Replies to Referee 1's comments.

We thank the Referee for the through review. Below we copy his/her comments (in italics) and provide point-by-point replies.

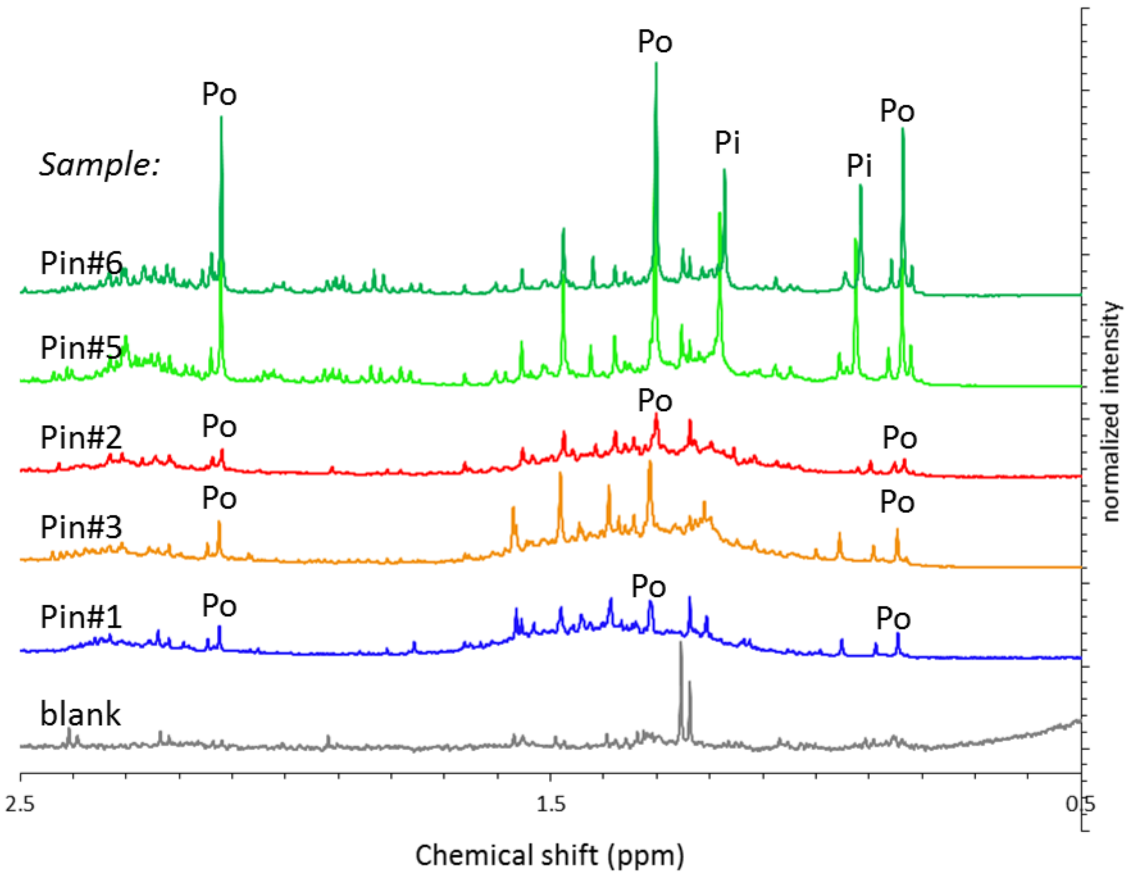
1. General comments: This article reports a very interesting study on the compositional features of secondary organic aerosol (SOA) samples generated using a Potential Aerosol Mass (PAM) oxidation flow reactor using organic precursors that are well-established source fingerprints: alpha-pinene (most studied biogenic VOC), naphthalene (proxy for anthropogenic aromatics), and isoprene (most abundant biogenic VOC). The generated SOA samples were characterized/analyzed by means of proton nuclear magnetic resonance (1H NMR) spectroscopy and ion-exchange chromatography coupled to a UV detector (on-line) and a TOC analyzer (off-line). SOA is one of the least understood constituents of fine aerosol particles; current widely-used models cannot predict its atmospheric loadings, oxidation state, or even the nature of the atmospheric ageing processes. Understanding, characterize, and (semi-)quantify the effects of SOA formation and ageing is challenging because it requires a different framework to capture and describe the continuous evolution of the structural features of organic compounds. Nonetheless, if those aims are attained, the outcomes will be very important for the atmospheric chemistry modelling community. In this Reviewer opinion, the topic of this paper is relevant to the journal's interests and will be of interest to readers. Studies such as this one are needed to advance our understanding on SOA oxidative aging mechanisms at ambient conditions. All in all, the quality of the measurements is excellent and the presentation and discussion of data is good. Nevertheless, this Reviewer has identified some issues requiring further clarification from the Authors. I recommend publication of this study after the Authors consider the specific comments (below).

2. Specific comments:

- Section 3.1.1, lines 198-200: Although there is a change in the intensity of NMR peaks between 1 and 3 ppm of alpha-pinene SOA with photochemical age, the 1H NMR spectra of alpha-pinene SOA at medium ageing still exhibits resonance at the same chemical shift regions of alpha-pinene SOA with low oxidation level. Therefore, I do not think that there is a sharp change of NMR fingerprinting of alpha-pinene SOA already at medium ageing.

