Qualification of an online device for the measurement of the oxidative potential of atmospheric particulate matter

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Revision Notes

Dear Pr. Francis Pope,

Thank you for giving us the opportunity to submit a revised draft of our manuscript "Qualification of an online device for the measurement of the oxidative potential of atmospheric particulate matter" to Atmospheric Measurement Techniques. We appreciate the time and efforts that you and the reviewers have dedicated to providing us with valuable feedback on our manuscript. We are grateful to the reviewers for their insightful comments on our paper. Their detailed feedback has been invaluable to improve the clarity, completeness, and scientific accuracy of the manuscript. We have been able to incorporate changes to reflect most of the suggestions provided by the reviewers.

These changes are denoted in red in the new manuscript, and reported here on a smaller font size. Here is a point-by-point response (in bold) to reviewers' comments and concerns (in italics).

We hope that these revisions and clarifications satisfactorily address the reviewers' concerns.

Sincerely, Albane Barbero on behalf of all co-authors

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Title: "Qualification of an online device for the measurement of the oxidative potential of atmospheric particulate matter"

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Referee #1(Kalberer Markus) comments: *Note: referee #1 will be referred to here as RC1.*

1. *Line 62: It is mentioned in the manuscript that "First, these methods **probably** underestimate PM redox activity due to the very short lifetime of some ROS…": In a recent paper by Campbell et al., 2025 (DOI: 10.1126/sciadv.adp8100) it is clearly shown that ROS and OP in a wide range of particle sources decay with half-lives of minutes to hours and that offline methods severely underestimate ROS and OP. Please add this reference.*

   Answer: RC1 has raised an important point here. We have added the reference to Campbell et al., 2025 and revised the sentence accordingly to emphasize the rapid decay of ROS and the limitations of offline methods.

   "First, these methods severely underestimate PM redox activity due to the very short lifetime of some ROS (such as hydroxyl radical ˙OH) and/or the loss of the most volatile compounds (such as formaldehyde HCHO) in a non-proportional and unsystematic way (Campbell et al., 2025; Jiang et al., 2019)."

2. *Line 70: The instruments described in Fuller et al. 2014 and Wragg et al., 2016 are fully automated instruments, please indicate this more clearly. The current text suggests these instruments are "semi-automated".*

   Answer: We would like to thank RC1 for catching this inconsistency. The text has been modified consequently.

   "Fuller et al. (2014) have designed a fully automated, portable and integrated DCFH technique, allowing the PM sampling and solubilisation before their subsequent analysis (Fuller et al., 2014)."

3. *Line 76: In a recent paper by Campbell et al. 2024 (https://doi.org/10.1016/j.envint.2024.109102) a more or less continuous OP(AA) data set over three months is shown. Please correct the statement that there are no published continuous OP measurements.*

   Answer: Indeed, the text written in this part of the manuscript was not up to date, thanks to RC1, it has now been modified consequently. We updated the sentence to reflect that such long-term continuous OP^{AA} measurements have indeed been published, referencing Campbell et al., 2024.

   "The sensitivity of this device was tested on several transition metals, biogenic and anthropic secondary organic aerosol, and a mix of them, concluding that the method allowed for OP measurement in polluted urban environments (Campbell et al., 2023). Puthussery et al., 2018, showed a quasi-continuous data set over 2.5 months of OP^{DTT} and in a recent paper by Campbell et al., 2024 a more or less continuous OP^{AA} data set over three months is shown. Nevertheless, the different OP assays being sensitive at varying levels to diverse ROS-generating compounds (Lin et al. 2022), using several assays is crucial to provide a wider picture of the redox processes at stake."

4. *Fig. 1: Upstream of the BioSampler, a "Filter holder with 20µM filter" is indicated. The function of this filter and the unit µM is not clear to me. Which particle sizes are removed here? Please describe in more detail.*

Answer: We'd like to thank RC1 for having seen the error, the "20 µM" refers to the particle cut-off diameter, it should be 20 µm and not µM as it is not a concentration. Furthermore, the diagram was not up to date because we don't use a filter after all, but a simple protective grid to prevent the intrusion of insects or other elements that might clog the sampler's nozzles. The diagram has been modified accordingly. Again, thank you to RC1 for pointing out this inconsistency. Additionally, the text was modified accordingly.

"To maximise the PMs collection, the instrument was set for a sample duration of 30 minutes at a flow rate of 10.5 $l.min^{-1}$. A protective grid was placed on the inlet line in order to protect the instrument from larger particles that might have clogged the BioSampler® or damaged the FCs."

5. *Fig. 1: Why was the milliQ water reservoir on a magnetic stirrer? Please explain shortly.*

Answer: Thanks to RC1 for pointing out the lack of explanation for this setup. It ensures homogeneous temperature distribution and prevents stratification. This has been briefly explained in the revised text.

"A 20 l Jerrycan (GDPE Nalgene®) of MQ water automatically feeds a heated glass tank (1 l) that delivers water for collection and reactions at physiological temperature, this tank is kept on a magnetic stirrer to ensure homogeneous temperature distribution and prevents stratification."

6. *Fig. 1: A reservoir containing DTT+DTNB+PM is shown. For the AA analysis, where are the AA and BioSampler flows mixed? Is there another reservoir bottle that is not shown in Fig. 1? Or is the sample/AA solution kept in the detector for 30min? The AA analysis section should be explained in more detail.*

Answer: Thank you to RC1 for highlighting the need for clarification. In the AA line, the AA solution and the sample are first mixed inside the syringe pump during withdrawal. The resulting mixture is then introduced into the flow cell (FC), where the reaction is monitored by spectroscopy over 10 minutes. In the DTT line, since DTT itself is not absorbing but its reaction product (TNB) is, the DTT and sample are also first mixed in the syringe pump and then transferred to a reservoir to allow the reaction to proceed for 15 minutes. Subsequently, DTNB is added—again mixed with the reactive mixture in the syringe pump—and the resulting TNB is introduced into the FC for absorbance measurement at 412 nm. The manuscript has been modified accordingly.

"In the AA line, the AA solution and the sample are first mixed inside the syringe pump during withdrawal. The resulting mixture is then introduced into the flow cell (FC), where the reaction is monitored by spectroscopy at 265 nm over 10 minutes. In the DTT line, since DTT itself is not absorbing but its reaction product (TNB) is, the DTT and sample are also first mixed in the

syringe pump and then transferred to a reservoir to allow the reaction to proceed for 15 minutes. Subsequently, DTNB is added—again mixed with the reactive mixture in the syringe pump—and the resulting TNB is measured in the FC for absorbance measurement at 412 nm."

7. *Eq. 3: I assume V_{BS} is variable over time, depending on humidity, temperature etc. How was V_{BS} determined? Was the dilution by the addition of AA considered for V_{FC}?*

Answer: As mentioned, Vassalia probes (T° and Rh) were installed before and after the BioSampler® to evaluate the evaporation during sampling. Therefore, in the calculations of OP, corrected volume from evaporation is used. And all the volumes are taken into account. A sentence has been added to clarify.

"V_{BS} is calculated from the initial and final volumes measured via humidity and temperature sensors. Dilution due to reagent addition is negligible but accounted for via volume correction in data processing."

