Response to Anonymous Referee #2

In this letter we try to briefly comment all the observations from Rev#1. The major changes can be read in the reviewed version of the manuscript and supplementary information (if a review is requested by the Editor).

Rev#2: García-Alix et al. studied long-chain diols extracted from sediments from 2 cores from Laguna de Rio Seco. Long chain diols are novel biomarkers in lacustrine environments that have not been used for paleo-reconstruction, but, from what is known from the marine realm, they could be a good paleo-thermometer. The authors used the LDI, which is the ratio of different long-chain diols, calculated following Rampen et al. (2012) and calibrated against air temperature from different instrumental stations from lower elevation corrected for altitude effect. From this calibration, based on the last 100 years of instrumental record, they extrapolate LDI temperature on the last 1500 years. From the diol distribution in LdRS the authors deduct a different source organism than though until then, i.e. freshwater eustigmatophytes.
Rev#2: Global comments: The study site is extremely interesting, as Mediterranean alpine environments are prone to rapid changes, and uncovering the causes of environmental changes in these high elevation sites would be helpful for understanding future climatic changes. Furthermore, long-chain diols are rarely used in lacustrine environments and developing a temperature calibration would be particularly interesting as long-chain diols are commonly found in lake sediments globally distributed (Rampen et al., 2014).

Thank you very much for your comments.

Rev#2: However, Rampen et al., 2014 did a thorough study (n=62 lake sediments) of possible correlation of the LDI and diol fractional abundances with annual mean air temperatures and/or GDGT-reconstructed lake temperatures and concluded that LDI does not seem applicable in lake environments. As such, why is only LDI tested for temperature and not any other diol fractional abundances mentioned in Rampen et al. (2014)? In particular, as the C32 1,15-diol seems to have a positive relation with temperature in cultures (as in Rampen et al., 2014, Goniochloropsis) and the author's dataset, why not test for the C32:0 1,15 over the C32:1 1,15? Only one m/z has to be added to the SIM mode (m/z=339). More details on seasonal temperature calibration would be interesting to mention as diol are subject to seasonality (Smith et al., 2013; Lattaud et al., 2018). Furthermore, the conclusion on the organisms producing the diols lacks concrete evidence, as the LDI (a ratio) gives no indication of diol-producer abundances. It would be better to compare the concentration of diols in the sediment with the number of cysts. As the lake studied is so specific a thorough study of all diol present are needed (especially if any source organism is hypothesized) and should be reported at least as results such as 1,14-diols, C32 1,16-diol, C34 1,17 etc.

One of the main conclusions from Rampen et al. (2014a) was that although the relative abundances of individual LCDs in lakes did not show correlation with temperatures, the GDGT deduced temperatures did show good correlation with the LDI (R^2=0.64). This correlation was even higher when using a multiple regression with the same LDI isomers (R^2=0.74). In both cases, one outlier of the dataset was removed to improve the correlation. Rampen et al. (2014a) also suggested that more tests are needed in freshwater environment to assess the application of LCDs in these environments and this is one of the main aims of our manuscript. In our paper we developed a LDI-temperature calibration because the relationship between LDI, and therefore the LCDs involved in the index, and instrumental temperatures was higher (R^2>0.8) than those of the different approaches performed in the above-mentioned paper. Anyway, Rev#2 is right and we are including a discussion of the distributions of the different LCDs identified, the different temperature calibrations based on different LCD indices, multiple regressions, etc. We have also included some new figures in supplementary information showing these LCD-temperature correlations.

We cannot re-run all the samples again, but we are now including different diols with the m/z that were previously measured, i.e. 1,14-diols. In addition, we did look for the C32:1 1,15-diol and the C32 1,16-diols in the TIC chromatograms, but they were not always present and in most of the samples the concentration was below the detection limit. This precluded us from getting any reliable data.

In the case of the short core we have provided the (very scarce) C28 and C30 1,14-diols, as well as the C34 1,17-diol, since these masses where previously measured in SIM mode.

Regarding the relationship between LCDs and cyst, Rev#2 is right, this is not the best way to discuss about the producers. Further molecular and sediment traps studies (currently in progress) are also required to identify the biological source. We have changed this section (see also comment Rev#1 9).

As far as seasonality is concerned, we had slightly explained it in Table S3 of the supplementary information. We agree with Rev#2, and we have discussed the seasonality in the new version of the main text.