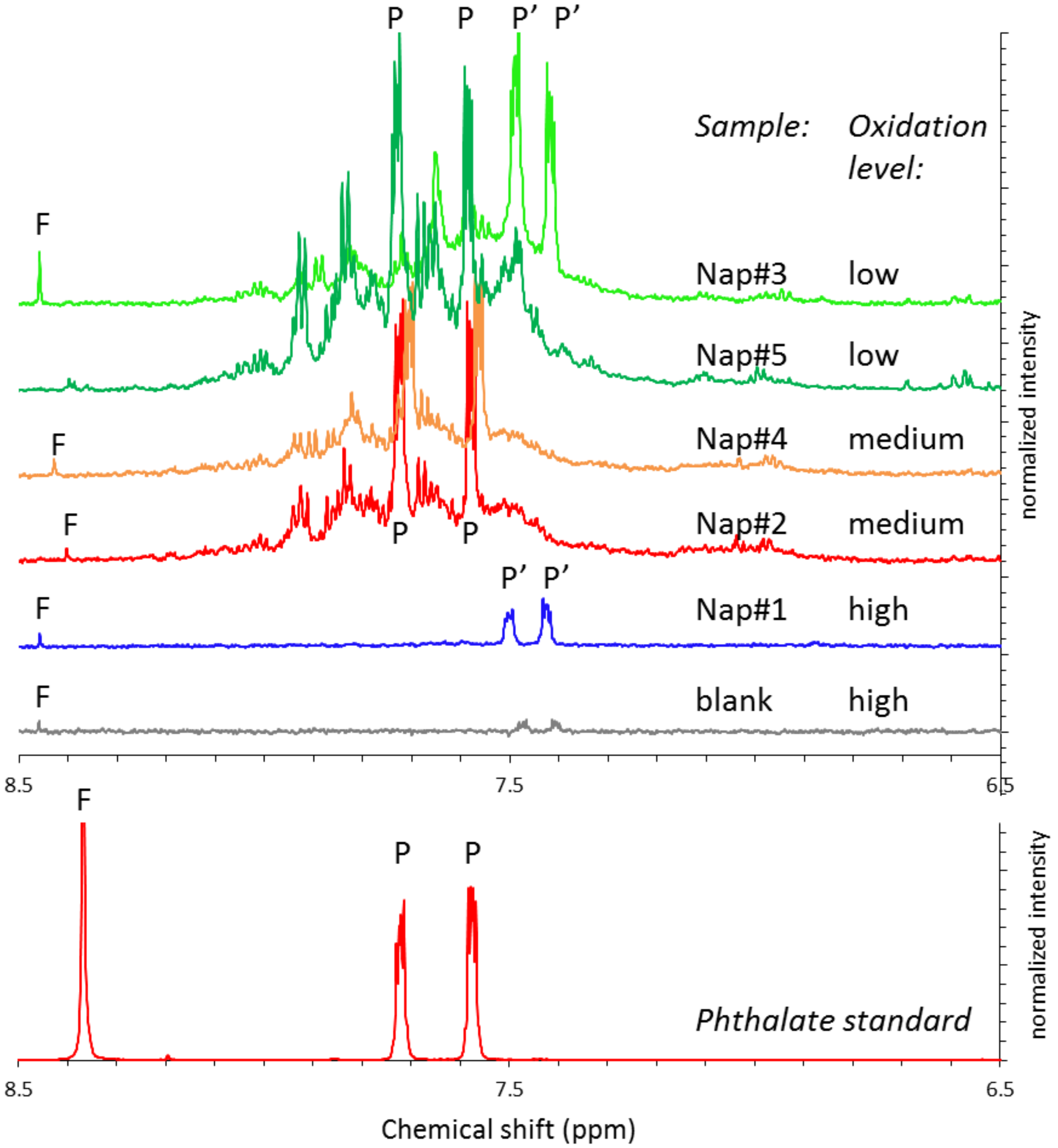
REPLY: There are indeed several specific resonances, some of which are known tracers of alpha-pinene oxidation, found in all alpha-pinene SOA samples, but their intensity decreases with ageing, especially between the fresh to the medium oxidation state samples. This shows up more clearly when using an y scale for Figure 1 illustrating the highest peaks (from pinic and pinonic acids in Pin#5 and Pin#6 samples) (see figure below). The integral of the resonance at 0.83 ppm arising from one of the two gem-methyls of pinonic acid changes from 2% and 3% of the total integral of the spectrum for samples Pin#5 and Pin#6, respectively, to 0.3% for all the other three samples. Therefore, the contribution of first generation oxidation products of alpha-pinene is one order of magnitude greater in fresh SOA than in the medium and high ageing state samples. This confirms that there is in fact a marked change in NMR fingerprinting already at medium ageing state. The figure below will be added in the Supplementary material. The text (lines 198-200 of the first submission) will be changed into:

"In the NMR spectra corresponding to a "medium" SOA oxidation level, the resonance at 0.83 ppm of chemical shift, arising from one of the two gem-methyls of pinonic acid, accounts for only 0.3% of the total integral of the spectrum, while it represented 2 – 3 % in the fresh SOA samples."