8. *Line 104: This paragraph discusses the offline and online method together, which is a bit confusing. Please separate the description of online and offline more clearly.*

Answer: We'd like to thank RC1 for pointing out the confusion. This section has been reorganized to first describe the offline method, then the online adaptation for better clarity.

"The offline method originally adapted from Cho et al., 2005 and Li et al., 2009a is fully described in Calas et al., 2017; Weber et al., 2018, 2021. Briefly, particulate matter (PM) extracts from atmospheric filters are introduced into two 96-well plates maintained under physiological conditions (pH = 7.4, T = 37 °C). To ensure homogeneity, the mixtures are shaken for 60 seconds for DTT and 10 seconds for AA. The absorbance of the matrix is read after 3 seconds for DTT and 10 seconds for AA. The reactions are initiated by adding AA or DTT to the respective plates, allowing the antioxidants to react with PM-induced reactive oxygen species (ROS). AA depletion is monitored continuously over 30 minutes at λ = 265 nm using a plate reader spectrophotometer (TECAN, model M1000 Infinite), and the depletion rate is determined by linear regression. DTT depletion is assessed indirectly by titrating the remaining DTT with dithionitrobenzoic acid (DTNB) at specific time points (0, 15, and 30 minutes). This reaction produces 5-thio-2-nitrobenzoic acid (TNB), which absorbs at λ = 412 nm and is measured using another plate reader (TECAN, model M200 Infinite). Both AA and DTT depletion are quantified using the Beer-Lambert law (Eq. 1) ... [] are calculated following Eq. 2… [] is the initial quantity of reagent [mol]. Blanks are performed using filter and field blanks to account for absorbance due to quartz fibre fragments or potential contamination.

The *ROS-Online* system automates the previously described offline assays for continuous ambient air monitoring. The flow-based system replicates the essential steps of the offline protocol while enabling time-resolved measurement of oxidative potential (OP). In the AA line, the AA solution and PM sample are first mixed during withdrawal into the syringe pump. This mixture is then transferred into the flow cell (FC), where the AA depletion is monitored by UV absorbance at 265 nm for 10 minutes. In the DTT line, since DTT does not absorb light directly but its reaction product TNB does, the DTT and sample are mixed in the syringe pump and stored in a reaction reservoir for 15 minutes. After this incubation, DTNB is added (again via mixing in the syringe pump), producing TNB. This final mixture is introduced into the FC, where absorbance at 412 nm is recorded to quantify the remaining DTT indirectly. For both lines, the Beer-Lambert law (Eq. 1) and the consumption rate

formula (Eq. 2) are applied, with the optical path *L* corresponding to the flow cell length in this case. Ultra-pure water (Type 1 Milli-Q®) is used in place of PM extract for blanks. The oxidative potential of ambient air is calculated using Eq. 3, following the approach by Fang et al. (2015b, 2016a) ... [] i.e. a modified BioSampler® (U.S Patent No. 5,902,385). V_{BS} is calculated from the initial and final volumes measured via humidity and temperature sensors. Dilution due to reagent addition is negligible but accounted for via volume correction in data processing. The sensitivity of ROS-Online enables one complete measurement of both OP^{AA} and OP^{DTT} over a 20-minute sampling period, repeated every 60 minutes in moderately polluted environments such as European urban background sites. ROS-Online is designed as a mobile, rack-mounted instrument (75 × 65 × 170 cm; 85 kg), requiring ~800 W of power and an operating environment of 15–30 °C. All components are temperature-controlled. The system is field-deployable and can be operational within a few hours. The current design supports autonomous operation for approximately two weeks, limited by ultra-pure water consumption and storage."

9. *Line 111/112: A 3-point measurement protocol was used to determine the consumption rates. How good is the linearity? Are any statistical analyses used to assess uncertainties of the kinetics determined?*

   Answer: Thanks to RC1 for raising this question. Indeed, only measurements with slopes having an R^2 between 0.9 and 1 are kept in the data post-treatment. Very few (< 0.1 %) measurements are discarded.

10. *Line 133: Measurements are repeated every 60 min. I assume this is the sum of 20min sampling and 30min analysis which leaves 10min. Please briefly mention what the 10min are used for.*

    Answer: As mentioned and clarified now in the text, the mixing is taking place in the syringe pump (line 152-155). Therefore, an automated in-line rinsing protocol is necessary between cycles to ensure there is no smoothing or memory effect between two cycles.

11. *Line 162/3: Same as above: the instruments described in Campbell 2019 and Wragg 2016 are fully automated. Please correct the respective wording.*

    Answer: We thank RC1 for catching this mistake. The text has been modified consequently.

    "Several automated and semi-automated prototypes characterising OP have been reported in the literature. Fang et al. (2015b) and Gao et al. (2017) adapted the widely used OP^{DTT} measurement, while Campbell et al. (2019) proposed a fully automated prototype based on AA chemistry following the design presented in Wragg et al. (2016)."

12. *Line 163: Eiguren-Fernandez et al. was published in 2017 instead of 2008.*

    Answer: We thank RC1 for catching this mistake. The text has been modified consequently.

    "Eiguren-Fernandez et al., 2017 developed an online monitor which combined a liquid spot sampler and a chemical module optimize. []"

13. *Fig. 3: Maybe this is a misunderstanding. I think the particle collection efficiency of the BioSampler was characterized by KCl particles. The respective size distribution is shown in Figure S1. It seems that there are no particles above ca. 200nm.*

Answer: Thanks to RC1 for their comments. Indeed, we use a KCl aerosol that contains few large particles but still contains some, with an initial particle concentration of 295.8 #/cm^3 at 300 nm and decreases to a level below 0.1 #/cm^3 at 5 µm, which allows us to calculate an efficiency for particles ≤ 5 µm but with greater variability, as shown by the error bars in Figure 3. Our results are consistent with the characterisation of SKC BioSampler® efficiencies as documented in recent literature (Su et al. 2020).

It shows U-shaped curves which are typically reported in literature for this type of bio aerosol sampler, thus our results are consistent with previously reported BioSampler collection efficiencies (Bøifot et al., 2024; Guo et al., 2024; Su et al., 2020).

14. *How was the particle collection efficiency determined up to 5 micrometres? If this was all done with KCl particles, is the presumably very low number concentrations at large sizes (i.e., micron-size) not too low to determine a reliable collection efficiency?*

Answer: As with the previous answer, we used a KCl aerosol containing a low number of particles larger than 5 µm. Therefore, the variability in efficiency is greater for these particle sizes. Nevertheless, the efficiencies obtained are very close to those reported in literature. To measure efficiencies up to 5 µm, it is necessary to use another aerosol, such as an Arizona Test Dust (ATD). This was not done in this study due to the unavailability of an ATD generator.

15. *Could you show a size distribution up to 5 micrometre of the particles used to calculate the collection efficiency as SI figure?*

Answer: Unfortunately, due to the use of KCl aerosol, it is not possible to obtain a particle size distribution greater than 5 µm, as the number of particles above this value is close to zero. This is why we only estimated collector efficiencies up to 5 µm; results above this value are uncertain and unreliable.

