^2H values of short-chain fatty acids, phytol, and the diatom biomarker brassicasterol in surface water particulate organic matter in two lakes in central Switzerland with different trophic states at six time points throughout the spring and summer of 2015. Measurements were paired with in situ incubations with ^{13}C-enriched DIC that allowed us to calculate lipid production rates.

In April in both lakes, lipid concentrations were at their lowest and lipid δ^2H values were at their highest. In the case of short-chain fatty acids, which are produced by both photoautotrophic and heterotrophic microbes, the relatively high fractionation factors observed in the spring are consistent with a greater proportion of these compounds being derived from heterotrophs (Zhang et al., 2009a; Osburn et al., 2011; Heinzelmann et al., 2015a). As phytoplankton productivity increased throughout

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the springtime, net $\alpha_{lipid\text{-}water}$ values declined to the range more commonly associated with photoautotrophs. The observed decline in $\alpha_{lipid\text{-}water}$ for fatty acids in oligotrophic Lake Lucerne was similar to that observed during the spring bloom in the North Sea (Heinzelmann et al., 2016), but was nearly three times as large in eutrophic Lake Greifen.

Changing contributions from heterotrophs cannot explain all of the decline in $\alpha_{lipid\text{-}water}$ from April to May, since this was also observed to a lesser extent in phytol and brassicasterol, compounds produced exclusively by photoautotrophs. Several factors could be responsible for changes in photoautrophic $\alpha_{lipid\text{-}water}$ throughout the spring, including temperature, growth rate, and species assemblage. Fractionation factors were inversely correlated with temperature for most fatty acids in each lake, and the slope of this relationship in Lake Lucerne (Fig. 3) was consistent with laboratory cultures, which suggest that $\alpha_{lipid\text{-}water}$ decreases with temperature by 0.002 – 0.004 per °C for acetogenic lipids (Zhang et al., 2009b; Wolhowe et al., 2009). Slower growth rates in the early spring could also result in higher $\alpha_{lipid\text{-}water}$ values at this time, as low growth rates correlate with higher $\alpha_{lipid\text{-}water}$ values in cultures (Schouten et al., 2006; Zhang et al., 2009b; Sachs & Kawka, 2015). Finally, changes in phytoplankton species assemblage could have contributed to changes in $\alpha_{lipid\text{-}water}$ over time, as hydrogen isotope fractionation has been observed to vary among eukaryotic algal species grown in culture (Schouten et al., 2006; Zhang & Sachs, 2007; Heinzelmann et al., 2015a)

While average fractionation factors for most lipids were consistent between the two lakes, average $\alpha_{lipid\text{-}water}$ values for brassicasterol were 0.047 ± 0.008 lower in Lake Greifen relative to Lake Lucerne, suggesting that sterol hydrogen isotopes may be more sensitive to nutrient availability than those of fatty acids and phytol.

Author contribution

S. N. Ladd designed the study with input from N. Dubois and C. J. Schubert. S. N. Ladd and N. Dubois collected the samples. S. N. Ladd processed and measured the samples. S. N. Ladd, N. Dubois, and C. J. Schubert contributed to data interpretation. S. N. Ladd prepared the manuscript with contributions from N. Dubois and C. J. Schubert. The authors declare that they have no conflict of interest.

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This research was funded by a National Science Foundation Earth Sciences Postdoctoral Fellowship (Award #1452254) to NL and Eawag internal funds. Alois Zwyssig and Alfred Lück assisted with sample collection. Serge Robert and Julian Stauffer assisted with sample preparation and laboratory analyses. Daniel Montluçon at ETH-Zurich measured the water isotopes. Algal counts were conducted by Esther Keller as part of Eawag's Department of Aquatic Ecology's long-term monitoring program. We had productive conversations with Ashley Maloney, Daniel Nelson, Julian Sachs, Blake Matthews, and Romana Limberger that improved the study design and interpretation of results. We are grateful for all of their contributions.

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References

Atwood, A. R. and Sachs, J. P.: Separating ITCZ-and ENSO-related rainfall changes in the Galápagos over the last 3 kyr using D/H ratios of multiple lipid biomarkers, Earth Planet. Sci. Lett., 404, 408-419, doi:10.1016/j.epsl.2014.07.038, 2014.

Bührer, H. and Ambühl, H.: Lake Lucerne, Switzerland, a long term study of 1961-1992, Aquat. Sci., 63, 432-456, doi:10.1007/s00027-001-8043-8, 2001.