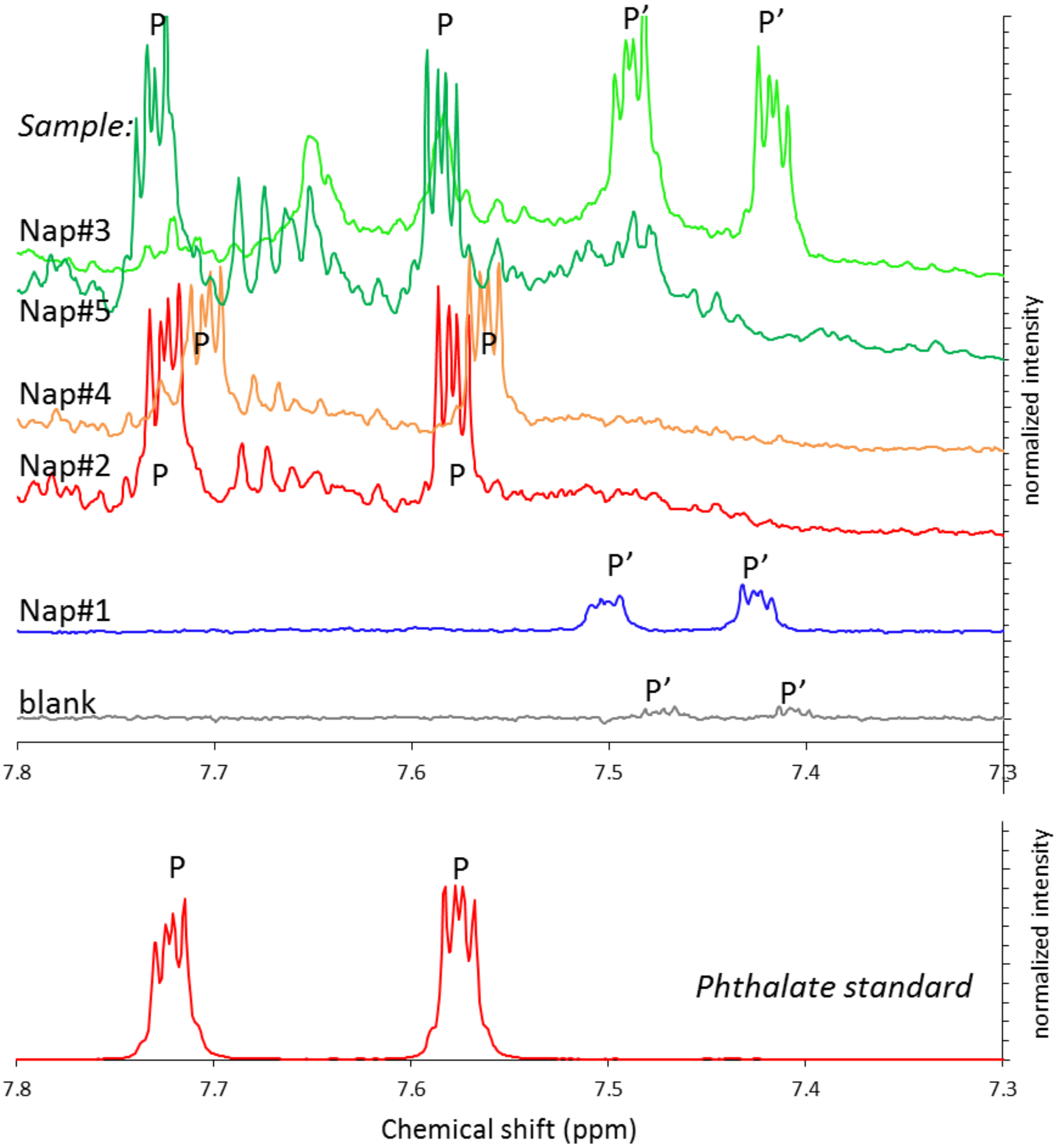


- Section 3.1.2, line 218: The Authors state that "moderately aged SOA show mainly the two singlets of phthalic acid" in the aromatic region. The singlets of phthalic acid should appear/resonate at approximately 8.1 ppm. This assignment is unclear in the spectra of naphthalene SOA (Figure 2) due to the presence of a broad resonance at approximately 7.2-8.3 ppm.

REPLY: Phthalic acid is a diprotic acid, and it can resonate in a range of chemical shifts depending on protonation sate, hence on pH of the aqueous solution. We have performed the NMR analysis of naphthalene SOA in unbuffered solution, therefore we expect the chemical shifts of carboxylic acids to differ to a certain extent between samples. Below, we show a blow-up of Figure 2 of the original manuscript showing the aromatic region in more details. Most of the spectra show a very small singlet at 8.40 – 8.46 ppm which must be attributed to formate (marked with "F" in the figure). The 0.06 ppm difference in chemical shift between samples must be caused by pH differences in the solutions. At the bottom of the figure, we added the spectrum of a phthalic acid standard (potassium hydrogen phthalate) in buffered solution (a potassium deuterated formate/formic acid buffer with pH~3.8, the same used for the isoprene experiments). The position of the formate peak indicates that the pH of the phthalic acid standard solution is approximately the same of sample Nap#2. Our test clearly indicates that the two complex resonances at 7.56 – 7.59 and 7.71-7.74 in sample Nap#2 - and of analogous peaks in samples Nap#4 and Nap#5 (marked with "P" in the figure) - must be attributed to phthalic acid. The other samples (Nap#1, Nap#3 and the blank) exhibit a system of peaks with a fine structure similar to that of the standard of phthalic acid but resonating a lower chemical shifts (between 7.4 and 7.5 ppm) and marked "P' " in the figure. On the basis of the position of the formate peak, approaching 8.46 ppm, in these samples, the peak systems P' are presumably from phthalic acid, while in different pH conditions with respect to samples Nap#2, #4 and #5 and the phthalic acid standard solution.