16. *In figure 3 11.5l/min are shown. Later in the text it is mentioned that 10.5l/min are used. Please comment on this difference.*

Answer: We thank RC1 for pointing out the inconsistency. There is no error, the BioSampler® tests were carried out between 9 and 11.5 l/min in order to frame the ROS-Online operating value of 10.5 l/min. A quick explanation has been added to the revised manuscript.

"In this study, efficiencies of BioSampler® were tested at sampling flow rates of 9 l.min^{-1} and 11.5 l.min^{-1} in order to frame the *ROS-Online* operating value of 10 l.min^{-1}."

17. *Line 244/5: When the particle collection efficiency decreases over time with the filling height of the BioSampler, how often does the BioSampler liquid have to be topped up? It is mentioned elsewhere in the manuscript, that the instrument presented here can run unattended for 2 weeks. Does the liquid content in the BioSampler stay constant over such a long time?*

Answer: It seems there was a misunderstanding. The BioSampler® is topped once at the beginning of the 20-min sampling with 20 ml of MQ water. It is then completely drained for the analysis and refilled with new MQ water for the next sample. During each 20-min sampling, the evaporation is calculated using the Vassalia probes (see answer N°7 above) in order to obtain the final volume for the OP calculation. Less than 2 ± 1 ml evaporation was observed under European conditions over one year. In addition, regarding the operation over two weeks, reservoirs are prepared in advance and progressively consumed throughout the campaign, ensuring a constant supply of MQ water for each sampling.

18. *Line 249: Probably a typo: "summarized", not "resumed"?*

Answer: Thanks for the correction that has been taken into account.

"Experimental conditions are summarized in Table 2. *ROS-Online* collected over 20 min at 0.6 m^3.h^{-1} (~ 10.5 l.min^{-1}) inside the main air flow at 20 m^3.h^{-1}"

19. *Line 299: It is mentioned that O_3 does not affect AA depletion. However, looking at figure S2, it seems that 50 ppb O_3 increases AA depletion by about 1.5 to 2 pmol/min. How does that relate to AA depletion rates at ambient concentrations (in units of pmol/min/m3)?*

Answer: We'd like to thank RC1 for the opportunity to discuss this particular experiment. As shown in Figure S2, an apparent increase in AA consumption can be observed when considering only the time series. To clarify this observation, Figure S2 has been added and modified. It demonstrates that, despite the introduction of elevated ozone (O_3) concentrations into the system, AA consumption remains unaffected. A closer inspection of the time series reveals that elevated AA consumption values were also recorded on days with relatively low O_3 levels (e.g., 15/11), while no increase in AA consumption was observed during a pronounced O_3 peak (07/11). This further supports the conclusion that O_3 does not significantly impact AA depletion under our experimental conditions. This is consistent with the low water solubility of ozone, which limits its reactivity in the aqueous phase. However, as mentioned in the manuscript, we think that further investigations are necessary

to assess the potential impact of other oxidizing gases. This point is now explicitly addressed in the revised section.

"Indeed, the oxidative potential of O_3 is not effectively captured via AA depletion in *ROS-Online*, potentially because: 1) ozone adsorbs or reacts with surfaces (e.g. pipes) before entering the sampler, or 2) ozone is not very soluble in water and/or the contact time between water and air is insufficient, so it cannot be trapped. In atmospheric conditions, it tends to adsorb / react with surfaces or airborne particles before it can dissolve in a liquid-phase assay (Bates et al., 2019; Bellini and De Tullio, 2019; Chang et al., 2021; Charrier and Anastasio, 2012). Other atmospheric oxidants, including more water-soluble inorganic and organic gaseous compounds, could also contribute to AA or DTT depletion, and further investigations are required to evaluate their role in the overall oxidative potential of the gas phase."

20. *To interpret for example ambient data in figure 9a, where OP^{AA} follows closely the diurnal ozone time trend such an assessment would be important, i.e., it would be interesting to know how much of the AA diurnal cycle can be attributed to direct oxidation of AA by ozone?*

Answer: As mentioned in section 3.2.1 and in answer N°19, the oxidative potential of O_3 is not effectively captured via AA depletion in *ROS-Online*, potentially because: 1) ozone adsorbs or reacts with surfaces (e.g. pipes) before entering the sampler, or 2) ozone is not very soluble in water and/or the contact time between water and air is insufficient, so it cannot be trapped. In atmospheric conditions, it tends to adsorb / react preferably with surfaces or airborne particles before it can dissolve in a liquid-phase assay (Bates et al., 2019; Bellini and De Tullio, 2019; Chang et al., 2021; Charrier and Anastasio, 2012).

21. *Other gaseous compounds (organic and inorganic) could also react with AA. If this was not investigated, please add a respective sentence that other gaseous components could potentially affect the measured AA depletion rates.*

Answer: We'd like to thank RC1 for pointing that out. A concluding sentence has been added at the end of Section 3.2.1 to explicitly acknowledge that only ozone (O_3) was tested in this study. It highlights that other, more water-soluble oxidising gases could also contribute to the overall atmospheric oxidative potential, in addition to particulate matter. The need for further research to assess and quantify the oxidative potential of these gaseous species is now clearly stated

"Other atmospheric oxidants, including more water-soluble inorganic and organic gaseous compounds, could also contribute to AA or DTT depletion, and further investigations are required to evaluate their role in the overall oxidative potential of the gas phase."

22. *Line 307: Please indicate what the Cu and PQN concentrations in nM in the calibration solutions correspond to in µg/m3 in air, taking into account the air sampling flow rates, the 20ml in the BioSampler etc.?*

Answer: The manuscript has been modified accordingly.

"$CuCl_2$ ([5– 50] nM) and PQN ([5 – 150] nM) standard solutions were selected to assess the OP^{AA} and OP^{DTT} sensitivities, respectively, of the system. Concentrations ranges for Cu(II) and PQN were chosen to be representative of concentration levels measured in the atmosphere of European cities, i.e., $CuCl_2$ [0.013 – 0.31] µg.m^{-3} and PQN [0.0013 – 0.1619] µg.m^{-3} (Delgado-Saborit et al., 2013; Denier van der Gon et al., 2013; Liu et al., 2024; Yang et al., 2018)."

23. *Fig. 5: Calibration curves with PM are shown down to 5 µg/m3. In the abstract it is mentioned that the LOD is about 20µg/m3. Please comment on this apparent discrepancy. How was the LOD in units of µg/m3 derived?*

Answer: RC1 is raising an important point here. Indeed, the LOD in Fig. 5 corresponds to the minimum detectable air concentrations using spiked samples. In the abstract, the 20 µg/m³ refers to the minimum mass concentration for reliable ambient detection. We added a sentence clarifying this distinction between direct sample and atmospheric sample.

"Each standard solution was analysed for OP in triplicate and the three replicates were used to calculate the standard deviations for the online method. It is important to note that the limits of detection (LODs) presented in Figure 5 correspond to the minimum detectable concentrations based on spiked samples, while the value of 20 µg.m^{-3} mentioned in the abstract refers to the minimum ambient PM mass concentration required for reliable detection under real atmospheric sampling conditions."

