

Seasonal survey of the composition and degradation state of particulate organic matter in the Rhone River using lipid tracers by Galeron et al.

This manuscript couples lipid and lipid degradation byproducts to explore the underlying dynamics to determine the origin of particulate organic matter in a major mixed-use river system. The authors sampled near the river outlet repeatedly throughout the year to explore the seasonal dynamics and change in the source and origin of POM sources.

There is relatively little preexisting work exploring these dynamics using lipids in mixed use rivers, and the seasonal aspect to this work is particularly valuable. The manuscript will be a welcome contribution to the literature after some minor revisions and considerations. Overall, the manuscript is fairly well written, although it contains some loose and colloquial language and could use some additional tightening. The paper could also be improved by creating a table of lipid tracer names, origins, and associated degradation products discussed in the study. However, these issues are minor, and my two main areas of concern are as follows:

The merit of the manuscript is presenting all of the data available in POM at the Rhone estuary. However, the conclusions made from this information ignore the significant gap in knowledge about the breakdown rates of these byproducts. Since sampling was only conducted at the mouth of the river, diverse breakdown or uptake rates of these compounds may heavily bias the interpretation of these results. The conclusions should be qualified with this concern and discuss the gap in knowledge and its implications.

The presentation and interpretation of fatty acid data is underdeveloped. There is unclear usage of fatty acid nomenclature conventions, particularly in regards to PUFA classifications. This is particularly important due to different potential origins of 16-18 C PUFA in green

plants and 20+ C forms only found in algae. See Taipale et al. 2013 regarding fatty acid profiles of various algal groups, and related literature for information about terrestrial plants. PUFA should be considered to be split between 16-18 C and 20+ C forms. In addition, fatty acid profile (percentage relative to sum of all fatty acids quantified) should be considered in the tables instead of simple fatty acid content by weight. More detailed comments are contained as annotations in the attached document.

Authors: In the discussion with reviewer #1, we addressed the assumption that the removal rate of the tracers used was similar to that of the associated parent sterol. In section 3.3.2 (p. 14212, lines 4-6), we added the following precisions: "This assumption is based on the fact that aerobic biodegradation of sterols generally involves initial attack on the side chain, which is similar in all the degradation tracers selected to that of the corresponding parent Δ^5 -sterol. Moreover, it may be noted that $3\beta,5\alpha,6\beta$ -steratriols, employed for autoxidation estimates are weakly affected by abiotic degradation processes. This is also the case for Δ^4 - $6\alpha/\beta$ -hydroperoxysterols (photooxidation tracers), which are much more stable than Δ^5 - $7\alpha/\beta$ - and Δ^7 - 5α -hydroperoxysterols (Christodoulou et al., 2009). Indeed, β -scission of the alkoxy radicals resulting from homolytic cleavage of Δ^5 - 7 -hydroperoxysterols and Δ^6 - 5 -hydroperoxysterols affords secondary and tertiary radicals, respectively, more stable than the primary radical resulting from the cleavage of Δ^4 - 6 -hydroperoxysterols (Christodoulou et al., 2009). Moreover, proton driven cleavage (Hock cleavage) of Δ^5 - 7 -hydroperoxysterols and Δ^6 - 5 -hydroperoxysterols involves a highly favored migration of vinyl group (Frimer, 1979), while only an unfavored migration of alkyl group is possible in the case of Δ^6 - 5 -hydroperoxysterols (Rontani et al., 2014)". These precisions on removal rates of degradation products should eliminate all interpretation bias.

Comments on fatty acid profiles and on the interpretation of their origins have been added

(see next comments), but their determination and quantification was not done with enough detail to be able to clearly identify a unique source.