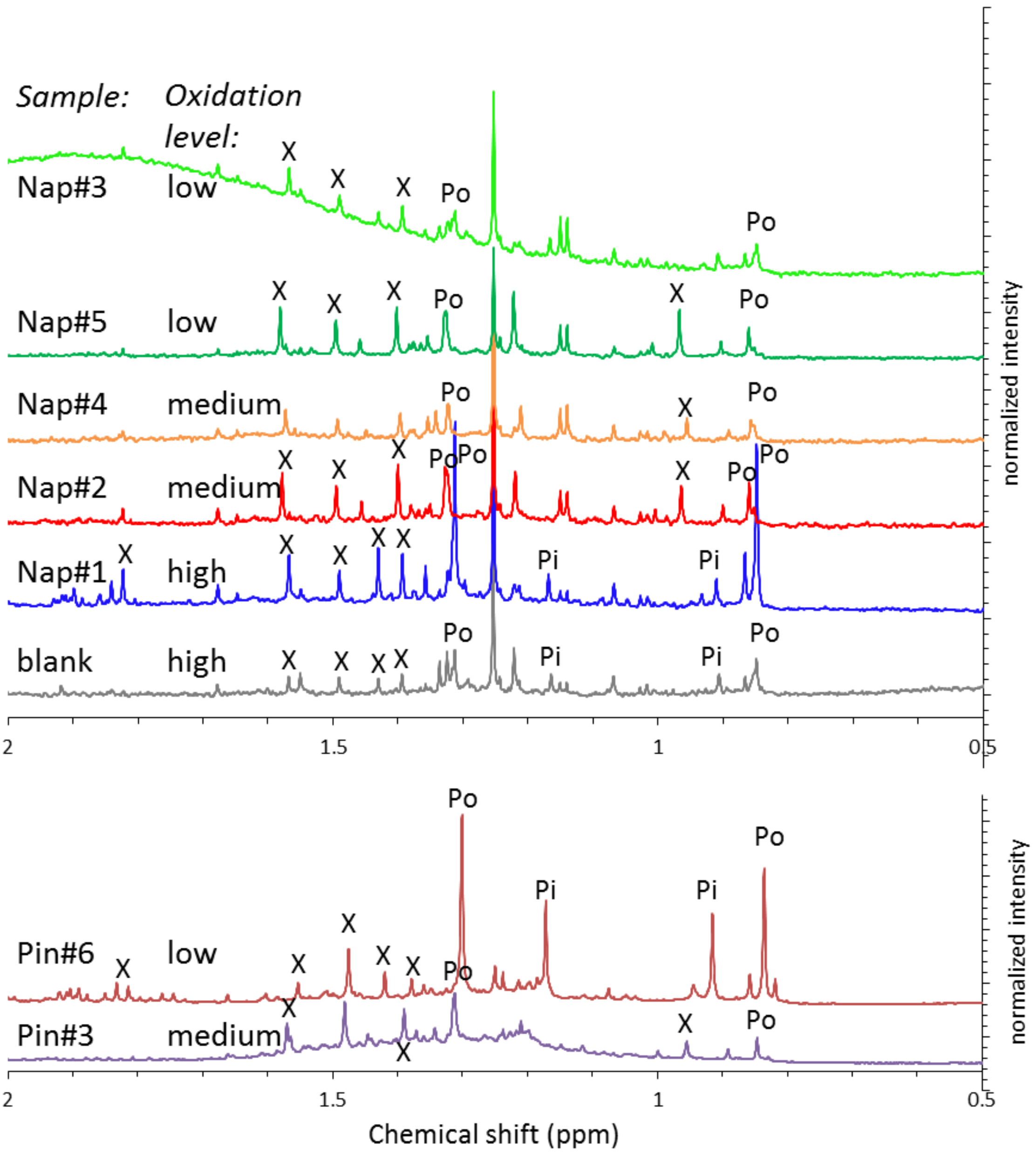


Below, we present the same figure, with chemical shift range between 7.3 and 7.8 ppm enlarged, showing the fine structure of the phthalate resonances.



- Section 3.1.2, lines 218-221: Besides exhibiting NMR peaks between 3.5 and 6.0 ppm, all spectra also exhibit noticeable NMR peaks between 1 and 2 ppm. Could these NMR resonances be still a consequence of the presence of colloidal hydrophobic material in solution, or they could be attributed to aliphatic structures derived from the ageing process?