24. *Line 344/346: It is mentioned that the solutions are illuminated continuously, if I understand correctly, in the flow cells for detection for 10min (for AA) or for 15min (for DTT). Was the degradation of AA not measured for 30min? Was the DTT solution not pumped from the reservoir (labelled "DDT+H2O+PM" in Fig. 1) into the flow cell after being reacted in the dark for 15 or 30 min? Or did the reaction of DTT+PM happen inside the illuminated flow cell?*

Answer: This appears to be a misunderstanding. In the online method, AA depletion is monitored over a 10-minute period, but the solution in the flow cell is not continuously illuminated. Instead, absorbance measurements are taken at regular intervals (e.g., at 2, 4, 6, 8, and 10 minutes), with each acquisition involving a short illumination period of approximately 2 seconds. Similarly, for the DTT assay, the titration of TNB is performed at three time points, and each step is monitored for 2 minutes with repeated short illuminations of 2 seconds each. This has been clarified in the revised manuscript through a reorganisation of Section 2.1.1, as referenced in response No. 8.

25. *Fig. 7a: Are the relatively large error bars for the offline data and AA expected and typical for this offline method? If yes, you could emphasize the much better repeatability of your online instrument compared to the offline method, which would be a big advantage of your instrument.*

Answer: RC1 raises a valid and relevant concern here. However, it should be noted that the offline method here was not used in its normal conditions but in the online conditions to allow the comparison, therefore, making it less repeatable for this range of PM concentration. Nevertheless, the text has been revised to emphasize the better repeatability as suggested.

"Quite large error bars on the TECAN microplate reader when measuring AA depletion can be noticed. It should be noted that the offline measurements presented here were performed under the same operational conditions as the online method to allow direct comparison, which may have affected the typical repeatability of the offline protocol. Despite this, the significantly lower variability observed in the online data highlights the improved repeatability and robustness of the *ROS-Online* instrument."

26. *Line 401: It is mentioned that a "20µm filter" was placed in-line. Is this an impactor with a cut-off of 20µm or an actual filter? Please describe this device and its characteristics in more detail, especially its behaviour with respect to retaining particles below 20µm.*

Answer: Comment N°4 allowed us to identify and correct an error in the manuscript. A protective grid was indeed used during the experiment, and the text has been revised accordingly, as already noted in Response N°4.

27. *Line 405: Please comment why the time resolution of the instrument increases when the DTT analysis is not done. I was under the impression that the two analyses (AA and DTT) are running in parallel.*

Answer: RC1 highlights a critical aspect that warrants clarification. A complete measurement cycle, with both AA and DTT lines operating in parallel, is constrained by the DTT assay, which requires 15 minutes for measurement — compared to 10 minutes for the AA assay — as well as additional time for titration point preparation and rinsing. Disabling the DTT line to perform AA-only measurements eliminates this dormant time, thereby enabling higher temporal resolution for the *ROS-Online* system. The text has been modified consequently.

"OP^{DTT} measurements were maintained from September 1st to September 6th only, allowing a higher resolution of OP^{AA} measurements after this date. Indeed, a complete measurement cycle, with both AA and DTT lines operating in parallel, is constrained by the DTT assay, which requires 15 minutes for measurement — compared to 10 minutes for the AA assay — as well as additional time for titration point preparation and rinsing. Disabling the DTT line to perform AA-only measurements eliminates this dormant time, thereby enabling higher temporal resolution for the *ROS-Online* system."

28. *Line 408: It is mentioned that 1.3ml of the BioSampler solution evaporates. Please indicate over what time span this evaporation was observed. Does that limit the time the instrument can be operated autonomously? Is the BioSampler liquid refilled automatically?*

Answer: RC1 appropriately highlights the need for clarification. The reported evaporation of 1.3 ml corresponds to the average volume loss observed during each 30-minute sampling period (see also Response N°17). This evaporation is measured and accounted for in every cycle to ensure accurate volume correction. The text has been revised accordingly to clarify this point.

"Finally, evaporation during sampling was evaluated to be (1.3 ± 0.1) ml with minimum and maximum values of 0.8 and 1.9 ml, respectively, mainly depending on the temperature difference between the outside and the shelter's thermostated temperature. The reported evaporation of approximately 1.3 ml corresponds to the average loss measured during each 30-minute sampling period and is systematically corrected for during each analytical cycle."

29. *Fig. 9/S8: It seems that the diurnal DTT data is noisier that the AA data. Please add confidence intervals to the average diurnal cycles shown in figure 9. This would allow us to estimate how much of the detailed DTT structure is due to day-to-day variability.*

Answer: To clarify this comment, we have modified Figures 8, 9, S4 and S8 accordingly.

30. *Line 448-452: The description that this instrument measures particle and gaseous OP at the same time is not consistent with the rest of the manuscript. As far as I could see Fig. S2 is the only place where gaseous responses of the instrument are characterised and I think the conclusion of this section is that gaseous components such as O_3 are not resulting in a strong instrument response (which I think needs more discussion, see comment above). If the conclusion section emphasises that gaseous OP is a main feature of the new instrument, then more data should be presented and discussed.*

Answer: Thanks to RC1 for this important observation. Our intention was to highlight that, unlike offline methods, the *ROS-Online* system takes samples from the BioSampler® solution, which may both contain soluble gaseous compounds alongside particulate matter. These gaseous species, although currently unquantified, could contribute to the observed OP responses (OP^{AA} and OP^{DTT}). However, comprehensive evaluation of the instrument's sensitivity to gaseous oxidative potential requires further investigation, ideally within controlled atmospheric chamber experiments where gaseous and particulate contributions can be independently assessed. Accordingly, the conclusion has been revised to clarify that while *ROS-Online* is capable of capturing both particulate and gaseous OP, only preliminary tests on gases have been conducted and additional research is necessary.

"Its innovative design, incorporating dual independent lines for simultaneous OP^{AA} and OP^{DTT} assays, allows for near-real-time measurements with high reliability and specificity. Notably, *ROS-Online* has the potential to capture oxidative potential contributions from both particulate matter and soluble gaseous species present in the BioSampler® solution, providing a more

comprehensive assessment of air quality compared to the traditional offline methods that collect PM only. However, current results on gaseous OP are preliminary, and further controlled experiments are required to fully characterize the instrument's response to atmospheric gases."

Referee #2 comments: *Note: referee #2 will be referred to here as RC2.*

General comments:

1. *This manuscript uses a standard and commercially available device to collect the ambient PM in suspension. The device operates at a fixed sampling flow rate, which is generally low (10.5). Given this, the instrument works great in a polluted environment where the high PM concentrations can easily yield concentrated slurry in the BioSampler. However, I am not clear how the instrument will work in the clean (low pollution) environment. The authors mention that the concentration of antioxidants can be decreased to enhance the sensitivity of the OP method. However, given antioxidant oxidation rate is proportional to initial concentration, this will change the activity. In such a case, how can one compare the activity obtained from different studies?*

   Answer: We thank RC2 for this valuable comment. However, it seems there was a misunderstanding as ROS-Online is working in a clean environment such as European background. Its LOD is ~ 20 µg. m^{-3} as mentioned in the introduction. Furthermore, it is mentioned in the conclusion that "the key strength of *ROS-Online* is its versatility across diverse environmental conditions. The possibility to adapt antioxidant concentrations allows OP measurements in both relatively clean European urban environments and highly polluted regions and or industrial sites, where particulate matter concentrations are significantly higher. This flexibility ensures accurate assessments regardless of pollution levels, making the device suitable for global deployment." Nevertheless, to make it clearer, we've added two sentences in the revised manuscript conclusion to ensure better clarity.

