

## Reply to anonymous Referee #2

We thank anonymous Referee #2 for the helpful comments, which will lead to a substantial improvement of our manuscript. In the following we like to address the different points raised by the referee. To facilitate the revision, the comments from Referee #2 are written in blue whereas our responses are in black.

Anonymous Referee #2: The observation that palmitic acid, a compound produced by all organisms on Earth, display nearly constant hydrogen isotopic fractionation across large environmental and salinity gradient should be discussed further. There are all kinds of different algae and bacteria living in these water masses with different water chemistry. This observation would mean palmitic acid hydrogen isotopic fractionation relative to environmental water is highly conserved in various organisms. A couple of previous papers also support this conclusion. For example, Li et al (2009, GCA, 73, 4803) shows that palmitic acid hydrogen isotopic values are constant in the sediment core when  $\delta D$  values of other compounds show large variations: the most probable explanation for the constant palmitic acid  $\delta D$  values is that various organisms living in the water column display the same hydrogen isotopic fractionation relative to the sea water. Although Li et al., argue, based branched fatty acid  $\delta D$  values, heterotrophic bacteria have different hydrogen isotopic fractionation values, the fact that the resulting sedimentary combined PA  $\delta D$  values show constant values (hence faithfully recording sea water isotopic ratios) indicate either the contribution of heterotrophic bacteria is small (even in the sediments), or the suggested difference between phototrophs and heterotrophic bacteria is not manifested in the real natural system. The sediment data are particularly important for supplementing the evidence presented in this paper, because sediment will have, undoubtedly, large heterotrophic bacteria input. One possibility is to consider if the newly produced PA from a heterotrophic bacteria during biodegradation may actually have the same hydrogen isotopic values as the PA in the decomposing organic matter. It is not impossible to consider a scenario that, because PA exists in all organisms, the enzymes leading to produce this compound may share such a great deal of similarity, and hence the hydrogen isotopic fractionation relative to source water is all constant across different organisms. For heterotrophs some of the hydrogen on PA would come from food rather than water, but perhaps that proportion is relatively small when all heterotrophs are considered.

Response: We agree that the discussion about the palmitic acid  $\delta D$  could be expanded and thank Referee #2 for his suggestions. Accordingly, we expanded section 4.3.2. *Palmitic acid  $\delta D$*  of our manuscript along the lines suggested by the referee. We especially emphasized the consistency of isotopic fractionation along the large variety of environments and palmitic acid producers.

Anonymous Referee #2: To say that when alkenone concentrations are higher than 10 ng/L, its hydrogen isotopic ratios are correlated to water  $\delta D$  and salinity is an overstatement. P values are too high, and if residual is plotted, it is too large across the salinity gradient.

Response: We agree that the p value of 0.05 for the  $\delta D_{H_2O} - \delta D_{C_{37}}$  correlation of high  $C_{37}$  concentration samples is high. We included a short discussion about the p value in the revised manuscript and also modified the abstract to that regard.

Anonymous Referee #2: If alkenone advection is the culprit, surely PA will also be affected. If the argument is the PA gets degraded faster hence the influence on hydrogen isotopic ratios is smaller than alkenones (which is more recalcitrant), one has to explain why regenerated PA from decomposers would not have been messed up for its H isotopic signal. I think overall alkenones simply do not track water hydrogen isotopic ratios or salinity trends. The main reason is probably the species effect and do not think the  $C_{37}/C_{38}$  ratio is a reliable indicator of species (the ratio changes at different grow rate and salinity, and in particular different strains of the same species). E Hux has much greater hydrogen isotopic fractionation than the coastal species I Galbana, and any water isotopic signal (about 40 per mil in modern Amazon plume) is simply overwhelmed by the species effect. Clearly, the percentage of galbana and E hux does not change linearly across the salinity gradient, otherwise one could still expect to see some kind of linear relationship between alkenone dD and salinity. This corroborate with the results from Chesapeake Bay where species effect basically cancels the salinity effect.

Response: We agree that alkenone advection is unlikely the dominant factor responsible for the lack of correlation between  $\delta D_{C_{37}}$  and  $\delta D_{H_2O}$ , since this explanation is insufficient to explain the deviations observed for the temperature reconstruction (see page 10, lines 8-12 of the original version of the manuscript). However, we also doubt that the species effect is the dominant factor. If species variability would be the dominating factor, the  $C_{37}$  concentration in the low salinity outflow plume would not be necessarily low. Furthermore, it would be expected that the fractionation factor would increase in proximity to the coast, which is not the case. Although the  $C_{37}/C_{38}$  ratio has its limitations, a strong species variation would arguably lead to large variations in the  $C_{37}/C_{38}$  ratio (M´Boule et al. 2014, Conte et al. 1998), which we do not observe. We have expanded the discussion in Section 4.3.1 *Alkenone  $\delta D$*  by further stressing why we doubt that advection is the dominant controlling factor of  $\delta D$  in  $C_{37}$ .

Anonymous Referee #2: I do not think paired measurement of dD values of PA and alkenones will improve paleosalinity reconstruction based on the results from this study: the chances are that more confusion will be generated when two disagrees.

Response: Indeed, we do not claim that paired  $\delta D_{PA}$  and  $\delta D_{C_{37}}$  analyses would necessarily improve salinity reconstructions. We rather suggest that the paired use could give indications if one of the proxies is biased due to one of the many potential issues discussed in the manuscript (see page 14, lines 24-26 of the original version of the manuscript). Conversely, a good match between the  $\delta D$  of two independent biomarkers would add additional confidence to the validity of a salinity reconstruction. We clarified this in the section 5. *Conclusions* of the revised manuscript version.

Anonymous Referee #2: However, I would suggest in future get the core top sediments across this salinity gradient, and measure the hydrogen isotopic ratios of PA and alkenones. Sediment would integrate all input sources, include heterotrophic bacteria, and can serve as a better test or calibration of paleosalinity reconstruction using PA dD values.

Response: We absolutely agree that the analysis of core top sediments would be an important addition to the study of  $\delta D$  in biomarkers from suspended particles. In the Amazon Plume, the study of core top sediments is however somewhat complicated by the marked temporal and spatial variations in the salinity gradient. Furthermore, there is little to no modern sedimentation in some areas on the continental slope ocean wards of the Amazon Plume.

## References

Conte, M. H., Thompson, A., Lesley, D., and Harris, R. P.: Genetic and physiological influences on the alkenone/alkenoate vs. growth temperature relationship in *Emiliana huxleyi* and *Gephyrocapsa oceanica*, *Geochim. Cosmochim. Ac.*, 62, 51–68, doi:10.1016/s0016-7037(97)00327-x, 1998.

M'Boule, D., Chivall, D., Sinke-Schoen, D., Sinninghe-Damsté, J. 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. Ac.*, 130, 126–135, doi:10.1016/j.gca.2014.01.029, 2014.