Bürgi, H. R., Heller, C., Gaebel, S., Mookerji, N., and Ward, J. V.: Strength of coupling between phyto-and zooplankton in Lake Lucerne (Switzerland) during phosphorus abatement subsequent to a weak eutrophication, J. Plankton Res., 21, 485-507, doi:10.1093/plankt/21.3.485, 1999.

Chivall D., M'Boule D., Sinke-Schoen D., Damsté J. S. S., Schouten S., and van der Meer M. T. J.: The effects of growth phase and salinity on the hydrogen isotopic composition of alkenones produced by coastal haptophyte algae, Geochim. Cosmochim. Acta 140, 381–390, doi:10.1016/j.gca.2014.05.043, 2014.

Craig H. and Gordon L.: Deuterium and oxygen 18 variations in the ocean and the marine atmosphere, in Proceedings of a Conference on Stable Isotopes in Oceanographic Studies and Paleotemperatures (ed E. Tongiori), CNR-Laboratorio di Geologia Nucleare, Pisa, Italy, 9-130, 1965.

Englebrecht, A. C. and Sachs, J. P.: Determination of sediment provenance at drift sites using hydrogen isotopes and unsaturation ratios in alkenones. Geochim. Cosmochim. Acta, 69, 4253-4265, doi:10.1016/j.gca.2005.04.011, 2005.

Gat, J. R., Oxygen and hydrogen isotopes in the hydrologic cycle. Annu. Rev. Earth Planet. Sci. 24, 225-262, doi:10.1146/annurev.earth.24.1.225, 1996.

Heinzelmann, S. M., Villanueva, L., Sinke-Schoen, D., Damsté, J. S. S., Schouten, S., and van der Meer, M. T. J.: Impact of metabolism and growth phase on the hydrogen isotopic composition of microbial fatty acids, Front. Microbio., 6, 408, doi:10.3389/fmicb.2015.00408, 2015a.

Heinzelmann, S. M., Chivall, D., M'Boule, D., Sinke-Schoen, D., Villanueva, L., Damsté, J. S. S., Schouten, S., and Van der Meer, M. T. J.: Comparison of the effect of salinity on the D/H ratio of fatty acids of heterotrophic and photoautotrophic microorganisms, FEMS microbiology letters, 362, fnv065, doi:10.1093/femsle/fnv065, 2015b.

Heinzelmann, S. M., Bale, N. J., Villanueva, L., Sinke-Schoen, D., Philippart, C. J. M., Damsté, J. S. S., Smede, J., Schouten, S., and van der Meer, M. T. J.: Seasonal changes in the D/H ratio of fatty acids of pelagic microorganisms in the coastal North Sea, Biogeosci., 13, 5527-5539, doi:10.5194/bg-13-5527-2016, 2016.

Henderson, A. K. and Shuman, B. N.: Hydrogen and oxygen isotopic compositions of lake water in the western United States, Geol. Soc. Am. Bull., 121, 1179-1189, doi:10.1130/B26441.1, 2009.

Hollander, D. J., McKenzie, J. A., and Ten Haven, H. L.: A 200 year sedimentary record of progressive eutrophication in Lake Greifen (Switzerland): implications for the origin of organic-carbon-rich sediments, Geology, 20, 825-828, doi:10.1130/0091-7613(1992)020<0825:AYSROP>2.3.CO;2, 1992.

Huang, Y., Shuman, B., Wang, Y., and Webb III, T.: Hydrogen isotope ratios of palmitic acid in lacustrine sediments record late Quaternary climate variations. Geology 30, 1103-1106, doi:10.1130/0091-7613(2002)030<1103:HIROPA>2.0.CO;2, 2002.

Nemiah Ladd 13/6/2017 11:43
Deleted: Bird, B. W., Abbott, M. B., Vuille, M., Rodbell, D. T., Stansell, N. D., and Rosenmeier, M. F.: A 2,300-year-long annually resolved record of the South American summer monsoon from the Peruvian Andes, P. Natl. Acad. Sci. USA, 108, 8583-8588, doi:10.1073/pnas.1003719108, 2011.

Nemiah Ladd 13/6/2017 11:42
Deleted: Flynn, K. J., Stoecker, D. K., Mitra, A., Raven, J. A., Glibert, P. M., Hansen, P. J., Granéli, and Burkholder, J. M.: Misuse of the phytoplankton–zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types, J. Plankton Res., 35, 3-11, doi:10.1093/plankt/fbs062, 2012. ... [32]

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Huang, Y., Shuman, B., Wang, Y. and Webb, T.: Hydrogen isotope ratios of individual lipids in lake sediments as novel tracers of climatic and environmental change: a surface sediment test, J. Paleolimnol., 31, 363-375, doi:10.1023/B:JOPL.0000021855.80535.13, 2004.