   "A key strength of *ROS-Online* is its versatility across diverse environmental conditions. The possibility to adapt antioxidant concentrations allows OP measurements in both relatively clean European urban environments and highly polluted regions and or industrial sites, where particulate matter concentrations are significantly higher. To improve sensitivity in low-PM environments, i.e. < 20 µg.m^{-3}, *ROS-Online* can operate with modified reagent concentrations. Any such adjustments require calibration and documentation to ensure comparability. This flexibility ensures accurate assessments regardless of pollution levels, making the device suitable for global deployment."

2. *How did the authors withdraw the extract from the BioSampler in an automated manner? I didn't see any description of the modification of the BioSampler.*

   Answer: Thanks to RC2 for pointing that out. In section 2.1.1, it is mentioned that the BioSampler is modified. However, we've adapted the sentence to insist on the fact that it was custom modified for this specific application.

   "where V_s is the ambient air volume sampled by *ROS-Online*; V_{FC} the FC volume; and V_{BS} the final sampled volume, considering evaporation in the sampling device, i.e. a modified BioSampler® (U.S Patent No. 5,902,385). The BioSampler® was specifically

modified by a glassblower to include a custom-fabricated bottom outlet, facilitating automated extraction of the collected liquid sample."

Specific comments

1. *Line 35: The reference "State of Global Air Report" is repeated.*

   Answer: Thanks RC2 for spotting this error, it has now been modified.

2. *Line 76: "none is currently qualified for long-term automatic online measurements". I think the instrument developed by Puthussery et al. 2018 (AMT) has been successfully deployed in several field campaigns. It is surprising that the manuscript doesn't even discuss th*at.

   Answer: We thank the reviewer for this observation. We have now modified the text to include the work of Puthussery in the discussion.

   "The sensitivity of this device was tested on several transition metals, biogenic and anthropic secondary organic aerosol, and a mix of them, concluding that the method allowed for OP measurement in polluted urban environments (Campbell et al., 2023). Puthussery et al., 2018, showed a quasi-continuous data set over 2.5 months of OP^{DTT} and in a recent paper by Campbell et al., 2024 a more or less continuous OP^{AA} data set over three months is shown. Nevertheless, the different OP assays being sensitive at varying levels to diverse ROS-generating compounds (Lin et al. 2022), using several assays is crucial to provide a wider picture of the redox processes at stake."

3. *Line 106: "AA and DTT are then respectively added in each plate to initiate their oxidation by PM induced ROS": I think the author is confused here. The oxidation of AA and DTT is initiated mostly by PM, leading to the formation of ROS. Although, the oxidation might be initiated by PM-induced ROS but to what extent is not known.*

   Answer: RC2 has raised an important point here. The section has been modified (see answer N°8 to RC1). Therefore, the text has been modified and no more confusion should be found.

4. *Line 147: It is a good strategy to install both temperature and humidity sensors at the inlet and outlet of the unit to assess the extent of evaporation in the BioSampler. However, the question remains how you take this evaporation into account for calculating the DTT activity. As I understand, the evaporation will increase the concentration of DTT in the reaction vial. It is OK if the extent of evaporation is constant every time. However, that is not the case. So, my question is that in an automated system like this, do you have to do post-processing of the data to adjust for this evaporation effect in the DTT activity calculation? And if so, how do you do it?*

Answer: We thank the reviewer for this important question. In fact, DTT (as well as DTNB and AA) is added after the air sampling step, during the controlled reaction phase, where fixed and known reagent and sample volumes are used. As a result, the concentration of DTT in the reaction cell is not affected by the evaporation occurring during sampling. However, what can vary due to evaporation is the actual volume of air-derived samples collected in the BioSampler. This impacts the calculation of OP when normalized to the volume of air sampled (in m^3). To account for this, ROS-Online continuously monitors temperature and humidity at both the inlet and outlet of the sampling system. These values are used to estimate the evaporation and determine the final collected sample volume (V_{BS} in Equation 3 of the manuscript). This corrected volume is then used in post-processing to accurately calculate the oxidative potential (OP) in standard units ($nmol.min^{-1}.m^{-3}$). We have clarified this point in Sections 2.1.2 of the revised manuscript.

We replace "Evaporation may occur during the 20 min pumping period, due to temperature and pressure differences between ambient air and air in the device. Temperature and humidity sensors installed at the inlet and outlet of the unit allow us to assess the actual water sample volume left at the end of each sampling period." by "During sampling, a fraction of the water in the BioSampler® reservoir can evaporate due to the temperature differential between the ambient air and the thermostated sampling chamber (37 °C). To monitor this, *ROS-Online* is equipped with temperature and relative humidity sensors at the inlet and outlet of the BioSampler®. These data allow the estimation of the actual volume of liquid remaining at the end of the sampling period. Since the oxidative potential (OP) is expressed per cubic meter of air sampled (Eq. 3), the corrected final sample volume is essential for accurate post-processing and normalization. It is important to note that DTT as well as AA and DTNB are added after the sampling step, during the controlled reaction phase, and always in fixed volumes. Therefore, evaporation during sampling does not affect the reagents concentrations in the reaction, but only the total sampled air volume used to calculate OP."

5. *Line 186: Is the slope of DTT and AA determined based on the measurements conducted only at two time points, i.e. t=10 and 15 minutes. Does this account for any non-linearity in case of very high DTT/AA activity?*

Answer: We thank the reviewer for this important clarification. The slopes for both AA and DTT assays are not based on only two time points. This point has been answered for RC1 (N°24): in the online method, AA depletion is monitored over a 10-minute period, but the solution in the flow cell

is not continuously illuminated. Instead, absorbance measurements are taken at regular intervals (e.g., at 2, 4, 6, 8, and 10 minutes), with each acquisition involving a short illumination period of approximately 2 seconds. Similarly, for the DTT assay, the titration of TNB is performed at three time points, and each step is monitored for 2 minutes with repeated short illuminations of 2 seconds each. This has been clarified in the revised manuscript through a reorganisation of Section 2.1.1, as referenced in response No. 8 to RC1.

6. *Figure 3: Did the authors compare the results of their characterization of the BioSampler with existing literature. BioSampler is a standard commercially available device and I am sure there should have been studies on characterizing its size-segregated collection efficiency.*

Answer: Yes, and we thank the reviewer for pointing this out. Text has been modified to make it clearer.

"The fractional collection efficiency at different flow rates calculated using Eq. 5 are reported in Fig. 3. It shows U-shaped curves which are typically reported in literature for this type of bio aerosol sampler, thus our results are consistent with previously reported BioSampler collection efficiencies (Bøifot et al., 2024; Guo et al., 2024; Su and Vincent, 2004)."

7. *Line 270: The oxidation of AA doesn't involve Fenton-type radical reactions. The superoxide radicals generated following the oxidation of AA can react with Fe, which is a Fenton reaction.*

Answer: We revised this sentence and clarified that transition metals may catalyse AA oxidation through redox cycling mechanisms, not necessarily via Fenton chemistry.

