Seasonal survey of the composition and degradation state of particulate organic matter in the Rhone River using lipid tracers (written by M.-A. Galeron et al.)

- **General comments:**

This paper provides detailed data on seasonal variation of a wide variety of lipid molecules contained in suspended particulate matter in the Rhone River estuary. Using these data, the authors discussed the origins and the biological and abiotic degradation processes of the lipids.

In particular, their approach for evaluating quantitatively the influence of abiotic oxidation on the removal of a few lipid compounds seems an interesting challenge. Even though similar studies have been published for a few other ecosystems recently (e.g. Rontani et al. 2009, 2014b), I think that their study has a sufficient merit to be published in Biogeosciences after appropriate revision, because this study focused on an environment with relatively high human impacts compared to the preceding studies. The argument concerning the provenance of POM based on the lipid composition is generally convincing.

My concern is that their conclusions, particularly those for the individual degradation processes, depend on numerous assumptions that are not verified enough. Above all, the estimation of the degrees of biodegradation and auto- and photo-oxidation undergone by specific lipids such as cholesterol and sitosterol depends on the assumption that the parent lipids and all the degradation intermediates used for the estimation have similar turnover rates in river water, which seems dubious. In fact, the authors noted that some of the intermediates (e.g. Δ6-5α-hydroperoxides, p.14213, line 5) are too unstable under certain conditions to be used as tracers. They used an alternative, apparently more stable species as a tracer; however, it seems quite difficult to confirm whether it is so stable as to conform to the above

assumption. The assumption that the yield ratios of different oxidation intermediates are constant is also questionable. Although they mentioned these assumptions only briefly (p.14212, lines 2-5; p.14213, lines 1, 10-13), I would like to request them to elaborate the validity of these assumptions in more detail and discuss how the following interpretations may be changed if the turnover rates and/or the yield ratios are variable.

Authors: Although each sterol and its degradation products may be potentially totally mineralized by marine bacteria, we assume that they should exhibit similar reactivity towards bacterial degradation. 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 3β,5α,6β-steratriols, employed for autoxidation estimates are weakly affected by abiotic degradation processes. This is also the case for Δ^4-6α/β -hydroperoxysterols (photooxidation tracers), which are much more stable than Δ^5-7α/β - and Δ^7-5α-hydroperoxysterols (Christodoulou et al., 2009). Indeed, β-scission of the alkoxyl 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 will be added into the manuscript, in the sterol degradation section.

- **Specific comments:**

• The use of the terms "plant-derived organic matter" and "organic matter of human-origin" in Abstract (lines 13-16) is an overgeneralization. This study investigated the degradation processes of only two sterols, one of which (sitosterol) is surely of plant origin but should not be regarded as a representative organic matter of plants. The other, cholesterol, is not limited to human (cf. p.14206, lines 7-9).

Authors: In the abstract, we propose to replace the sentence "terrigenous contribution to the plant-derived particulate organic matter" by "terrestrial higher-plant contribution to the particulate organic matter". We also propose to replace the sentence "Plant-derived organic matter appears to be mainly affected by photo-oxidation and autoxidation" with "higher-plant-derived organic matter appears to be mainly affected by photo-oxidation and autoxidation (…) while organic matter of mammal or human origin…". Cholesterol is indeed a sterol that can have multiple sources, whether human or not, but we did include coprostanol and epicoprostanol in the degradation products studied (these 2 sterols being exclusively of mammal or human origin). The point here is mainly to point the difference in degradation patterns between these 2 sterols (and the types or organic matter they are mainly found in)

• The authors mentioned that the sampling station at Arles was an estuarine station (p.14201, line 1). If so, the mixing between seawater and river water would play an important role in the dynamics of particulate matter. But they didn't argue this point in this paper. I would request them to show the salinity data in Fig. 2 and discuss briefly possible influence of salinity on the behavior of suspended particles. Water temperature data are also worthwhile to show here.