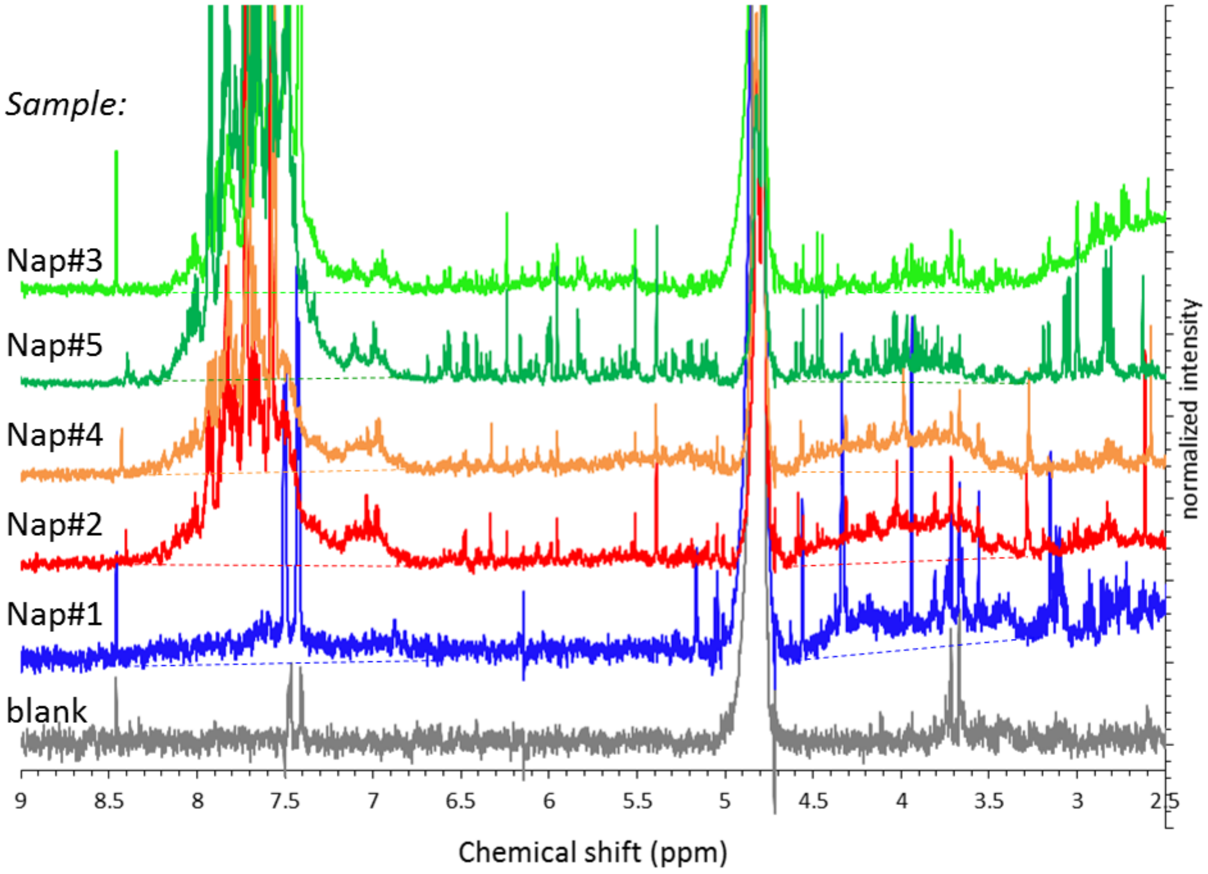
REPLY: The referee is right in pointing out the presence of multiple resonances between 1 and 2 ppm in the spectra of naphthalene SOA. It should be noted, however, that most of them are also found in the blank (spectrum in grey in the Figure 2). The inspection of such peaks in the blank suggests that α-pinene oxidation products, such as pinonic acid (peaks marked with “Po” in the figure below) and pinic acid (peaks marked with “Pi”) together with other unidentified compounds (marked “X”) from previous experiments have caused contaminations of the reactor during the naphthalene experiments (see figure below). Based on these results, it is doubtful that any of the naphthalene SOA samples exhibited genuine resonances in the range between 1 and 2 ppm of chemical shift.



- Section 3.1.2, lines 224-229: In my opinion, the broad aromatic band between 6.5 and 8.5 ppm is totally indiscernible in the spectrum of the most aged naphthalene SOA sample. I am also compelled to disagree when the Authors conclude that "there is no clear trend in the formation/disappearance of aromatic and aliphatic bands with ageing". In my opinion, the 1H NMR spectrum of the most aged sample clearly indicate the disappearance of resonance in the aromatic region, whereas a few NMR peaks resonate in the aliphatic region (chemical shifts = 3.5 and 5.0 ppm). The Authors should include additional explanations to support their statement/conclusion.

REPLY: The inspection of the spectral feature of sample Nap#1 (the most aged naphthalene SOA sample) is challenging due to the very small loadings and the interference from the reactor blank (see comment above). If we restrict our analysis to chemical shifts higher than 2.5 ppm – to get rid of most blank signals – and enlarge the y scale of the spectra, we can have closer inspection of the resonances in sample Naph#1 (see figure below). In spite of the poor signal-to-noise ratio of this spectrum, a very broad aromatic band between 6.7 and 8.2 ppm is still visible in Nap#1, whereas the baseline is completely flat in the blank spectrum. The aromatic band is actually structured into two sections, with one from phenolic compounds (from 6.7 to 7.2

ppm) and a second one arising from aromatic structures substituted with electron-withdrawing groups (from 7.2 to 8.2 ppm). The latter is relatively much less intense than in the other naphthalene SOA samples. In conclusion, the small sample amount for Nap#1 prevents an accurate quantification of aromatic compounds. The Referee is right in noticing that the aliphatic groups between 3.5 and 5 ppm are much more concentrated in this sample with respect to aromatics; however, it is not entirely true that aromatic groups have completely disappeared in this sample.