"This result is consistent with the commonly reported sensitivity of ascorbic acid (AA) to transition metals. Although the oxidation of AA itself does not proceed via Fenton-type radical reactions, the superoxide radicals generated during its oxidation can subsequently react with iron through Fenton chemistry (Bates et al. 2019; Fang et al. 2016; Bresgen et Eckl 2015; Pietrogrande et al. 2022)."

8. *Section 3.2.1: The ozone interference tests were conducted only for AA assay and not for the DTT assay, why? Also, why didn't the authors consider using activated charcoal to remove ozone?*

Answer: We thank RC2 for this relevant observation. At the time of the experiment, our focus was solely on the AA assay, as it exhibited profiles comparable to O_3 during the Chamonix campaign. Regarding ozone removal, we deliberately chose not to use activated charcoal or other ozone scrubbing techniques, as our objective was to maintain atmospheric conditions as representative as possible of what humans actually inhale.

9. *Figure 7: The horizontal error bars in this figure are quite large. Though, the authors mention them in the text below, what is the cause of such large uncertainty in the offline measurements.*

   Answer: RC2 raises a valid and relevant concern already commented by RC1. Please refer to answer N°25 for RC1.

10. *Line 402: Use of 20 µm filter: The filter by filtration theory can affect much smaller particles than 20 µm (e.g. by diffusion). Did the authors characterize the penetration efficiency of this filter before deployment? Also, if the authors intended to remove larger particles, why didn't they consider the use of PM10 impactors which will affect only larger particles and not smaller?*

    Answer: We thank RC2 for the opportunity of clarification. As answered to RC1 concerns (N°4 - N°26), no filters were actually used in this study. The decision to include size-selective inlets (e.g., PM_{10} or $PM_{2.5}$ impactors) is made depending on the objectives and constraints of each campaign. For instance, during a separate campaign in Marseille — currently under analysis as part of an ongoing PhD project — a $PM_{2.5}$ sampling head was installed at *ROS-Online'*s inlet to enable intercomparison between different online analysers. These results are still under investigation.

Referee #3 comments: *Note: referee #3 will be referred to here as RC3.*

1. *The abstract should mention the results of the comparison between the online device and the offline measurements.*

   Answer: We agree and have added the following sentence at the end of the abstract:

   "Comparison of *ROS-Online* measurements with established offline methods showed an excellent correlation for both AA and DTT assays (r > 0.96), supporting its reliability for atmospheric monitoring. These preliminary results mark an important step towards establishing *ROS-Online* as a viable and effective tool for OP assessment in future research and monitoring endeavours."

2. *Line 35: please fix the citation, it looks like it is repeated twice*

   Answer: Thank RC3 for noticing. The duplicate reference has been removed.

3. *Line 44: trace metals should also be mentioned as a potential cause for oxidative stress and Line 46: transition metals do not act only as ROS producers, they can also do redox chemistry themselves depending on their oxidation state. Some relevant literature on OP has been omitted in the introduction:*

   Answer: We agree with RC3 who has raised an important point here. We have included trace metals in the discussion of causes of oxidative stress.

   "More specifically, these mechanisms are driven by oxidative stress and involve reactive oxygen species (ROS) carried or induced by PM (Campbell et al., 2019; Delfino et al., 2013; Strak et al., 2012), and redox-active transition metals which, depending on their oxidation state, may participate in ROS production or act as direct oxidants, but also by certain trace gases (Dovrou et al., 2021a). Trace metals are also recognized contributors to oxidative stress due to their catalytic redox properties."

4. *Some relevant literature on OP has been omitted in the introduction.*

   Answer: The reviewer has raised an important issue that we have now addressed in the revised manuscript by adding a new short paragraph.

   "Recent advances in the field have emphasized the role of short-lived reactive species in OP (Campbell et al., 2025) and the impact of aerosol acidity and ligand-mediated metal solubility on OP (Shahpoury et al., 2019, 2021, 2024a, b). Furthermore, newer antioxidant assays are being explored to complement traditional metrics (Shahpoury et al., 2019)."

5. *In the paragraph starting at line 68, it would appear that the methods developed by Wragg, Campbell and Utinger are semi-automatic but they have been deployed for online/real-time measurements.*

   Answer: We thank RC3 for his observation. The manuscript has been modified following RC1 and RC2 similar concerns. (N°2 for both RC1 and RC2)

6. *Line 73, the prototypes by Campbell and Utinger do not combine two assays in one instrument, they are actually two different instruments: one for ascorbic acid and one for DCFH.*

Answer: We appreciate the reviewer's comment and have addressed it as follows:

"More recently, two separate prototypes were developed to assess online OP using fluorescence methods, one based on the OP^{AA} assay and the other on the OP^{DCFH} assay (Campbell et al., 2019; Utinger et al., 2023)."

7. *Line 105, what filters have been used, and what was the extraction procedure?*

Answer: We thank the reviewer for this relevant question and appreciate the opportunity to clarify. The manuscript has been revised accordingly to provide additional information on the filters used and the extraction procedure. As the focus of the paper is on the online method, we have kept the description of the offline method concise to maintain the overall balance of the manuscript. However, in response to the reviewer's comment, we have added further details to the brief explanation in the text. For a more comprehensive description of the offline procedure, we kindly refer the reviewer to the references cited in the manuscript.

"Briefly, particulate matter (PM) extracts from atmospheric filters (150 mm-diameter pure quartz fibre filters) during 75 minutes are introduced into two 96-well plates maintained under physiological conditions (pH = 7.4, T = 37 °C)."

8. *Please discuss possible interferences from brown carbon in the absorbance measurements.*

Answer: We thank the reviewer for this pertinent comment regarding the potential interference from brown carbon (BrC) in our absorbance-based measurements. BrC is known to absorb light in the UV–visible range, and therefore may contribute to the signal at the wavelengths used in our study (265 nm and 412 nm). As shown by Wu et al. (2023), BrC can contribute significantly to light absorption during biomass burning events, particularly at shorter wavelengths, with its absorption decreasing toward the visible range. Similarly, Basnet et al. (2024) demonstrated that emissions from residential biomass combustion in Europe contain BrC with strong absorption between 300 and 500 nm, which could influence absorbance measurements if not properly accounted for. In our method, absorbance is used to monitor the kinetic consumption or production of reagents, not just the absolute absorbance of the solution. Furthermore, we systematically include blanks and controls to correct for background absorbance, including that potentially attributable to BrC. This approach minimizes the influence of static absorbance contributions from sample matrix components. Nevertheless, we agree that BrC could represent a source of background interference, especially at 265 nm. To address this, we have added a brief discussion of this potential limitation to the revised manuscript, along with citations of the above studies in the conclusion.

“Potential interference from brown carbon (BrC) in our absorbance-based measurements. BrC is known to absorb light in the UV–visible range, and therefore may contribute to the signal at the wavelengths used in our study (265 nm and 412 nm). As shown by Wu et al., (2023), BrC can contribute significantly to light absorption during biomass burning events, particularly at shorter wavelengths, with its absorption decreasing toward the visible range. Similarly, Basnet et al., (2024) demonstrated that emissions from residential biomass combustion in Europe contain BrC with strong absorption between 300 and 500 nm, which could influence absorbance measurements if not properly accounted for. In our method, absorbance is used to monitor the kinetic consumption or production of reagents, not just the absolute absorbance of the solution. Furthermore, we systematically include blanks and controls to correct for background absorbance, including that potentially attributable to BrC. This approach minimizes the influence of static absorbance contributions from sample matrix components.”