Jones, A. A., Sessions, A. L., Campbell, B. J., Li, C., and Valentine, D. L.: D/H ratios of fatty acids from marine particulate organic matter in the California Borderland Basins, Org. Geochem., 39, 485-500, doi:10.1016/j.orggeochem.2007.11.001, 2008.

Kasper S., van der Meer M. T. J., Metz A., Zahn R., Damsté J. S. S., and Schouten S.: Salinity changes in the Agulhas leakage area recorded by stable hydrogen isotopes of C37 alkenones during Termination I and II. Clim. Past. 10, 251–260, doi:10.5194/cp-10-251-2014, 2014.

Keller, B., Wolinska, J., Manca, M., and Spaak, P.: Spatial, environmental and anthropogenic effects on the taxon composition of hybridizing Daphnia, Philos. T. Roy. Soc. B., 363, 2943-2952, doi:10.1098/rstb.2008.0044, 2008.

Leduc, G., Sachs, J. P., Kawka, O. E., and Schneider, R. R.: Holocene changes in eastern equatorial Atlantic salinity as estimated by water isotopologues. Earth Planet. Sci. Lett., 362, 151-162, doi:10.1016/j.epsl.2012.12.003, 2013.

Li, C., Sessions, A. L., Kinnaman, F. S., and Valentine, D. L.: Hydrogen-isotopic variability in lipids from Santa Barbara Basin sediments, Geochim. Cosmochim. Acta, 73, 4803-4823, doi:10.1016/j.gca.2009.05.056, 2009.

Maloney, A. E., Shinneman, A. L., Hemeon, K., and Sachs, J. P.: Exploring lipid 2 H/1 H fractionation mechanisms in response to salinity with continuous cultures of the diatom *Thalassiosira pseudonana*, Org. Geochem., 101, 154-165. doi:10.1016/j.orggeochem.2016.08.015, 2016.

M'boule, D., Chivall, D., Sinke-Schoen, D., Sinninghe Damsté, J. S. S., Schouten, S., and van der Meer M. T. J.: Salinity dependent hydrogen isotope fractionation in alkenones produced by coastal and open ocean haptophyte algae, Geochim. Cosmochim. Acta 130, 126–135, doi:10.1016/j.gca.2014.01.029, 2014.

McKenzie, J. A.: Carbon-13 cycle in Lake Greifen: a model for restricted ocean basins, in: Nature and origin of Cretaceous carbon-rich facies (eds. Schlanger, S. O. and Cita, M.), Academic Press, London, UK, 197-208, 1982.

Nelson, D. B. and Sachs, J. P.: The influence of salinity on D/H fractionation in dinosterol and brassicasterol from globally distributed saline and hypersaline lakes, Geochim. Cosmochim. Acta, 133, 325–339, doi:10.1016/j.gca.2014.03.007, 2014.

Nelson, D. B. and Sachs, J. P.: Galápagos hydroclimate of the Common Era from paired microalgal and mangrove biomarker $^{2}H/^{1}H$ values, P. Natl. Acad. Sci. USA, 113, 3476-3481, doi:10.1073/pnas.1516271113, 2016.

Osburn, M. R., Sessions, A. L., Pepe-Ranney, C., and Spear, J. R.: Hydrogen-isotopic variability in fatty acids from Yellowstone National Park hot spring microbial communities, Geochim. Cosmochim. Acta, 75, 4830-4845, doi:10.1016/j.gca.2011.05.038, 2011.

Osburn, M. R., Dawson, K. S., Fogel, M. L., and Sessions, A. L.: Fractionation of hydrogen isotopes by sulphate- and nitrate-reducing bacteria, Front. Microbio., 7, 1166m doi:10.2289/fmicb.2016.01166, 2016.

Nemiah Ladd 13/6/2017 11:41
Deleted: Leng, M. J. and Marshall, J. D.: Palaeoclimate interpretation of stable isotope data from lake sediment archives, Quaternary Sci. Rev., 23, 811-831, doi10.1016/j.quascirev.2003.06.012, 2004.

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Pahnke, K., Sachs, J. P., Keigwin, L., Timmermann, A., and Xie, S.: Eastern tropical Pacific hydrologic changes during the past 27,000 years from D/H ratios in alkenones, Paleoceanography 22, PA4214, doi:10.1029/2007PA001468, 2007.