Rev#2: Nit-picking and other comments.

1. **Rev#2:** L27: what do you define as extreme responses?

We meant abrupt environmental responses, for example amplification of natural trends due to human pressure, i.e. Garcia-Alix et al. (2017). We did not explain that at this point since this is the abstract section. Anyway, we have changed the sentence: *"While major environmental shifts have occurred over the last ~1500 years in these alpine ecosystems, only changes in the recent centuries have led to extreme environmental responses…"*

2. **Rev#2:** L29: Rather than "algal lipids" the study calibrated algal lipid proxies

Done.

3. **Rev#2:** L30: Rephrase "extending alpine temperatures backward 1500 years", I suggest: "extending alpine temperature reconstructions to 1500 years before present"

Done.

4. **Rev#2:** L60: Instead of "this is the case", "as it is the case"

Done.

5. **Rev#2:** L87: Willmott et al., 2010 would be a reference to mention in term of nutrient proxies

Done. We have also included (Rampen et al., 2008) as Rev#1 suggested.

6. **Rev#2:** L182-183: In Table S7 21 samples are reported for the long core.

Rev#2 is right. We meant the samples that we really used. We have now mentioned the 21 samples in that sentence, and we have included a new sentence explaining that one of the samples fell below quantification limits: "*The sample at 19.5 cm depth in the long core was discarded because its concentration fell below quantification limits.* "

7. **Rev#2:** L197: How was the concentration evaluated as no internal/external standard is mentioned?

In this step we only wanted to roughly know the concentration of the different compounds of the samples with the GC-FID so that the signal would not saturate the MS detector when we measured them in the GC-MS. We measured an external standard of cholesterol every five samples. We have included this explanation in the revised version.

8. **Rev#2:** L215: spacing between "from 1965 to 2011" and "(Spanish National: : :"

Done.

9. **Rev#2:** L218-220: Is there any GDGT/alkenones detected? As they could provide another independent temperature for calibration.

Sadly, our tests showed that there are not alkenones in the alpine lakes of the Sierra Nevada. The analyses of the polar fraction (to assess the GDGTs presence) is a new project that will develop once our equipment is properly set up for these kinds of heavy compounds.

10. **Rev#2:** L265: "(C28, C30 and C32 1,13- and 1,15-diols)" do you find the C32 1,13-diol? Furthermore, there is no significant difference between the diol distribution of the short and long core recent samples (last 200y) and what has been previously published, that should be stated in the manuscript or a statistical test should be provided if the authors think otherwise. Only the samples from the LIA seems to fall close to the marine sediment distribution and might point toward

a shift in producer but could also be an adaptation to the cold from the same organism, a more detailed discussion is needed.

We did not find the C32 1,13-diol in our samples. In the new version we have specified "*C28 and C30 1,13-diols and C30 and C32 1,15-diols"*. As commented before, we cannot re-run all the samples again, but we have included the 1,14-diols identified with the m/z previously measured and we have also looked for other diols in the TIC chromatograms (see previous comment). Following Rampen et al. (2014a), we had plotted *C28 1,13-diol, C30 1,13-diol, C30 1,15-diol and C32 1,15*-diol distributions in a ternary plot including the published diol distributions. We found that the LCDs distribution in LdRS, even though close to that of riverine material, fitted with a blank region in the diagram published by Rampen et al. (2014a). Although we still think that the LCD distribution in LdRS may differ from the published ones, we are including a brief discussion about the LCDs distribution identified in the text, and a new double-ternary diagram.

11. **Rev#2:** L273: due to their small size (<3 um), eustigmatophytes are usually overlooked during planktonic study that does not include DNA sequencing. DNA sequencing on the modern lake water would bring stronger evidence.

Rev#2 is right. We are currently recovering material from sediment traps and suspended particulate material in order to conduct both geochemical and DNA sequencing analyses. This is an ongoing work, since this is an oligotrophic lake we would like to have at least two-annual cycles in order to catch up the different algal blooms and the LCDs producers and better understand the relationship between LCDs distribution and lake temperatures.

12. **Rev#2:** L275: quite a bold statement without explanation in the text, explain the method to obtain the figure S3 (cyst count and identification). As the diol distribution is not significantly different from the previously published distribution a more thorough discussion is needed on why Chromulina spp. are potential diol producers and not freshwater eustigmatophytes. The comparison on fig S3 between LDI and Chromulina cyst is not an evidence as LDI do not correlate with diol abundance (it is actually independent), nor with diol-producer biomass (Balzano et al., 2016). Are any long-chain alcohols present? Or Long-chain ketones? As they would give idea on the producers (Volkman et al., 1999) and the possible state of degradation of the sediments (Versteegh et al., 2000).

