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Interactive comment on "Interplay of temperature, productivity, and community assemblage on hydrogen isotope signatures of algal lipid biomarkers" *by* S. Nemiah Ladd et al.

Anonymous Referee #1

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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

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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.

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.

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).

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.

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?

p16-1 – Can you calculate and compare a weighted average isotope value (as in Osburn et al., 2016) between these two?

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 mid-chain fatty acids as these are often used to describe paleolake water.

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.

Figure 4: Divide out sediment traps and surface sediment data then report all measured rather than just C16:0

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. 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? p6-5-8 – You don't report compositional information for other lipids. This is ok, but is there anything noteworthy? p6-15 – What compound classes are eluting in each of these fractions? p6-22 – Do any of the aforementioned methods need citations or are they all original to this study? p7-24 – Please confirm that reported errors include propagated errors from replicate measurements, standards, and derivatization processes. p8 section 3.1 – this section could probably be condensed, it is quite repetitive. p9-2 – lowest here means fastest right? I would suggest going with time designations when referring to residence times p11, sec 4.1.2. this has been studied in plants p12-29 – lower alpha is confusing here p13-2 – The ultimate explanation should be the same for all FAs unless you are suggesting a different source. 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. p13-27 – Can

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a statistical analysis help you here? Does any taxa change correlate with changes in fractionation? 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. 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. p14-18 – Be careful, there are plenty of bacteria that make primarily 16:0, 16:1, and 18:1. p17-14 – Reporting an offset in permil would be helpful to the paleo community p17-19 – isoprenoid change to sterol, you haven't made the case for isoprenoids more generally.

Minor editorial comments: p1-9 – isotope → isotopic p1-21 – in situ should be italicized here and throughout the manuscript p1-22 – increased magnitude of fractionation needs a direction term as the fractionation factor for lipid-water can be both above and below 1. p2-22-23 – This is stating the obvious, rephrase or combine with the following sentence. p2-25 – also metabolism see Zhang et al., 2009 or Osburn et al. 2016 p3-1 – I think that should be permil per degree C p3-5 – I am not sure what 'more' fractionation means p3-13 – remove 'the' p4-10 – What is the depth at this sampling point? p5-24&25 – something is wrong with these sentences. Perhaps a 'was' after 'sample' p7-10 – Initially? Were they subsequently converted to a different scale? p7-31 13 needs superscript p7-32 – lipid p8-15-16 – these numbers are the same (1.0 and 1.1) but the text suggests that one should be lower. p9-19 – add 'relative' after depleted p14-26 space before 2010

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