Pinker, R. T. and Laszlo, I.: Modeling surface solar irradiance for satellite applications on a global scale, J. Appl. Meteorol., 31, 194-211, doi:10.1175/1520-0450(1992)031<0194:MSSIFS>2.0.CO;2, 1992.

Popp, B. N., Prahl, F.G., Wallsgrove R. J., and Tanimoto, J. K.: Seasonal patterns of alkenone production in the subtropical oligotrophic North Pacific, Paleoceanography, 21, PA1004, doi:10.1029/2005PA001165, 2006.

Rampen, S. W., Abbas, B. A., Schouten, S., and Damsté J. S. S.: A comprehensive study of sterols in marine diatoms (*Bacillariophyta*): implications for their use as tracers for diatom productivity. Limnol. Oceanogr. 55, 91–105, doi:10.4319/lo.2010.55.1.0091, 2010.

Randlett, M. E., Bechtel, A., van der Meer, M. T., Peterse, F., Litt, T., Pickarski, N., Kwiecien, O., Stockhecke, M., Wehrli, B., and Schubert, C. J.: Biomarkers in Lake Van sediments reveal dry conditions in eastern Anatolia during 110.000–10.000 years BP, Geochem. Geophys. Geosy., 18, doi:10.1002/2016GC006621, 2017

Richey, J. N. and Sachs, J. P.: Precipitation changes in the western tropical Pacific over the past millennium, Geology, 44, 671-674, doi:10.1130/G37822.1, 2016.

Roessler, P. G.: Environmental control of glycerolipid metabolism in microalgae: commercial implications and future research directions, J. Phycol., 26, 393-399, doi:10.1111/j.0022-3646.1990.00393.x, 1990.

Sachs, J. P.: Hydrogen isotope signatures in the lipids of phytoplankton, in: Treatise on Geochemistry, 2nd Edition, eds: Holland, H. D. and Turekian, K. K., Elsevier, Oxford, UK, Vol. 12, 79-94, doi:10.1016/B978-0-08-095975-7.01007-X, 2014.

Sachs, J. P. and Kawka, O. E.: The influence of growth rate on 2 H/1 H fractionation in continuous cultures of the coccolithophorid Emiliania huxleyi and the diatom *Thalassiosira pseudonana*, PloS one, 10, e0141643, doi:10.1371/journal.pone.0141643, 2015.

Sachs, J. P. and Schwab, V. F.: Hydrogen isotopes in dinosterol from the Chesapeake Bay estuary, Geochim. Cosmochim. Acta, 75, 444–459, doi:10.1016/j.gca.2010.10.013, 2011.

Sachs, J. P., Sachse, D., Smittenberg, R. H., Zhang, Z., Battisti, D. S., and Golubic S.: Southward movement of the Pacific intertropical convergence zone AD 1400–1850, Nat. Geosci., 2, 519–525, doi:10.1038/ngeo554, 2009.

Sachs, J. P., Maloney, A. E., Gregersen, J., and Paschall, C. Effect of salinity on $^{2}H/^{1}H$ fractionation in lipids from continuous cultures of the coccolithophorid Emiliania huxleyi, Geochim. Cosmochim. Acta, 189, 96-109, doi:10.1016/j.gca.2016.05/041, 2016.

Sachs, J. P., Maloney, A. E., and Gregersen, J.: Effect of light on 2H/1H fractionation in lipids from continuous cultures of the diatom *Thalassiosira pseudonana*, Geochim., Cosmochim., Acta, 209, 204-215, doi:10.1016/j.gca.2017.04.008, 2017.

Sachse, D. and Sachs, J. P.: Inverse relationship between D/H fractionation in cyanobacterial lipids and salinity in Christmas Island saline ponds, Geochim. Cosmochim. Acta, 72, 793–806,

Nemiah Ladd 13/6/2017 11:36
Deleted: Perry, G. J., Volkman, J. K., Johns, R. B., and Bavor, H. J: Fatty acids of bacterial origin in contemporary marine sediments, Geochim. Cosmochim. Acta, 43, 1715-1725, doi:10.1016/0016-7037(79)90020-6, 1979.

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doi:10.1016/j.gca.2007.11.022, 2008.