Rev#2 is right. See also response to comment 9 by Rev#1. We have removed this sentence regarding other potential biological producers since we do not have strong evidences of this fact. Regarding the other organic compounds, the algal productivity is not too high in this alpine oligotrophic lake. We have found *n*-alkanes and fatty acids in the sediments (from semi aquatic plants and from small plant patches in the catchment), but we have not found the shorter chains of these *n*-alkanes and fatty acids that might be related to algae/bacteria. We have not found long chain ketones either (see Rev#2 comment 9). Regarding the keto-ols occurrence, as possible degradation product of diols, we have only identified the C32 1,15-keto-ol (in TIC) in some of the samples, although in very low abundance. A further identification of these compounds would be very interesting for future analyses of current surface sediments, sediment traps, and suspended particulate matter (on going project: see Rev#2 comment 11).

13. **Rev#2:** L298: In figure 3 there is a group of points (LDI between 0.23-0.27) that deviates from the general correlation, are they all from the same period? Such as the LIA? If so, the LIA seem to be significantly different from the rest of the core and need to be handled independently.

In this figure we only showed data from 1908 to 2008 CE in order to calibrate the LDI vs historical temperatures. In the text we mentioned that the group of data referred by Rev#2 (corresponding to 1973: 1 LDI point vs. four temperature reconstructions at 3020 masl) might be an outlier.

14. **Rev#2:** L304-306: doing an outlier test would provide significance to this statement on the 1973 samples.

We have used the residuals in order to assess the outliers, since an outlier is a point with an unusually large residual, at least 2.5 standard deviations from the mean value (mean annual temperatures in our case). Data for 1973 show residuals that are 2.5 times higher than the standard deviations for two temperature reconstructions and lower than that value for the other two reconstructions. This is why we suggested a potential outlier. We have clarified this in the result section.

15. **Rev#2:** L350: Fig4 should be inversed with Fig5 as Fig5 is discussed before in the manuscript.

Figures 4 and 5 were mentioned at the same time in the manuscript, but Rev#2 may be right and the short core figure would make more sense as Fig. 4. We have done this change.

16. **Rev#2:** L352: The argument is reverse, the tree ring record supports the LDI data as it is a more known and used proxy.

We have changed the sentence: *"tree ring data from the Pyrenees and Iberian range show minor temperature variations, and even a slight temperature decrease from ~2000 to 2008 similar to the one observed in LdRS diol record (Fig. 4c).*"

17. **Rev#2:** L352-354: Are the warming rate from Southern Europe/Spain also stabilizing?

We mentioned this at the end of the next section 4.2: There is a similar trend in the Pyrenees area, Iberian range and marine platforms in the western Mediterranean.

18. **Rev#2:** L433: The LDI record does not have a sufficient resolution to recognize a 1 year-long event.

Although the eruption of the mentioned volcano occurred in 1963-1964, volcanic aerosols in the atmosphere can cause decadal-timescale effects (Sigl et al., 2015). We mentioned this in the same paragraph that is referred by Rev#2. These kinds of effects could (potentially) be recorded in our record with 5-7 years of time averaging (i.e. last 180 years). However, our intention with this volcano discussion was pointing that we are not showing this direct cooling effect in our lakes (according to the correlations and figures). This is indicated in the second sentence of that paragraph, but we cannot exclude its potential influence at some specific times, such as the related with the eruption of 1963-1964.

19. **Rev#2:** L437-438: The cooling in the LDI of 1450-1500 and 1690 CE could also be attributed to solar minima rather than volcanic eruption. What about the volcanic aerosol from 1200-1300 CE that do not seem to impact the LDI in LdRS?

Actually, in these lines we mentioned that although reconstructed-LDI cold temperatures occasionally seem to occur coevally with volcanic eruptions, for example, at ~1450-1500 and 1690 CE, there is **not** a direct relationship between intensity of number of large eruptions and the reconstructed coolings in LdRS records. In addition, in lines 422-423 of the former draft we mentioned that "*volcanic forcing do not show a significant correlation with LDI-derived temperatures from LdRS over the last 1500 year* " and that most of the cooling events recorded in LdRS, such as those during the LIA, were coeval with low solar activity periods (former lines 441-442). Therefore, we are discarding a strong impact of volcanic aerosols in our paleoclimate records.