9. *Line 141, please provide details of the filters used in the BioSampler®.*

Answer: We thank RC3 for the opportunity to clarify the operation of the BioSampler®. A sentence has been added in the revised manuscript.

“Airborne PM and soluble gases are collected into a BioSampler® filled with 20 ml MQ water by pumping ambient air at a constant flow rate of ~ 10.5 l.min-1. This airflow is monitored by using a Venturi flow meter. The BioSampler® nozzle section contains three tangential 0.630 mm nozzles that act as sonic orifices that maintains a pressure drop of ~ 0.5 atm or more across the sampler at normal atmospheric conditions (sonic flow) (BioSampler, 2024). Nebulisation of the water is generated via the nozzles during vacuuming ensuring optimal gas/liquid exchange and homogenisation of the solution. No physical filters are used inside the BioSampler®; particles are directly collected into MQ water.”

10. *Section 2.1.2, please provide more details on the reaction mixtures used, such as pH and buffer used, and any other additives/components.*

Answer: We appreciate the reviewer’s careful reading and thoughtful suggestion. The text has been modified accordingly.

“Tanks containing AA (185 µM), DTT (2.12 mM) and DTNB (2.12 mM) solutions, the last two being in phosphate buffer at pH = 7.4, are sheltered from the light and stored at T = 4 °C in a cooler and refreshed every 10 to 12 days.”

11. *Section 2.1.4 is not really a methodology section. It should come after the validation section.*

Answer: We acknowledge the structural suggestion. As the journal encourages chronological presentation, we chose to retain the current order but added clarification that Section 2.1.4 is intended for comparison with other devices.

Added sentence: "This section is intended to highlight how ROS-Online compares with existing instruments previously deployed for real-time OP measurements."

12. *Line 238-239, please provide a reference to support this statement. Smaller particles can contribute significantly to PM mass in some environments (e.g. traffic sites) and therefore a discussion on the collection efficiency of the instrument in this case should be added*

Answer: We thank the reviewer for this important comment. We agree that in certain environments, particularly near traffic sources or during specific atmospheric events, the contribution of sub-300 nm particles to PM mass can be non-negligible. In response, we have revised the text to include supporting references and a more nuanced discussion of the potential limitations of our collection efficiency in such cases. Specifically, we now cite observational studies showing that, under typical ambient conditions, most of the aerosol mass is associated with particles larger than 300 nm. For example, Brock et al. (2021) report that in remote or urban background sites, more than 90 % of the aerosol mass lies in the accumulation and coarse modes (particles > 300 nm). However, in high-emission environments such as roadside or tunnel locations, fine-mode particles (including < 300 nm) can contribute more significantly to PM mass (Vu et al., 2015; Cheng et al., 2010). In our study, the lower collection efficiency measured for particles < 0.3 µm is therefore not expected to significantly bias total aerosol mass collection under most atmospheric conditions. However, we now explicitly acknowledge in the revised manuscript that in environments dominated by freshly emitted ultrafine particles, such as traffic sites, this limitation may lead to an underestimation of the total collected mass.

"Indeed, KCl atomization produces relatively few large particles, with approximately 70 % of the total mass carried by particles larger than 300 nm. In contrast, ambient atmospheric aerosols are typically dominated by accumulation and coarse mode particles, with studies showing that more than 90 % of the mass is often found in particles larger than 300 nm in urban background or remote environments (Brock et al., 2021; Keuken et al., 2013). Therefore, the reduced collection efficiency observed for particles smaller than 0.3 µm is expected to result in only a limited loss of total collected mass across the 10 nm to 10 µm range. However, we acknowledge that in specific environments such as traffic sites, where freshly emitted ultrafine particles can contribute more significantly to PM mass, this limitation may be more relevant (Cheng and Lin, 2010; Gillies et al., 2001)."

13. *Line 249: resumed -> summarized*

Answer: Modified accordingly.

14. *Table 2, please add particle size and concentration*

Answer: We thank the reviewer for this suggestion. Unfortunately, the particle size distribution and concentration data were not recorded or available during the measurements reported in Table 2. Therefore, we are unable to provide these details.

15. *Line 271: Fenton-like reactions are not the only reactions that transition metals can do that contribute to OP. Some metals can oxidise directly for example.*

Answer: We appreciate the attention to detail shown by RC3. The text has been modified

"As shown in Fig. 4 (a) and (b) for $CuCl_2$ experiments 1 and 2, OP^{AA} confirmed its high metal-sensitivity (Calas et al., 2018a; Fang et al., 2016; Godri et al., 2011) with OP values of (21.6 ± 3.9) and (12.3 ± 0.8) nmol.min^{-1}.m^{-3}. This result is consistent with the commonly reported sensitivity of ascorbic acid (AA) to transition metals. Although the oxidation of AA itself does not proceed via Fenton-type radical reactions, the superoxide radicals generated during its oxidation can subsequently react with iron through Fenton chemistry (Bates et al. 2019; Fang et al. 2016; Bresgen et Eckl 2015; Pietrogrande et al. 2022). OP^{DTT} results show significant but less heterogeneous values, (14.5 ± 3.7) and (15.8 ± 3.6) nmol.min^{-1}.m^{-3}, respectively, as already evidenced in the literature (Charrier and Anastasio, 2012; Lin and Yu, 2011; Pietrogrande et al., 2022a)."

16. *Line 272, what do you mean by "heterogenous values" in this sentence?*

Answer: We thank the reviewer for bringing this to our attention. We have now revised the sentence to provide a clearer explanation.

"OP^{DTT} results show lower variability across experiments, (14.5 ± 3.7) and (15.8 ± 3.6) nmol.min^{-1}.m^{-3}, respectively, suggesting a less concentration-dependent response than AA, as already evidenced in the literature (Charrier and Anastasio, 2012; Lin and Yu, 2011; Pietrogrande et al., 2022a)."

17. *Line 277: how does the tested concentration compare to that you would expect in the ambient?*

Answer: We thank the reviewer for this relevant question. The concentrations of 1,4-naphthoquinone (NQ) and $CuCl_2$ used in our bench tests were indeed significantly higher than those typically observed in ambient air in European urban environments. However, this choice was intentional: our goal at this stage was to evaluate the instrument's performance well above the limit of detection (LOD), in order to validate its linearity and response behaviour under controlled conditions. These tests were not designed to simulate ambient concentrations, but rather to establish robust analytical performance prior to deployment in real atmospheric conditions.