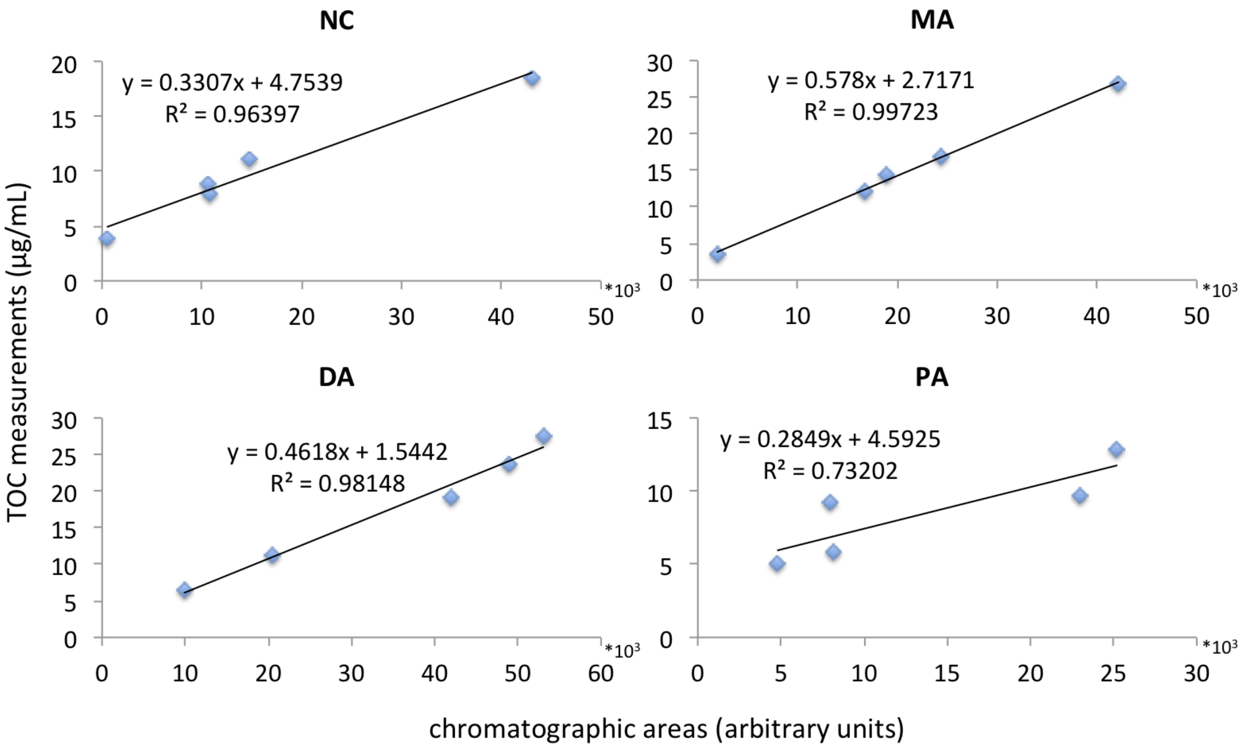


The text in the manuscript will be revised and implemented as follows:

"Despite the low naphthalene SOA concentration, a broad aromatic band between 6.5 and 8.5 ppm and the same signals found between 3.5 and 6.0 ppm seen in the samples with a medium O/C ratio are still visible in this most aged naphthalene SOA spectrum. However, the band from oxygenated functional groups between 3 and 4.5 ppm becomes relatively more intense with respect to aromatics compared to SOA samples of smaller ageing state. Compared to α-pinene SOA, the ^{1}H-NMR fingerprint of naphthalene SOA appears less sensitive to variations in the OH exposure between the low and the medium level of exposure. More substantial changes can be found for the most oxidized sample, which are only partly visible due the low signal-to-noise ratio of the spectrum."

- *Section 3.2, lines 275-276: The chromatograms in Figure 5 suggest that the signal intensity of the chromatographic peaks corresponding to mono- and di-acids increases with increasing photochemical age, which seems to contradict the statement "that the TOC mass fraction of mono- and di-acids decreases from 33% to 18%" with increasing photochemical age. Besides, in lines 273-374, the Authors conclude that "a net increase in acidic compounds with photochemical age can be clearly observed". Additional explanations should be provided to clarify these apparent conflicting conclusions.*

REPLY: There was a mistake in the text. The TOC mass fraction decreasing from 33% to 18% is only for mono-acids, not for total mono-/di-acids. In fact, the fractional content of di-acids remained fairly stable during ageing in naphthalene SOA. The HPLC classes distributions for the naphthalene SOA samples are reported in Fig. S4 where the concentrations units are derived by the measured TOC content in the eluted chromatographic fractions instead of the integrals of chromatographic peaks. On the other hand, peak area and TOC content are clearly correlated for each HPLC fraction type (see figure below).



The text will be revised as follows:

"However, the HULIS content, initially small, increases substantially and progressively with ageing. With increasing photochemical age, the TOC mass fraction of mono- and di-acids decreases from 33% to 18% and from 34% to 33% respectively, while the fraction of PA/HULIS increases from 11% to 30%".

Below we report Figure 5, reformatted also taking into account Referee 2's suggestions. We believe that the reduction of mono-acids and the relatively stable content of di-acids during ageing is clear from both the TOC measurements and the evolution of chromatographic peaks in Fig. 5.