Specific comments:

- P 14198: "likely dominated by diatoms" - This could be more directly addressed by the fatty acid profiles

Authors: Additional comments will be added in the manuscript in the fatty acid section regarding the fatty acid profiles and their potential sources: "More recently, C_{16:1 ω 7} and C_{20:5 ω 3} (eicosapentaenoic acid) were identified as 2 of the main fatty acids in Bacillariophyceae (Taipale et al., 2013). These 2 markers are present in our samples, and together, form 40.5 and 34.1% of the 6 March 2012 and 12 March 2013 samples (versus an average of 13,2% across all samples), which, when coupled with our sterol analysis, concurs with our hypothesis that diatoms are major contributors in the algal blooms identified. It is worth noting that in the 3 October 2012 sample, C_{16:1 ω 7} forms 53.6% of all quantified fatty acids, while C_{20:5 ω 3} is completely absent (0%), and therefore these markers alone cannot be considered to be specific enough in natural river water samples which can contain a number of fatty acids from various sources."

- p. 14200 : "it would be logical" - Casual language. Why?

Authors: On page 14200 line 8, this will be rephrased in the revised manuscript and changed to " we propose to confirm the hypothesis according to which the POM sampled in the Rhone is mainly constituted of terrestrially-produced particulate organic matter travelling with the runoff, while also identifying other sources of OM, sometimes significantly aquatic/planktonic.

- p. 14201 : "40 km upstream from the river mouth" - Is there strong tidal influences and what is salinity range?

Authors: We had identified an issue here, where the sampling station was qualified as estuarine, which is something that was also picked up by reviewer #1: This will be corrected in the manuscript, and "riverine" will replace "estuarine" in the revised manuscript, as the sampling station sees no tidal influences, and the salinity is constant and at 0.

- p. 14203 : "standards" - Need detail about what standards were used. Singles/FAME mix?

Authors: Standards used were single standards. This precision will be added in the revised manuscript on page 14203, line 27: "calibration with external single standards"

- p. 14205: "A we" - Grammar/typo? Should this be "As we also see an increase..."

Authors: Yes, this will be corrected in the revised manuscript

- p14205: "less important" - Less important how? Rather subjective and loose wording

Authors: This refers to figure 3a, where a slight increase in planktonic sterols is visible but is much less important than the one in march 2012. We wonder here if this slight increase is important enough to consider these potential blooms a yearly event.

On page 14205, this will be revised and will read: "As we see an increase, although less important in the proportion of planktonic sterols in the Spring 2013 samples (20 times less in quantity when compared to the 6 March 2012 sample, but still constituting 31 and 27% of the total of all sterols quantified in the 12 March and 21 March 2013 samples respectively)"

- p. 14208 : "Polyunsaturated fatty acids" - Was this defined here or in the methods? Including both sum of 16-18 and 20+ C polyunsaturated fatty acids? See Galloway et al and Taipale et al 2013 for the different origins of various carbon length PUFA and HUFA.

Authors: The manuscript did not provide details on how the proportion of PUFA was calculated. We have added the information on p.14208 with "(quantified using the total of all PUFA quantified between C₁₄ and C₂₆)". As discussed previously, we have added a comment discussing the origin of different fatty acid profile.

- p. 14208: "These high contributions support the presence of a high proportion of fresh algal material in these samples." - Examination of total fatty acid profile and indicator FAs could give additional detailed information into fatty acid origin (algal class, terrestrial, bacterial)

Authors: See previous comment

- p. 14209: "harmless" - Rather strong statement. Harmless in what way?

Authors: "Harmless" was added when we were concerned that readers would tend to conclude that this industrial input was dangerous to the environment. This is also why we use the word contamination and not pollution. Since we do not have sufficient data to prove that it is harmful, or harmless for that matter, we will remove the word "harmless" from the revised manuscript leaving only "This industrial contamination" on p. 14209.

- p. 14212: Would be useful to have a singular table that summarizes all examined lipid products, their origins, and degradation byproducts products by process

Authors: Upon initial submission, we were required to reduce the length of the manuscript,

along with the number of figures and table. We agree this could be a useful table, but if we include all quantified lipids, degradation products and origins, this could become a rather large table. If deemed necessary by both reviewers, we can add it into the manuscript.