18. *Section 3.2.1, my understanding is that the instrument is designed to capture both soluble gases and PM. In this respect, ozone would not be an interference. Please clarify.*

Answer: **We thank RC3 for this relevant observation. During the Chamonix campaign, OP^{AA} profiles exhibited similarities with ozone levels, which raised the question of a potential contribution of O_3 to the measured oxidative potential. To clarify this, we performed dedicated laboratory experiments to assess the response of *ROS-Online* to ozone. While O_3 is a strong oxidant, our results showed that *ROS-Online*'s OP^{AA} signal was not significantly influenced by the presence of O_3, even at concentrations up to 50 ppbv. These findings support the interpretation that ozone is not efficiently**

trapped or detected by the *ROS-Online* system, likely due to its limited solubility in water and/or rapid surface reactivity before entering the liquid-phase assay. We have now clarified this point and revised the corresponding section of the manuscript to better reflect the instrument's behaviour with respect to gaseous oxidants.

19. *Line 299, is the AA depletion comparable to that of the blank measurements or just constant over the O_3 concentration range tested? Is AA stable over 2-weeks of unattended operation? In my experience, AA solutions are not stable for more than a couple of days.*

    Answer: We thank RC3 for the opportunity to clarify the experimental findings. The AA depletion observed during ozone exposure experiments was comparable to that of the analytical blanks, with values typically around 5 pmol.min^{-1}. Furthermore, the AA solution is stored at 4 °C in a light-proof container to minimize degradation. Stability tests conducted over a 15-day operational period confirmed the reproducibility and consistency of the blank signal, indicating minimal spontaneous AA degradation under these storage and handling conditions.

20. *Why is ozone interference not discussed for the DTT assay?*

    Answer: We thank RC3 for this relevant observation. As already answered to RC2 comment (N°8), at the time of the experiment, our focus was solely on the AA assay, as it exhibited profiles comparable to O_3 during the Chamonix campaign.

21. *Line 313, how long was the extraction?*

    Answer: We thank RC3 for this question and respectfully refer to our response to comment N°7 for further details.

22. *Line 320, R^2 values look decent but by looking at the plot I would say that the linearity is at an acceptable level for the intended purpose rather than state that the response is linear. Fig 5b does not look linear by eye. And others, like Figure 5a, 5d and 5f also look like they have some deviations from linearity. I suggest running a statistical test.*

    Answer: We thank RC3 for this valuable observation. As reported in Table 5, we already provide the p-values, Pearson correlation coefficients, and R^2 values for each calibration curve. These statistical indicators collectively support that the observed relationships are sufficiently linear for the intended application, particularly considering the variability typically encountered in environmental and experimental samples. Nonetheless, we acknowledge the visual deviations noted by the reviewer and

have clarified our wording in the manuscript to reflect that the linearity is acceptable for the purpose rather than strictly linear.

"Except for PQN in the AA assay (Fig. 5(c)), the calibration responses were found to be acceptably linear for the intended application, as supported by the R^2 values, Pearson correlation coefficients, and p-values reported in Table 5. While some deviations from perfect linearity are observed, the statistical indicators confirm that the linear fits are adequate for use with environmental and experimental samples."

23. *Figure 5e and 5f, why were the filters from the two environments tested only one assay each rather than both? It would have been nice to see both.*

Answer: We thank RC3 for this insightful comment. The choice to test filters from the two environments using only one assay each was driven by limited extract volume and the priority to maintain compatibility between assay and sample source (e.g., AA assay for traffic-related samples, DTT assay for winter background samples). Additionally, the offline assay conditions were adjusted to align with the online measurement method, which resulted in lower sensibility.

24. *Line 342, the non-zero intercept looks to me that it could be due to a deviation from linearity.*

Answer: We thank RC3 for this thoughtful observation. However, we respectfully disagree with the interpretation that the non-zero intercept indicates a deviation from linearity. Our interpretation, supported by the discussion in the manuscript, is that the intercept is primarily due to the blank response, and the observed non-linearity is caused by self-consumption effects from anti-oxidants with oxygen. We believe this explanation more accurately accounts for the observed data.

25. *Line 357, why use amounts in mol rather than concentrations?*

Answer: We thank RC3 for this important question. The use of moles rather than concentration was chosen to ensure comparability between the online and offline protocols by maintaining equal reagent quantities. This approach helps keep the reaction kinetics similar in both methods, allowing for a meaningful comparison.

26. *Figure 6, I can see a deviation from linearity at low and high concentrations in a and b. Part c does not look linear to me.*

Answer: We thank RC3 for this careful observation. While we acknowledge the deviations from linearity at low and high concentrations in Figures 6a and 6b, and the appearance of non-linearity in Figure 6c, we consider the linearity to be sufficient for the purpose of comparison. It is also important

to note that the offline methods were modified from their original versions to align with the online protocol, which may contribute to these observations

27. *Figure 7, why not test both filters with both assays? Why do you have such a large horizontal error bar in panel a?*

Answer: We thank RC3 for this question. As addressed in our response to comment N°23, the decision to test each filter with only one assay was driven by practical considerations, including limited sample volume and assay compatibility. Additionally, the offline method was adapted from its standard protocol to align with the online measurements, as explained in our response to RC1's comment N°25. Regarding the large horizontal error bar in panel a, this reflects the variability inherent to the measurement under these adapted conditions. The text has been modified to account for those comments.

28. *LODs are missing from the validation.*

Answer: We thank RC3 for this comment. If the question refers to the limits of detection (LODs) related to blanks, we would like to clarify that the blanks for the two methods were deliberately not reported. This is because self-consumption effects differ between the two types of analysers due to variations in their spectroscopic components, such as lamps, spectrometers, and illumination protocols. These differences prevent a direct comparison of blank values between methods.

29. *It would have been nice to see an apportionment of the contribution of soluble gases vs. PM to the OP. Why was that not considered?*

Answer: We thank the reviewer for this insightful suggestion. Due to limitations in resources and time, we were unable to perform a detailed apportionment of the contributions from soluble gases versus particulate matter to the oxidative potential. Moreover, the primary purpose of this prototype is to provide OP values that closely reflect the actual exposure of humans to ambient air, making it suitable for integration into air quality monitoring stations.

30. *The ozone concentrations measured during the field campaign were higher than the level tested in the lab as it can be seen from Figure S9. Could there be interference there? It would be nice to expand the tested range in the lab.*

Answer: We thank RC3 for raising this point. However, we respectfully disagree with the concern regarding the ozone concentration range tested. It appears there was some confusion between units (ppbv and µg.m^{-3}), as 50 ppbv corresponds approximately to 100 µg.m^{-3}. In fact, the ambient ozone

concentrations measured during the field campaign fall within the range already tested in the laboratory.

31. *Line 461, I would argue that linearity was not always "good".*

Answer: We thank the reviewer for this observation. The term "good" has been revised in the manuscript to "acceptable" to better reflect the calibration results.

"Calibration using $CuCl_2$ and 9,10-phenanthrenequinone (PQN) demonstrated acceptable linearity for environmental applications for both OP^{AA} and OP^{DTT} assays, highlighting its sensitivity to both inorganic and organic classes of atmospheric oxidants."

32. *There are several duplicate references, such as Dominutti et al 2024/2025, Dovrou et al 2021a/b, Fang et al 2016a/b, Fang et al 2015a/b, Pietrogrande et al 2022a/b, Rao et al 2020a/b, Yu et al 2020a/b*

Answer: We thank the reviewer for pointing this out. All duplicate references have been carefully reviewed and removed to ensure accuracy and clarity in the reference list.