Sachse, D., Billault, I., Bowen, G. J., Chikaraishi, Y., Dawson, T. E., Feakins, S. J., Freeman, K. H., Magill, C. R., McInerney, F. A., van der Meer, M. T. J., Polissar, P., Robins, R. J., Sachs, J. P., Schmidt, H. L., Sessions, A. L., White, J. W. C., West, J. B., and Kahmen, A.: Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. Annu. Rev. Earth Planet. Sci. 40, 212-249, doi:10.1146/anurev-earth-042711-1055353, 2012.

Sauer, P. E., Eglinton, T. I., Hayes, J. M., Schimmelmann, A., and Sessions, A. L.: Compound-specific D/H ratios of lipid biomarkers from sediments as a proxy for environmental and climatic conditions, Geochim. Cosmochim. Acta, 65, 213–222, doi:10.1016/S0016/7037(00)00520-2, 2001.

Schimmelmann, A., Sessions, A. L., and Mastalerz, M.: Hydrogen isotopic (D/H) composition of organic matter during diagenesis and thermal maturation. Annu. Rev. Earth Planet. Sci., 34, 501-533, doi:10.1146/annurev.earth.34.031405.125011, 2006.

Schmidt, H. L., Werner, R. A., and Eisenreich, W.: Systematics of ^{2}H patterns in natural compounds and its importance for the elucidation of biosynthetic pathways, Phytochem. Rev., 2, 61-85, doi:10.1023/B:PHYT.0000004185.92648.ae, 2003.

Schouten, S., Ossebaar, J., Schreiber, K., Kienhuis, M. V. M., Langer, G., Benthien, A., and Bijma, J.: The effect of temperature, salinity and growth rate on the stable hydrogen isotopic composition of long chain alkenones produced by *Emiliania huxleyi* and *Gephyrocapsa oceanica*, Biogeosci. 3, 113–119, doi:10.5194/bg-3-113-2006, 2006.

Sessions, A. L., Burgoyne, T. W., Schimmelmann, A., and Hayes J. M.: Fractionation of hydrogen isotopes in lipid biosynthesis, Org. Geochem., 30, 1193–1200, doi:10.1016/S0146-6380(99)00094-7, 1999.

Sessions, A. L., Sylva, S. P., Summons, R. E., and Hayes, J. M.: Isotopic exchange of carbon-bound hydrogen over geologic timescales, Geochim. Cosmochim. Acta, 68, 1545-1559, doi:10.1016/j.gca.2003.06.004, 2004.

Smittenberg, R. H., Saenger, C., Dawson, M. N., and Sachs, J. P.: Compound-specific D/H ratios of the marine lakes of Palau as proxies for West Pacific Warm Pool hydrologic variability, Quatern. Sci. Rev. 30, 921–933, doi:10.1016/j.quascirev.2011.01.012, 2011.

Steinman, B. A., Abbott, M. B., Nelson, D. B., Stansell, N. D., Finney, B. P., Bain, D. J., and Rosenmeier, M.F.: Isotopic and hydrologic responses of small, closed lakes to climate variability: Comparison of measured and modeled lake level and sediment core oxygen isotope records, Geochim. Cosmochim. Acta, 105, 455-471, doi:10.1016/j.gca.2012.11.027, 2013.

Taipale, S. J., Hiltunen, M., Vuorio, K., and Petomaa, E.: Suitability of phytosterols alongside fatty acids as chemotaxonomic biomarkers for phytoplankton, Front. Plant Sci., 7:212, doi: 10.3389/fpls.2016.00212, 2016.

Thevenon, F., Adatte, T., Poté, J., and Spangenberg, J. E.: Recent human-induced trophic change in the large and deep perialpine Lake Lucerne (Switzerland) compared to historical geochemical variations, Palaeogeogr., Palaeocl., 363, 37-47, doi:10.1016/j.palaeo.2012.08.010, 2012.

Nemiah Ladd 20/6/2017 18:17

Deleted: Schwab, V. F., Garcin, Y., Sachse, D., Todou, G., Séné, O., Onana, J. M., Achoundong, G., and Gleixner, G.: Dinosterol δD values in stratified tropical lakes (Cameroon) are affected by eutrophication, Org. Geochem., 88, 35-49, doi:10.1016/j.orggeochem.2015.08.003, 2015.