Authors: This is a good point, and one we became aware of as well. Arles has often been referred to as an estuarine station, when it actually is a riverine station – the salinity in Arles is 0, and it is too far to receive any seawater inputs. On p. 14201 – line 1, the term "estuarine"

will be replaced by “riverine” in the manuscript. There is no salinity data to show, and the automated measurement of water temperature at the station is highly unreliable, which is why we did not include it in this study.

• Acidification with sulfuric acid (p.14201, line 13) is relatively rare for the treatment of POM samples. Can the authors refer to some reference paper? I guess excess sulfur may cause rapid deterioration of catalysts in the instrument.

Authors: Generally hydrochloric or phosphoric acids are used to remove inorganic carbon. We didn't use phosphoric acid because of the risk for phosphorus contamination. We chose sulfuric acid (4%) instead of hydrochloric acid due to the large proportion of carbonate in the suspended matter. Also, this acidification procedure is similar to the one used for the wet-oxidation method which gives excellent results. On p.14201, line 13, the following reference will be added to the manuscript: (Raimbault et al., 1999)

Raimbault P., Diaz F., Boudjellal B., Simultaneous determination of particulate forms of carbon, nitrogen and phosphorus collected on filters using a semi-automatic wet-oxidation procedure. Mar. Ecol. Progr. Ser., 180: 289-295, 1999.

• Measuring silicate using the GF/F filtrates (p.14201, lines 15-16) often leads to an overestimation due to leaching from the GF/F.

Authors: Sampling procedure for silicate has not been described in the paper. In fact, samples for silicate analysis were filtrated through a 0.45 µm polycarbonate membrane and kept at 5°C until analysis. This will be added in the Materials & Methods section of the manuscript.

• A mixture of ethyl acetate/BSTFA (p.14202, line 24): what proportion?

Authors: Dry samples were taken up in 100µL BSTFA and an appropriate amount of ethyl acetate, depending on the concentration in lipids in each sample, in order to get the best possible GC-EIMS reading. On p.14202, line 24, this will be added into the manuscript: "After evaporation to dryness under a stream of N_2, the derivatized residues were taken up in 100µL BSTFA (to avoid desilylation of fatty acids) and an appropriate amount of Ethyl Acetate, depending on the concentration in lipids in each sample, in order to get the best possible GC-EIMS reading"

• The combined DCM extracts (p.14203, line 4): Did it include also the chloroform phase of the initial phase separation, or not?

Authors: Yes, it included the chloroform phase of the initial phase separation.

• The authors mentioned the minimum and maximal daily flow rates of the Rhone River recorded on 8 Oct 2011 and 19 May 2013, respectively (p.14204), but I could not find such sampling dates in Fig. 2. In addition, the time axis in Figs. 2-6 seems a bit confusing. Please consider adopting a real time scale where it is possible.

Authors: The minimal and maximal flow rates dates mentioned are the days during the entire sampling period on which flow rates were the highest and the lowest. The original manuscript included a figure where daily flow rates were shown for the entire period, not just on sample dates. The figure has since been removed, and we will correct the dates and water flows mentioned so that they reflect minimal and maximal flow rates on actual sample days. On p. 14204, the revised manuscript will read: "During our sampling period the daily flow rate fluctuated between 680 (19 September 2012) and 4661 $m^3.s^{-1}$ (5 November 2011; Figure 2)." Also, we did consider using a real time scale, but since our sampling dates were not regular (some months have multiple samples, and other months none), using a real time scale made

the figures more confusing since we could have gaps in the data presented. We chose to be consistent in the time axis used across all figures, even though it is not a real time scale, so that readers would not be confused by missing data or multiple points on one month.

• "Typical for river systems" (p.14204, line 18): Literature should be referred to here.

Authors: p.14204, line 18: the following reference will be added into the manuscript (Jansson, 1982)

Jansson, M.B. Land erosion by water in different climates. UNGI Rapport (Sweden), 1982

• On page 14207 (lines 5-8), the authors suggested that 5 April 2011, 2 May 2013, and 4 Nov 2011 were flood dates. However, Fig. 2 shows that the river was under the base-flow conditions on 5 April 2011, and that the flow rate was not recorded on 4 Nov 2011.