20. **Rev#2:** L473: Precise the number of samples analyzed for the MCA. The MCA baseline seems to be only represented by one samples, the rest of the MCA samples are much cooler. An average temperature of all the MCA samples is a better representation of the MCA temperature and can be used as MCA baseline.

We did not intend to create a temperature baseline for the MCA, but we wanted to stablish the highest temperature recorded in a non-industrial period during the Common era. This cannot be stablished with averaged temperatures. We agree with Rev#2, and the expression MCA temperature background in figures and text is not the best choice, since it is confusing and does not reflect our discussion. We have modified it accordingly.

21. **Rev#2:** L477: Precise the number of samples analyzed for the LIA

We have also added the oldest samples of the short core (from 1820s to 1850) to this discussion; therefore, we have a total of 8 samples for the LIA.

22. **Rev#2:** L497-498: Provide a reference for the statement: "Future scenarios are not optimistic for Sierra Nevada alpine areas either as projected temperature may rise at least _1.4 ºC by the end of the 21st century"

We have modified this paragraph (see Rev#1 comments 19 and 20), removed the potential overinterpretations, and added references.

23. **Rev#2:** L544: Is the temperature records mentioned from this study or from instrumental data, is there any precipitation reconstruction existing for this region?

Temperature data are those deduced from the LCD calibration, since long temperature records are lacking from southern Spain alpine areas. Precipitation (instrumental +

reconstructed) data showed in Fig. 7b are from Rodrigo et al. (1999). We have rephrased the sentence in order to clarify so: *"Precipitation data from southern Iberia (Rodrigo et al., 1999) along with the reconstructed temperatures in LdRS suggest ....."*

24. **Rev#2:** Fig 2b: the dashed line is almost not visible, either change colors or thickness. Add the timing of LIA and MCA to the figure. Add the temperature records from the instrumental data to help comparison.

Done. The instrumental temperature data have been added instead to current figure 4, as Rew#1 suggested, since the scale of the temperature instrumental data (100 years), would be too small for this figure (scale of 1500 years).

25. **Rev#2:** Fig S1: Correct "row" by "raw" in (a) (c) (e) (g) (i) and (k). Add unit for axis y

Done.

26. **Rev#2:** Fig S3: Please, add legend to the y axis. The authors use r and not r2 like in other figures, homogenize.

We have removed this figure. See previous comments.

Cited literature

Garcia-Alix, A., Jimenez Espejo, F.J., Toney, J.L., Jiménez-Moreno, G., Ramos-Román, M.J., Anderson, R.S., Ruano, P., Queralt, I., Delgado Huertas, A., Kuroda, J., 2017. Alpine bogs of southern Spain show human-induced environmental change superimposed on long-term natural variations. Scientific Reports 7, 7439

Rampen, S.W., Datema, M., Rodrigo-Gámiz, M., Schouten, S., Reichart, G.-J., Sinninghe Damsté, J.S., 2014a. Sources and proxy potential of long chain alkyl diols in lacustrine environments. Geochimica et Cosmochimica Acta 144, 59-71.

Rampen, S.W., Schouten, S., Koning, E., Brummer, G.-J.A., Sinninghe Damsté, J.S., 2008. A 90 kyr upwelling record from the northwestern Indian Ocean using a novel long-chain diol index. Earth and Planetary Science Letters 276, 207-213.

Rodrigo, F.S., Esteban-Parra, M.J., Pozo-Vázquez, D., Castro-Díez, Y., 1999. A 500-year precipitation record in Southern Spain. International Journal of Climatology 19, 1233-1253.

Sigl, M., Winstrup, M., McConnell, J.R., Welten, K.C., Plunkett, G., Ludlow, F., Buntgen, U., Caffee, M., Chellman, N., Dahl-Jensen, D., Fischer, H., Kipfstuhl, S., Kostick, C., Maselli, O.J., Mekhaldi, F., Mulvaney, R., Muscheler, R., Pasteris, D.R., Pilcher, J.R., Salzer, M., Schupbach, S., Steffensen, J.P., Vinther, B.M., Woodruff, T.E., 2015. Timing and climate forcing of volcanic eruptions for the past 2,500 years. Nature 523, 543-549.