Nemiah Ladd 13/6/2017 11:35

Deleted: Talbot, M. R.: A review of the paleohydrological interpretation of carbon and oxygen isotopic ratios in primary lacustrine carbonates, Chem. Geol., 80, 261-279, doi:10.1016/0168-9622(90)90009-2, 1990. ... [34]

van der Meer, M., Baas, M., Rijpstra, W., Marino, G., Rohling, E., Damsté, J. S. S., and Schouten S.: Hydrogen isotopic compositions of long-chain alkenones record freshwater flooding of the Eastern Mediterranean at the onset of sapropel deposition, Earth Planet. Sci. Lett., 262, 594–600, doi:10.1016/j.epsl.2007.08.014, 2007.

van der Meer, M., Sangiorgi, F., Baas, M., Brinkhuis, H., Damsté J. S. S., and Schouten, S.: Molecular isotopic and dinoflagellate evidence for Late Holocene freshening of the Black Sea, Earth Planet. Sci. Lett., 267, 426-434, doi:10.1016/j.epsl.2007.12.001, 2008.

van der Meer, M. T., Benthien, A., French, K. L., Epping, E., Zondervan, I., Reichart, G. J., Bijma, J., Damsté J. S. S., and Schouten, S.: Large effect of irradiance on hydrogen isotope fractionation of alkenones in *Emiliania huxleyi*, Geochim. Cosmochim. Acta, 160, 16-24, doi:10.1016/j.gca.2015.03.024, 2015.

Vasiliev, I., Reichart, G. J., and Krijgsman, W.: Impact of the Messinian Salinity Crisis on Black Sea hydrology—Insights from hydrogen isotopes analysis on biomarkers, Earth Planet. Sci. Lett., 362, 272–282, doi:10.1016/j.epsl.2012.11.038, 2013.

Vasiliev, I., Mezger, E. M., Lugli, S., Reichart, G. J., Manzi, V., and Roveri, M.: How dry was the Mediterranean during the Messinian salinity crisis?, Palaeogeogr. Palaeocl., 471, 120-133, doi:10.1016/j.palaeo.2017.01.032, 2017.

Volkman, J. K.: Sterols in microorganisms, Appl. Microbiol. Biotechnol., 60, 495–506, doi:10.1007/s00253-002-1172-8, 2003.

Volkman, J. K., Barrett, S. M., Blackburn, S. I., Mansour, M. P., Sikes, E. L., and Gelin F.: Microalgal biomarkers: a review of recent research developments. Org. Geochem., 29, 1163–1179, doi:10.1016/S0146-6380(98)00062-X, 1998.

Williams, P. J. L. B., and Laurens, L. M.: Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics, Energy & Environmental Science, 3, 554-590, doi:10.1039/B924978H, 2010.

Wolhowe, M. D., Prahl, F. G., Probert, I., and Maldonado M.: Growth phase dependent hydrogen isotopic fractionation in alkenone-producing haptophytes, Biogeosci., 8, 1681–1694, doi:10.5194/bg-6-1681-2009, 2009.

Wolhowe, M. D., Prahl, F. G., Langer, G., Oviedo, A. M., and Ziveri, P.: Alkenone δD as an ecological indicator: A culture and field study of physiologically-controlled chemical and hydrogen-isotopic variation in C 37 alkenones, Geochim. Cosmochim. Acta, 162, 166-182, doi:10.1016/j.gca.2015.04.034, 2015.

Zarrouk, W., Carrasco-Pancorbo, A., Zarrouk, M., Segura-Carretero, A., and Fernandez-Gutierrez, A.: Multi-component analysis (sterols, tocopherols and triterpenic dialcohols) of the unsaponifiable fraction of vegetable oils by liquid chromatography– atmospheric pressure chemical ionization–ion trap mass spectrometry, Talanta 80, 924–934, doi:10.1016/j.talanta.2009.08.022, 2009.

Zhang, Z. and Sachs, J. P.: Hydrogen isotope fractionation in freshwater algae: 1. Variations among lipids and species, Org. Geochem, 38, 582–608, doi:10.1016/j.orggeochem.2006.12.004, 2007.

Nemiah Ladd 15/6/2017 19:44

Deleted: Volkman, J. K., Johns, R. B., Gillan, F. T., Perry, G. J., and Bavor, H. J.: Microbial lipids of an intertidal sediment—I. Fatty acids and hydrocarbons, Geochim. Cosmochim. Acta, 44, 1133-1143, doi:10.1016/0016-7037(80)90067-8, 1980.

Zhang, X., Gillespie, A., and Sessions, A.: Large D/H variations in bacterial lipids reflect central metabolic pathways, P. Natl. Acad. Sci. USA, 106, 12580-12586, doi:10.1073/pnas.0903030106, 2009a.