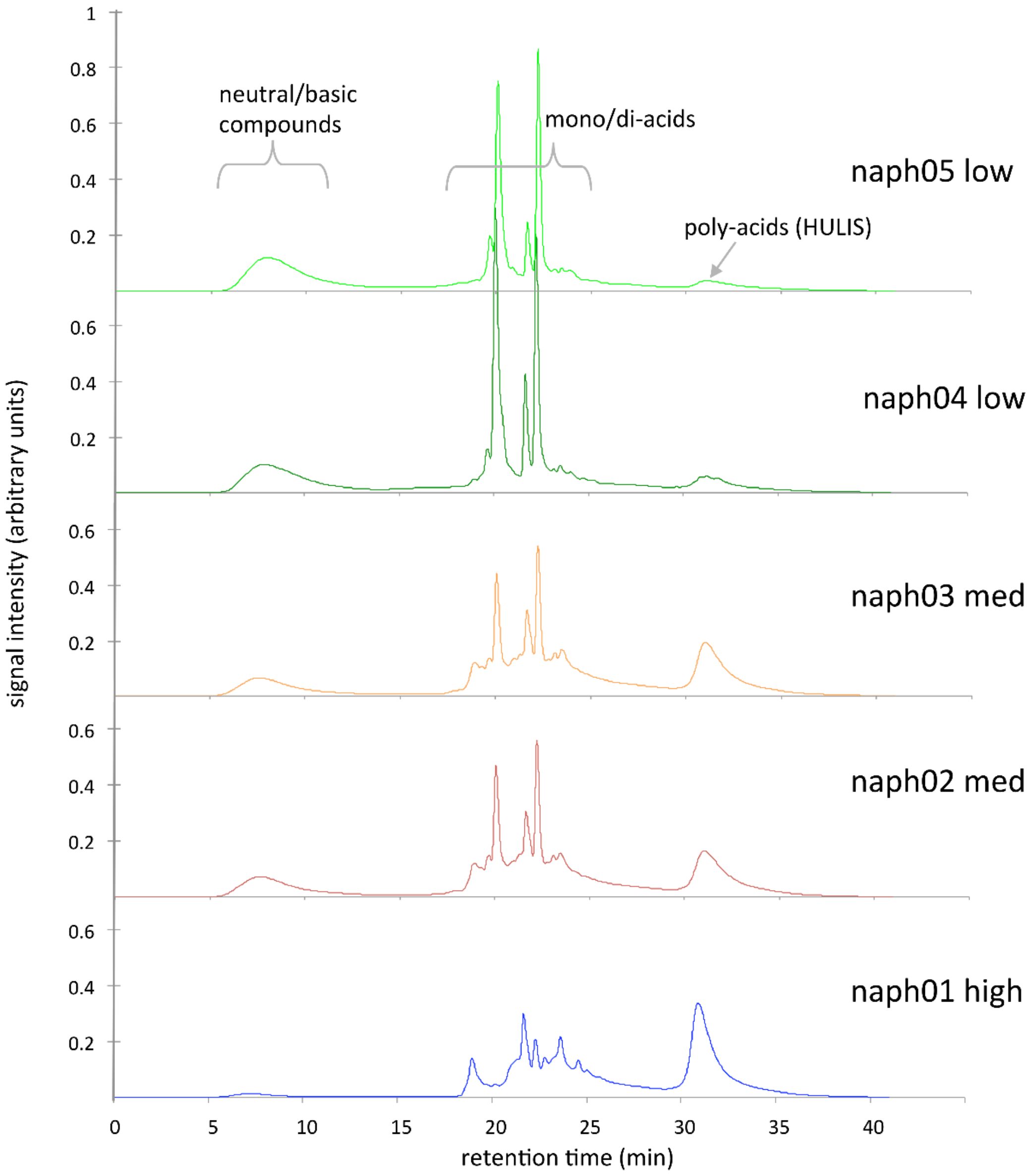


Figure 5.

Finally, we improved Section 3.2 with more clear references to the TOC data of the HPLC fractions presented in Figures S3 and S4 of the Supplementary. Additional observations on the distribution of HPLC classes is now reported at the end of Section 3.2.

- Section 3.2, line 282: the abbreviature “NC” for “neutral compounds” should have been previously defined in line 259.

REPLY: It is in fact defined in the Methods section (2.4).

- Section 4, lines 303-305: In Figure 7, it is unclear which marker correspond to ambient PEGASOS WSOC for the different oxidation levels.

REPLY: The figure reports correlation coefficients. Each value (one point in the figure) represents the Pearson correlation coefficient for one individual SOA spectrum with respect to the reference spectrum for ambient

WSOC (i.e., the same spectrum in Figure S1 upon binning). We have re-formatted the figure (taking into account also the comments by Referee 2):

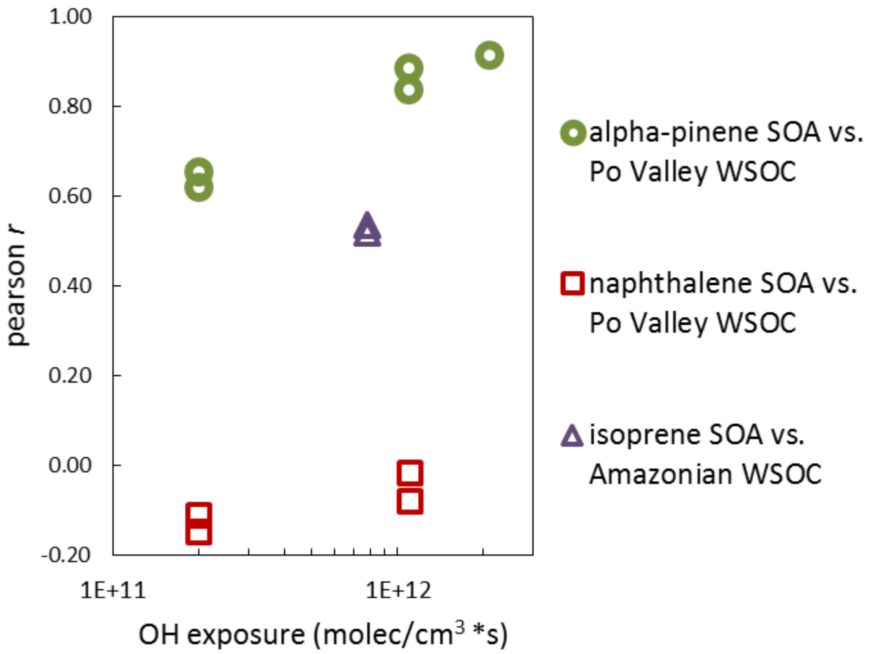


Figure 7.