Authors: That's right, there is a mistake about April 5, 2011 and we will correct that in the manuscript. However, November 4 was a flood date (this was actually a major recent flood), but on major floods, our measurement instrument is sometimes overflowed and could not record flow rates for a few days. Flow rates were only recorded again on Nov 7. On page 14207 (lines 5-8), the revised manuscript will read "The amount of cuticular waxes is variable amongst samples, between 0.02 and 3.8 $\mu g.mg^{-1}$(dry weight), with the highest in the 5/4/2011, 2/5/2013 and 4/11/2011 samples (3.8, 2.2 and 1.7 $\mu g.mg^{-1}$ respectively). Two of these sample dates (2 May 2013 and 4 November 2011) happen to be flood dates."

• What do the authors mean by "content variability" (p.14208 line 5)?

Authors: By "content variability" we meant that the amount of chlorophyll quantified on our different samples dates was highly variable.

• Section 3.2.4 (p.14208-9): The authors may explicitly mention here that the provenance analysis depending only on the fatty acid composition likely leads to an underestimation of higher plant contribution.

Authors: In section 3.2.4 (p.14208-9), on line 18, the revised manuscript will include a comment stating that "Due to the high degradability of fatty acids, a number of them could not be quantified, potentially leading to an underestimation of higher plant contribution."

• I recommend that the definitions and calculation methods for CPPI (p.14211), biodegradation % (p.14212), and auto- and photo-oxidation % (p.14213) may be described in the Materials and Methods section.

Authors: Since all calculations methods for CPPI, biodegradation, photo- and autoxidation heavily depend on the compound descriptions preceding it, adding it all into the M&M section might make this section too long and descriptive. Adding only CPPI methods might make for too short of a dedicated section in the M&M?

• On page 14216 (lines 26-29), the authors mentioned the detection of cis and trans allylic 18,(8-11)-dihydroxyoleic acids (auto- and photo-oxidation intermediates) referring to Table 2 and Fig. 5, but no such data can be found in this figure. Table 2 only shows 18,(8-11)-dihydroxy-C16:0. They suggested a high proportion of cis isomers (line 29) and larger amounts of oxidation products than the parent ω-hydroxyoleic acid on a few sampling events (p.14217, lines 1-3), but these are not confirmed by the presented data.

Authors: Since the manuscript had to be downsized, a number of figures were removed, and it's true that there no longer is a figure presenting this data, for size and clarity purposes. On page 14216 (lines 26-29) the "Table 2 and Fig. 5" reference shall be replaced by (Galeron & Rontani, unpublished data) in the manuscript.

• On page 14217 (lines 3-5), the authors mentioned "the previously discussed yearly variability in cuticular wax content", but it is a bit unclear what part of this manuscript they indicated by this phrase. Fig. 3b may be referred to here.

Authors: On page 14217 (lines 3-5) the reference (See section 3.2.2 and Fig. 3b) will be added in the manuscript after the sentence "the previously discussed yearly variability in cuticular wax content".

• What do the authors mean by "high compartmentalization effects" (p.14218, line 2)? Is it same as the protection by waxy materials from degradation suggested on p.14213 (lines 24-25)?

Authors: By “high compartmentalization effects” we mean that these compounds are structurally protected by rigid structures that physically protect them from degradation. This is different from the waxy material protection suggested on p. 14213.

- **Technical corrections:**

• Line 10 of page 14199: et -> and

Authors: Both instances of “et” on that line will be corrected to “and”

• Line 28 of page 14205: A -> As (?)

Authors: Indeed, we will correct the “A” into “As”

• Line 16 of page 14214: please remove "a" from McCalley et al., 1981a.

Authors: This will be corrected in the manuscript

• Cauwet et al. (1990) that appears in the reference list (p.14220) does not appear in the main text.

Authors: Although we carefully checked references after downsizing, this one must have slipped through the cracks! It will be removed from the reference list in the manuscript.

• Please remove "a" from Kolattukudy, P.E. 1980a (p.14222).

Authors: This will be corrected in the manuscript