Zhang, Z., Sachs, J. P., and Marchetti, A.: Hydrogen isotope fractionation in freshwater and marine algae: II. Temperature and nitrogen limited growth rate effects, Org. Geochem, 40, 428-439, doi:10.1016/j.orggeochem.2008.11.002, 2009b.

Zhang, Z., Leduc, G., and Sachs, J. P.: El Niño evolution during the Holocene revealed by a biomarker rain gauge in the Galápagos Island, Earth Planet. Sci. Lett., 404, 420-434, doi:10.1016/j.epsl.2014.07.013, 2014.

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Table 1 Summary of expected changes in $\alpha_{Lipid\text{-}Water}$ in response to different environmental variables, based on laboratory cultures and field studies in marine settings

Variable	Sign of correlation with $\alpha_{Lipid\text{-}Water}$	Magnitude	References
Temperature	Negative	2 – 4 ‰ per °C	Zhang et al., 2009b; Wolhowe et al., 2009
Growth Rate	Negative	~30 ‰ per division day^{-1}	Schouten et al., 2006; Zhang et al., 2009b; Sachs and Kawka, 2015; Wolhowe et al., 2015
Nutrient Availability	Negative	~40 ‰ difference between nutrient limited and nutrient replete cultures	Zhang et al., 2009b; Wolhowe et al., 2015
Light Availability	Positive	Below ~250 µmol photons m^{-2} s^{-1}, ~0.2‰ per µmol photons m^{-2} s^{-1}	van der Meer et al., 2015; Wolhowe et al., 2015; Sachs et al., 2017
Salinity	Positive	0.5 – 3 ‰ per practical salinity unit (PSU)	Schouten et al. 2006; Sachse and Sachs, 2008; Sachs and Schwab, 2011; Chivall et al., 2014; M'boule et al., 2014; Nelson and Sachs, 2014; Heinzelmann et al., 2015b; Maloney et al., 2016; Sachs et al., 2016
Species Assemblage	Variable	Differences up to 160 ‰ observed for *n*C16:0 fatty acid among species growing under identical conditions	Schouten et al., 2006; Zhang & Sachs, 2007

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Table 2 Summary of linear regression statistics for all water isotopes and various fractionation factors with salinity; bolded relationships are significant at the $p < 0.05$ level.

	LAKE GREIFEN					LAKE LUCERNE				
α Lipid-Water vs. Temperature										
Lipid	**Slope**	**y-intercept**	**R^2**	**p**	**n**	**Slope**	**y-intercept**	**R^2**	**p**	**n**
All Fatty acids	**-0.006 ± 0.002**	**0.95 ± 0.04**	**0.32**	**0.004**	**24**	**-0.003 ± 0.001**	**0.85 ± 0.02**	**0.24**	**0.015**	**24**
*n*C14:0 Fatty acid	**-0.004 ± 0.001**	**0.86 ± 0.03**	**0.75**	**0.03**	**6**	-0.0012 ± 0.0009	0.80 ± 0.02	0.28	0.28	6
*n*C16:0 Fatty acid	**-0.008 ± 0.002**	**0.98 ± 0.03**	**0.87**	**0.006**	**6**	**-0.003 ± 0.001**	**0.84 ± 0.02**	**0.66**	**0.049**	**6**
*n*C16:1 Fatty acid	-0.005 ± 0.003	0.98 ± 0.07	0.35	0.22	6	**-0.004 ± 0.001**	**0.89 ± 0.02**	**0.74**	**0.028**	**6**
*n*C18:x Fatty acid	**-0.006 ± 0.0003**	**0.97 ± 0.007**	**0.99**	**<0.0001**	**6**	**-0.004 ± 0.001**	**0.89 ± 0.02**	**0.70**	**0.037**	**6**
Phytol	-0.001 ± 0.001	0.66 ± 0.03	0.14	0.47	6	0.002 ± 0.001	0.61 ± 0.03	0.47	0.20	5
Brassicasterol	-0.002 ± 0.001	0.74 ± 0.02	0.67	0.09	5	0 ± 0.000	0.76 ± 0.02	0.005	0.90	6
α Lipid-Water vs. Lipid Production Rate										
Lipid	**Slope**	**y-intercept**	**R^2**	**p**	**n**	**Slope**	**y-intercept**	**R^2**	**p**	**n**
All Fatty acids	-0.01 ± 0.01	0.83 ± 0.02	0.10	0.18	20	-0.04 ± 0.03	0.81 ± 0.01	0.09	0.19	20
*n*C14:0 Fatty acid	0.00 ± 0.02	0.76 ± 0.02	0.0002	0.98	5	-0.2 ± 0.2	0.82 ± 0.05	0.25	0.39	5
*n*C16:0 Fatty acid	0.01 ± 0.02	0.78 ± 0.05	0.02	0.83	5	-0.01 ± 0.04	0.83 ± 0.03	0.46	0.21	5
*n*C16:1 Fatty acid	**-0.17 ± 0.04**	**0.89 ± 0.01**	**0.84**	**0.03**	**5**	0.6 ± 0.6	0.76 ± 0.06	0.22	0.42	5
*n*C18:x Fatty acid	-0.01 ± 0.02	0.85 ± 0.04	0.08	0.64	5	-0.09 ± 0.05	0.86 ± 0.02	0.57	0.14	5