- Section 4, lines 321-322: In the sentence "the H-NMR spectra of alpha-pinene SOA most closely mimic the functional group distributions of the ambient WSOC sample obtained in PEGASOS", are the Authors referring to the spectrum in Figure S1? If so, please redirect the reader to Figure S1.

REPLY: True. We will correct the text.

- Section 4, lines 331-335: The Authors conclude that the correlation coefficients shown in Figure 7 for the NMR spectra of alpha-pinene vs. ambient WSOC are smaller than those between the HR-ToF-AMS spectra of PAM-generated SOA vs. ambient OOA reported in reference Lambe et al. (2011). Could this difference be explained by the fact that Lambe et al. (2011) is focusing on ambient OOA, which is a fraction of total organic aerosols (OA) and a proxy for SOA, whereas in this study the Authors are comparing the NMR spectra of alpha-pinene with those from the whole WSOC fraction which probably also includes a small fraction of primary OA? Secondly, I did not find Figure 9 in reference Lambe et al. (2011).

REPLY: The Referee is correct, the correlation coefficients between lab-generated SOA and ambient OOA AMS were in fact reported in Fig. 5 in Lambe et al. (2011) not Fig. 9. We have changed the text. We acknowledge that the lack of full overlap between OOA and WSOC in ambient aerosol complicates the interpretation of the different results obtained in this study with respect to Lambe et al. (2011). Regarding the Referee's comment on the overlapping between OOA and WSOC, we have reasons to believe that OOA are not a subclass of WSOC: recent findings reported by Xu et al. (2017) indicate that ambient OOA can be fully or only partially water-soluble depending on their oxidation degree. We will add a sentence in the text to make the reader more aware of the OOA vs. WSOC issue:

“It should be noted, finally, that a comparison of the AMS and NMR techniques with respect to their ability to trace chemical ageing in laboratory SOA and ambient oxidized aerosols is challenged by the incomplete overlap between the classes of organic compounds contributing to OOA and to WSOC (Xu et al., 2017).”

3. Technical corrections:

- Section 2.1, Tables 1 and 2: There is some information missing from the “Oxidation Level” column for Pin#3 (Med.) and Pin#5 (Low) in Table 1, and for Iso#2 (med) in Table 2. Could you please also clarify the meaning of “f44” in these Tables? Is this corresponding to the m/z marker of COOH formation withdrawn from the AMS data? If so, this should be clarified in the manuscript.

REPLY: “f44” refers to the fraction of the m/z 44 signal with respect to total OA in the AMS electron impact MS spectra. We will include a statement of explanation in Section 2.1:

“By varying the concentrations of OH inside the PAM reactor, SOA with different oxidation state could be obtained. For instance, the OH exposure varied from 2.0E11 molec./cm^3*s to 2.1E12 molec./cm^3*s between the α-pinene experiments and the resulting SOA oxidation degree – traced by the “f44” parameter (i.e., the fraction of the m/z 44 signal with respect to the total OA) – increased from 0.05 to 0.24.”

We thank the Referee for pointing out missing f44 values from Table 1: Pin#3 oxidation level = med (f44 = 0.11), and Pin#5 oxidation level = low (f44 = 0.05). The AMS was instead not operative during experiment Iso#2 (Table 2).

- Section 2.2, line 144: Figure S1 shows the 1H NMR spectrum of an ambient WSOC sample collected in San Pietro Capofiume and not the 1H NMR spectra of methanol extracts from isoprene SOA samples. Instead, it could be Figure S2?

REPLY: We apologize for the wrong reference and will correct it in the revised manuscript.

- Section 2.4, line 167: I did not find the full HPLC-UV-TOC analytical protocol in the Supplementary material.

REPLY: The full description of the analytical protocol can be found in Mancinelli et al. (2007). We will change the sentence “The full HPLC-UV-TOC analytical protocol is reported in the Supplementary material” into “The HPLC column and chromatographic conditions used in this study were the same as in Mancinelli et al. (2007)”

References:

Lambe, A. T., Chhabra, P. S., Onasch, T. B., Brune, W. H., Hunter, J. F., Kroll, J. H., Cummings, M. J., Brogan, J. F., Parmar, Y., Worsnop, D. R., Kolb, C. E. and Davidovits, P.: Effect of oxidant concentration, exposure time, and seed particles on secondary organic aerosol chemical composition and yield, Atmos. Chem. Phys., 15, 3063–3075, 2015.

Xu L., Guo H., Weber R. J., Ng N. L.: Chemical characterization of water-soluble organic aerosol in contrasting rural and urban environments in the southeastern United States, Environ. Sci. Technol., 51, 78−88, 2017.