- p. 14215: "we will logically be only looking at unsaturated fatty acids here." - Loose and colloquial language.

Authors: This will be corrected in the revised manuscript on p. 14215 into "We will only be looking at unsaturated fatty acids here"

- p. 14218: "suspect diatoms to be major contributors." - This can be more closely examined using the fatty acid profiles. See Taipale and Galloway.

Authors: See previous comments. We have not been able to determine the double bond position with enough precision on all quantified FA to precisely identify contributors using the references provided. They have however been discussed in the revised manuscript (see previous comments).

- p. 14218: " Using specific lipidic degradation products, we were able to identify for the first time the part that bacterial degradation, autoxidation and photo-oxidation play in organic matter degradation in a Mediterranean river." - Discussion needed re: differing degradation rates and its implications

Authors: See first comment. Precisions have been added regarding the removal rates of these degradation products, according to reviewer 1 comments as well.

- p. 14218: "underestimated" - Needs more and clearer explanation

Authors: The underestimation of photo-oxidation was discussed in section 3.3.2

- Table 2a: Consider using fatty acid profile as a percentage - No C_{22:6ω3}?

Authors: The table has been modified to show Fatty Acid and Hydroxy Acid contents in percentage of the total FA and HA quantified. C_{22:6ω3} has not been detected.

- Figure 2: X axis should be standardized in time rather than equally display sampling dates

Authors: This has also been discussed with reviewer #1. We chose to represent the data this way since a real time scale would mean months with multiple data points and months with no data points at all. This would make for confusing figures with gaps in the data, and would make it difficult for the reader to get a clear picture. We have tried to make the X-axis as readable as possible, with a font size as large as possible.

- Figure 3a: Same comment about X axis as Fig 2. X-axis labels are small and hard to read. Y axis in Fig 3A appears bold and non-consistent with previous. Y-axis lines in figure are inconsistent.

Authors: See previous comment for x-axis. We will alter figure 3 in order to make the x-axis labels larger and easier to read. We will also fix the y-axis, which shouldn't appear bold. We are not sure what is meant by " Y-axis lines in figure are inconsistent". If this is about the horizontal grid lines, they are actually all present but for some reason sometimes only appear when the PDF file is zoomed in.

- Figure 3C: If March 6th 2012 is associated with the algal bloom and evidence in sterols, what about the PUFA spike in March 2013?

Authors: On p. 14208 (lines 21-22) we mentioned that "Polyunsaturated fatty acids are

present in very low proportions in our samples (quantified using the total of all PUFA quantified between C14 and C26), apart from the 06/03/2012 and 12/03/2013 samples where they contributed to 44 and 40% of total fatty acids (Figure 3C). These high contributions support the presence of a high proportion of fresh algal material in these samples. " We previously emitted "the hypothesis of a yearly phytoplanktonic spring bloom, with a larger magnitude for the 2012 event" and the PUFA data goes towards such an hypothesis, but as previously said, we did not go into enough detail in the PUFA analysis to be able to clearly identify contributors on PUFA data alone.

- Figure 4a: No error or replicate samples for Chlorophyll-a? - Is this supposed to be Jan 22nd? Or was this just sampled on a different date than everything else, including its photodegradation?

Authors: This data has been collected by the MOOSE observation system, and there is no replicate sample data, hence no error calculated. The data represented here is all the available data during our entire sampling period (no measurements were made in 2011), and if a number of points fall on the same dates as our own samples, not all of them do (Jan 21 is an example). This only applies to chlA quantification, as the data in Fig. 4b was calculated using our own samples.

- Figure 4b: Could this be lined up so that the dates are in the same position for both?

Authors: See previous comment. Since the dates are not the same, we chose not to align these 2 figures. There are dates in figure 4a that do not appear on figure 4b and aligning them could be misleading as readers might make a parallel between different dates.