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Phytol	0 ± 1	0.62 ± 0.03	0.02	0.80	5	0 ± 7	0.65 ± 0.02	$6*10^{-6}$	0.99	5
Brassicasterol	2 ± 3	0.69 ± 0.01	0.14	0.32	4	1 ± 43	0.75 ± 0.02	$3*10^{-4}$	0.98	5

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Table 3: Mean residence times in hours of lipids in lake surface water, calculated according to equation 2

Date	*n*C14:0	*n*C16:0	*n*C16:1	*n*C18:x	Brassicasterol	Phytol
			Lake Greifen			
May 11, 2015	30 ± 9	19 ± 4	60 ± 13	19 ± 3	59 ± 26	42 ± 10
June 5, 2015	9 ± 2	9 ± 1	21 ± 2	9 ± 1	58 ± 34	32 ± 8
July 2, 2015	19 ± 1	20 ± 1	49 ± 5	20 ± 3	165 ± 51	40 ± 6
Aug. 11, 2015	15 ± 3	17 ± 2	41 ± 5	16 ± 2	81 ± 34	123 ± 23
Sept. 8, 2015	10 ± 3	12 ± 2	31 ± 8	12 ± 2	76 ± 37	127 ± 26
			Lake Lucerne			
May 13, 2015	16 ± 6	16 ± 5	27 ± 8	17 ± 2	124 ± 22	99 ± 19
June 3, 2015	20 ± 12	18 ± 6	62 ± 27	16 ± 4	208 ± 56	106 ± 17
July 7, 2015	19 ± 8	19 ± 5	34 ± 18	17 ± 5	114 ± 39	94 ± 31
July 31, 2015	22 ± 9	19 ± 4	38 ± 8	18 ± 4	164 ± 59	399 ± 36
Aug. 31, 2015	20 ± 4	22 ± 3	41 ± 6	12 ± 3	258 ± 61	248 ± 42

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Figure Captions

Figure 1: Map of Switzerland with locations of Lake Greifen and Lake Lucerne indicated. Base map from d-maps (http://www.d-maps.com/carte.php?num_car=2648&lang=en).

Figure 2: Time series of lipid concentrations in µg/L (panels a and b), lipid production rates in µg L^{-1} hr^{-1} (panels c and d), lipid δ^2H values in ‰ relative to VSMOW (panels e and f), $\alpha_{lipid\text{-}water}$ values (panels g and h), and lake surface temperature (panels i and j) for Lake Greifen (left column) and Lake Lucerne (right column) during the spring and summer of 2015. Panels a-d are plotted on an exponential scale to accommodate the large range in lipid concentrations and production rates. Error bars represent 1 standard deviation of replicate measurements, and are propagated to include uncertainties from multiple sources in calculated production rates and $\alpha_{lipid\text{-}water}$ values. In cases where error bars are not visible, they are smaller than the marker size.

Figure 3: Relationships between $\alpha_{lipid\text{-}water}$ values and lake surface temperatures in Lakes Greifen (panel a) and Lucerne (panel b) throughout the spring and summer of 2015. Error bars are propagated 1σ uncertainty from replicate measurements of surface water and lipid δ^2H values. In cases where error bars are not visible, they are smaller than the marker size. Shading represents 95% confidence intervals of the linear regression. Statistics associated with each curve are summarized in Table 2, and plots of individual compounds are available in the supplemental file.

Figure 4: Cell counts (individuals per liter) for all algae and for most common taxa of algae in (a) Lake Greifen throughout 2015 and (b) Lake Lucerne in 2014. The scale of the y-axis differs between the two panels. Data from long-term monitoring program run by the department of Aquatic Ecology at Eawag.

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